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## Effect of biomaterials on angiogenesis during vital pulp therapy

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### Abstract

This review intended to provide an overview of the effects of dental materials, used in dentin-pulp complex and dental pulp regeneration, on angiogenesis processes during regenerative endodontic procedures. An electronic search was performed in PubMed and MEDLINE databases *via* OVID using the keywords mentioned in the PubMed and MeSH headings for English language published articles from January 2005–April 2014 that evaluated the angiogenic properties of different dental materials used in regenerative endodontic procedures. Of the articles identified in an initial search, only 40 articles met the inclusion criteria set for this review. Vital pulp therapy materials might have positive effects on angiogenesis events, while most of the canal irrigating solutions and antibiotic pastes have anti-angiogenic activity except for EDTA. Future clinical studies will be helpful in defining the mechanisms of action for dental materials that promote or inhibit angiogenesis events at applied areas.

### Keywords

Angiogenesis; Dentin-pulp complex; Regeneration; Stem cells

## INTRODUCTION

Regenerative endodontic has gained widespread interest in recent years as it is attempting to fill the root canal space with living tissues instead of artificial materials<sup>1</sup>). Most of dental materials release inorganic trace elements<sup>2–5</sup>), and most of inorganic trace elements regulate angiogenesis<sup>6,7</sup>). In the field of regenerative endodontics the most important goal is to provide a suitable environment for the regeneration of healthy tissue and restore the lost biological tissues<sup>8</sup>). Based on the treatment site, this field is divided into two distinct categories of dentin-pulp complex regeneration and dental pulp regeneration<sup>1,8</sup>). The dentin-pulp complex regenerative procedures or vital pulp therapies include the direct pulp capping, indirect pulp capping, and pulpotomy. Direct pulp capping is defined as covering an exposed dental pulp with a protective agent and indirect pulp capping is referred to the application of

a protective agent, on a thin layer of dentin over the nearly exposed dental pulp<sup>4</sup>). The other treatment in vital pulp therapies is the pulpotomy. It is defined as the surgical removal of inflamed coronal part of the dental pulp in the exposed pulpal tissue to save the remaining healthy tissue<sup>5</sup>). In dentin-pulp complex regeneration, clinicians attempt to provide an effective pulp capping with appropriate sealing ability<sup>5</sup>), and maintain the vitality of irritated pulp tissues and promote the formation of a dentinal bridge<sup>8–10</sup>) and other tissues including neural cells<sup>11</sup>). In these procedures, the progenitor dental pulp stem cells (DPSCs) are migrated, recruited, and differentiated into odontoblast-like cells, which have the ability to produce reparative dentin<sup>9,12</sup>). Although, this issue seems very simple in theory, in reality the whole process is possible if the homeostasis of pulp is reestablished<sup>8</sup>). In other words, restorations of the vascular network, through up- or down regulation of pro- or anti-angiogenic growth factors, is a key determinant component that guides the regenerative procedure toward survival or necrosis of pulp tissue<sup>13</sup>).

The second field of regenerative endodontics deals with regeneration of dental pulp tissue in necrotic teeth<sup>14,15</sup>). In this procedure the treatment is initiated with the complete removal of necrotic dental pulp tissue, which is referred to as pulpectomy, by instrumentation and irrigation that is followed by disinfecting the root canal space<sup>16</sup>). After canal preparation, the regenerative treatment, the revascularization process, begins with instrumentation of periapical tissue to cause bleeding into the canal space. The blood clot formed inside the canal provides a provisional matrix scaffold for the recruited stem cells from apical papilla<sup>17</sup>). It is also demonstrated that complete disinfection plays a key role in the successful treatment outcomes<sup>18,19</sup>).

Beside the stem cells derived from apical papilla, other investigators have used tissue engineered DPSCs for transplantation into the empty canal<sup>20</sup>). In this treatment protocol, the establishment of a functional vascular network in transplanted tissue is the challenging goal for a successful result<sup>21</sup>). The formation of this vascular structure is possible through angiogenesis, which is defined as the formation of new blood vessels from pre-existing vasculature<sup>22</sup>). In addition to regeneration of dental pulp tissue, apexogenesis and apexification are other endodontic procedures that are performed in immature permanent teeth<sup>23</sup>). Apexogenesis is the procedure that enables the immature permanent teeth to continue root end development, while the apexification provides a calcified barrier at the end of immature root by biocompatible material next to periapical tissue<sup>23</sup>). It has been reported that the revascularization process occurs through the angiogenesis events derived from the periapical tissues that grow into the engineered pulp tissue. Furthermore, the immature teeth with open apices are the best candidates for these regenerative procedures<sup>24</sup>).

These facts emphasize angiogenesis as an important factor involved in homeostasis of dentin-pulp complex and dental pulp regeneration<sup>25</sup>). In addition, angiogenesis has a pivotal role in dentinal and dental pulp tissues' regenerative and reparative procedures<sup>1,9</sup>). The present study attempts to review the effects of dental materials and treatment modalities used in dentin-pulp complex and dental pulp regeneration on angiogenesis. The possible influences of dental procedures and materials on angiogenesis events are reviewed.

## MATERIALS AND METHODS

### The review purpose

In this review, the effects of dental treatments and materials used in direct or indirect pulp capping, pulpotomy, pulpectomy, apexogenesis, and apexification were overviewed on dental pulp tissue regeneration and revascularization. The main aspect of this review is to evaluate the possible influence of dental procedures and materials used in regenerative procedures, which can either promote or inhibit angiogenesis, during regeneration or revascularization.

### Inclusion and exclusion criteria

The inclusion criteria were: 1) studies accepted and published in English language between January 2005–April 2014; 2) the scientific *in-vivo*, *ex-vivo*, or *in-vitro* articles, reviews, systematic reviews, case reports, and clinical trials with controlled study design; 3) studies that had evaluated the effect dental materials used in regenerative endodontics, direct or indirect pulp capping, pulpotomy, pulpectomy, apexogenesis, and apexification treatments on angiogenesis processes occurring in the applied area.

The exclusion criteria were: 1) studies that were published before January 2005 or after April 2014; 2) studies that did not evaluate the direct angiogenic potentials of the dental materials used in regenerative endodontics, direct or indirect pulp capping, pulpotomy, pulpectomy, apexogenesis, and apexification treatments; 3) studies that mainly focused on other aspects of dental materials, which have no effect on angiogenesis process.

### Search methodology

An electronic search was performed in PubMed and MEDLINE databases *via* OVID using the keywords mentioned in the PubMed and MeSH headings for English language published articles from January 2005–April 2014 that evaluate the angiogenic properties of different dental materials used in regenerative endodontic procedures.

### Search strategy

The electronic searching key words in PubMed and MEDLINE databases included: angiogenesis, pulp stem cells, regenerative endodontics, direct or indirect pulp capping, pulpotomy, pulpectomy, apexogenesis, apexification treatments and dental materials including calcium hydroxide, mineral trioxide aggregate, Bioaggregate, Cem cement, Biodentine, glass ionomer, adhesive resin, and enamel matrix derivatives in evaluation of dental pulp tissue regeneration and revascularization. Some of the most relevant article's full texts and reference lists were evaluated for eligibility. A flowchart of mentioned activities is presented in Fig. 1 to clarify the number of relevant articles used for this review.

# THE EFFECT OF DENTAL TREATMENTS AND MATERIALS ON ANGIOGENESIS IN DENTIN-PULP COMPLEX REGENERATION

## Direct or indirect pulp capping and pulpotomy

**1. Calcium hydroxide**—The introduction of vital pulp therapies including direct or indirect pulp capping date back to 1939 by Zander<sup>26</sup>. An ideal pulp capping material should provide easy handling, infection control, good sealing ability, and induce dentinal bridge formation<sup>27</sup>. Among several materials, calcium hydroxide (Ca(OH)<sub>2</sub>) was one of the most common material used in pulp capping<sup>28</sup>. Schröder indicated that Ca(OH)<sub>2</sub> can induce a limited necrotic zone on the surface of pulp tissue at the application sites<sup>28</sup>. Due to its alkalinity, Ca(OH)<sub>2</sub> has antibacterial activity and stimulates dentin formation<sup>29</sup>.

The effect of Ca(OH)<sub>2</sub> on dentin-pulp complex regeneration has been evaluated by several authors. Ji *et al.* indicated that Ca(OH)<sub>2</sub> increases the recruitment, migration, proliferation, and mineralization of DPSCs, and periodontal ligament stem cells (PDLSCs) through the expression of STRO-1 and CD146 markers<sup>30</sup>. Sangwan *et al.* reviewed the mechanisms of action of Ca(OH)<sub>2</sub> in tertiary dentinogenesis<sup>31</sup>. They indicated that the regenerative effects of Ca(OH)<sub>2</sub> are due to calcium ion release and the high pH value<sup>31</sup>. Calcium ions promote the migration of pulp progenitor cells, increase the synthesis of biomolecules such as fibronectin and bone morphogenic proteins (BMPs), and participate in mineralization<sup>31</sup>.

The alkaline pH can present antibacterial and anti-inflammatory effects, activate transforming growth factor  $\beta$  (TGF- $\beta$ ), increase the activity of alkaline phosphatase (ALP), and enhance the dissolution of dentine extracellular matrix (ECM)<sup>31</sup>.

The pro-angiogenic effects of Ca(OH)<sub>2</sub> is mainly attributed to the release of growth factors preserved in the dentin matrix including TGF- $\beta$ , platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and insulin-like growth factor (IGF)<sup>31</sup> (Fig. 2B). This issue was also discussed by Chun *et al.* where they demonstrated that chelating agents such as ethylenediaminetetraacetic acid (EDTA) can dissolve the dentin matrix and release pro-angiogenic factors, and promote the regenerative process<sup>32</sup>. Roberts-Clark and Smith demonstrated that in addition to PDGF and FGF-2, dentine matrix also contains vascular endothelial growth factor (VEGF)<sup>33</sup>, which is one of the most important factors in angiogenesis events<sup>34</sup>. Løvschall *et al.* introduced another mechanism that Ca(OH)<sub>2</sub> can influence angiogenesis process<sup>35</sup>. They suggested that this pulp capping material can activate notch signaling pathway, which is implicated in cell-cell interactions involved in many regenerative processes such as angiogenesis<sup>36</sup>. In dental pulp tissue, this signaling pathway is activated due to injury, and participates in differentiation of stem cells to odontoblast-like and perivascular cells<sup>35</sup>. Previously, Iso *et al.* reported that notch signaling plays an active role in angiogenesis including the proliferation and migration of endothelial cells, smooth muscle, and arterial-venous differentiation<sup>36</sup> (Fig. 2D).

**2. MTA**—Mineral trioxide aggregate (MTA) is another commonly used dental material in pulp capping treatments with more advantages over Ca(OH)<sub>2</sub><sup>37</sup>. MTA has been recently optimized in many aspects including the setting time<sup>3</sup> and calcium ion release<sup>38</sup>. Al-

Hezaimi *et al.* compared the regenerative effects of Portland cement and MTA with Ca(OH)<sub>2</sub> and indicated that the thickness of reparative dentin was thicker in MTA and Portland cement, while the quality was not different<sup>37</sup>). Cavalcanti *et al.* showed that MTA, Ca(OH)<sub>2</sub>, and Single Bond adhesive system can increase the release of IL-8, while the release of interleukin-1 $\beta$  (IL-1 $\beta$ ) was only increased in samples treated with MTA<sup>39</sup>). IL-1 $\beta$  is a pro-inflammatory cytokine which is secreted after tissue injury and induces the release of chemokines such as IL-8, which is considered a pro-angiogenic factor<sup>40</sup>). Similar results were noticed by Ferreira *et al.*, which evaluated the effect of pulpotomy agents including Ca(OH)<sub>2</sub>, MTA, adhesive resin, and formocresol on dental pulp tissue fibroblasts<sup>41</sup>). These authors reported that MTA was the only pulpotomy material which increased the release of IL-1 $\beta$  and IL-8 by fibroblasts. Calcium hydroxide only stimulated the release of IL-1 $\beta$ , while adhesive resin and formocresol could increase IL-8 levels<sup>41</sup>) (Fig. 2D).

Zhang *et al.* reported that MTA had a greater potential for expression of TGF- $\beta$ 1 in rat dental pulp<sup>42</sup>). Paranjpe *et al.* tissue compared with Ca(OH)<sub>2</sub> demonstrated that MTA had positive effects on angiogenesis and differentiation of dental pulp cells when it was placed in direct contact with dental pulp<sup>43</sup>). These authors reported that MTA, as a direct pulp capping agent, can induce the expression of VEGF, osteocalcin and dentin sialoprotein<sup>43,44</sup>).

Zhang *et al.* evaluated the effect of MTA and Ca(OH)<sub>2</sub> on expression of inducible nitric oxide synthase (iNOS) in rat dental pulp tissue<sup>45</sup>). These pulp capping materials increased the expression of iNOS 3 days after their application<sup>45</sup>). Ziche *et al.* indicated that NO plays an important role in regulating the angiogenesis process through the enhancement of endothelial cells proliferation and migration<sup>46</sup>). Huang *et al.* indicated that MTA could activate mitogen-activated protein kinase (MAPK), specifically the p38 signaling pathway in hDPSCs<sup>47</sup>). The *in vitro* culture of hDPSCs with MTA facilitated their differentiation, and also increased the expression of angiogenic factors including von Willebrand factor (vWF) and angiopoietin-1 (Ang-1)<sup>47</sup>) (Fig. 2D).

**3. CEM cement**—Calcium-enriched mixture (CEM) cement is one of the pulp capping agents, which has osteogenic, cementogenic and dentinogenic functions<sup>48</sup>). Asgary *et al.* compared the ability of CEM and MTA as capping agents. They concluded that CEM could increase the expression of FGF-4 and bone morphogenic protein 2 (BMP-2), while MTA positively affected the expression of TGF- $\beta$ 1 in pulp tissue<sup>48</sup>) (Fig. 2D).

**4. Biodentine**—Biodentine (Septodont, Saint-Maur-des-Fossès, France) is a tricalcium silicate-based cement, which has been recently introduced as a pulp capping material<sup>49</sup>). Luo *et al.* reported that the induction effect of Biodentine cement on differentiation of DPSCs is through the mitogen-activated protein kinase (MAPK) and calcium/calmodulin-dependent protein kinase II (CaMKII) pathways<sup>49</sup>). The angiogenic effect of this cement was evaluated by Laurent *et al.* who suggested that Biodentine can induce early mineralization in dental pulp due to an increase in release of TGF- $\beta$ 1, a pro-angiogenic factor produced by pulp cells<sup>50</sup>) (Fig. 2D).

**5. Glass ionomer and adhesive resins**—Lutfi *et al.* showed that glass ionomer cement (GIC) as a lining material can act similar to Ca(OH)<sub>2</sub> in inducing proliferative activity in

dental pulp of exfoliated deciduous teeth<sup>51</sup>). Dammaschke *et al.* compared the effect of dentine adhesive Gluma Comfort Bond (GCB) and Ca(OH)<sub>2</sub> on the proliferation of pulp cells and concluded that GCB, due to its missing antibacterial efficacy and foreign body reactions, causes the inflammation of pulp tissue. Direct contact of GCB with dental pulp could increase the number of fibroblasts and endothelial cells in granulation tissue and negatively influence the Höhl cells<sup>52</sup>) (Fig. 2D).

Adhesive systems have also been used as dental pulp capping agents. Tran-Hung *et al.* evaluated the effect of HEMA on secretion of pro-angiogenic factors in dental pulp after mechanical injury<sup>53</sup>). They indicated that HEMA can increase the level of VEGF, decrease the expression of FGF-2, and has no effect on platelet derived growth factor-AB (PDGF-AB) in mechanically injured human dental pulp tissue<sup>53</sup>). Similar results were reported by Mantellini *et al.* who demonstrated that HEMA or SingleBond adhesive resin could increase the expression of pro-angiogenetic factor VEGF in mouse odontoblast-like cells (MDPC-23) and macrophages, while this up-regulation was not observed in the undifferentiated mouse pulp cells and gingival fibroblasts<sup>13</sup>) (Fig. 2D). However, other investigators demonstrated that in case of mechanically exposed dental pulp, the application of dentine adhesive systems cannot induce the formation of an acceptable tertiary dentine bridge at applied area<sup>54</sup>).

**6. Enamel matrix derivative (EMD)**—Güven *et al.* demonstrated that enamel matrix derivative (EMD) can also be used as a pulp capping material<sup>55</sup>). These authors showed that EMD was more capable of inducing the differentiation and proliferation of human tooth germ stem cells (hTGSCs) compared with calcium hydroxide-containing cement (DYCAL) and mineral trioxide aggregate (MTA)<sup>55</sup>). Even the EMD-coated DYCAL was shown to be less toxic, which emphasizes the biocompatible nature of EMD<sup>55</sup>). Yuan *et al.* demonstrated that EMD can exhibit angiogenic effects by presenting chemotactic effect on human umbilical vein endothelial cells (HUVEC) *in vitro*<sup>56</sup>). These authors reported that more endothelial cells and new blood vessels were detected in cell cultures treated with EMD than in control group<sup>56</sup>). Several authors have indicated that EMD has angiogenic activity at the applied sites<sup>57</sup>), and it can stimulate periodontal cells to produce VEGF<sup>58</sup>). However, Darwish *et al.* suggested that enamel matrix derivative, could be preferable material for periodontium, while it is not suitable for dentin-pulp complex regeneration<sup>59</sup>). Olsson *et al.* reported similar results by comparing the effect of calcium hydroxide and Emdogain Gel (Biora AB, Malmö, Sweden), consisting of an enamel matrix derivative (EMD) in a propylene glycol alginate (PGA) vehicle, on the postoperative symptoms of the experimentally exposed human dental pulps<sup>60</sup>). It was shown that the application of Emdogain Gel to the exposed dental pulp causes more inflammation with no effective formation of hard tissue barrier compared with samples subjected to calcium hydroxide application<sup>60</sup>).



## THE EFFECT OF DENTAL TREATMENTS AND MATERIALS ON ANGIOGENESIS IN DENTAL PULP REGENERATION AND REVASCULARIZATION

The summit goal of the regenerative endodontic procedures is to provide a suitable environment for the regeneration of healthy tissue to restore the lost biological tissues<sup>61</sup>. The challenge, however, is to create pulp tissue in a vacant canal, which is exactly identical to the pulpal tissue before necrosis and can function properly<sup>8,9</sup>. This issue is one of the main differences between regenerative attempts and the conventional treatments including apexification. In conventional modalities, the treatment is ended with obturation of empty canal rather than giving it a chance to be filled with biological tissue. The expected clinical and radiographic outcomes of regenerative procedures are the resolution of apical periodontitis, continuity of root development including the length and thickness, and normal responding of regenerated pulp tissue to different pulp tests<sup>62,63</sup> (Fig. 3C).

According to current status, previous authors presented a general protocol for pulp regeneration or root revascularization procedures. However, some modifications and improvements have been suggested as well. The overall steps in these procedures included: 1) canal irrigation and disinfection; 2) blood clot formation; and 3) coronal seal to provide appropriate environment for regeneration<sup>61,64,65</sup> (Fig. 3). In the following section, the effects of canal irrigating solutions and disinfecting agents on angiogenesis are discussed.

### NaOCl

The first step in dental pulp regeneration is the complete disinfection of root canal system. At first, root canal space should be irrigated with sodium hypochloride (NaOCl). NaOCl has proteolytic and antimicrobial activities which dissolves the organic debris and eliminate microorganisms inside the dental canal<sup>66</sup>. Bennett *et al.* evaluated the effect of different antiseptic or antimicrobial agents including 5% mafenide acetate (Sulfamylon solution), 10% povidone with 1% free iodine (Betadine), 0.25% sodium hypochlorite (“half-strength” Dakin), 3% hydrogen peroxide, and 0.25% acetic acid on wound healing. It was indicated that 0.25% sodium hypochlorite and sulfamylon could significantly increase neodermal thickness and sulfamylon was the most effective agent on promoting angiogenesis<sup>67</sup>. However, the dilution of sodium hypochlorite can decrease the disinfecting efficiency of this solution. Kozol *et al.* also suggested that NaOCl has toxic effects on cells and did not recommend it for use in open wounds<sup>68</sup>.

Jaimes *et al.* indicated that HOCl can inhibit nitric oxide production during inflammation and suggested that this effect might attribute to dysregulation of vascular events and negatively affect the interactions between leukocytes and endothelial cells<sup>69</sup>. Alkahtani *et al.* reported that NaOCl solution has toxic effects on human bone marrow mesenchymal stem cells (MSCs)<sup>70</sup>. Martin *et al.* also reported that high concentrations of NaOCl can drastically effect the survival and differentiation of stem cells of the apical papilla (SCAPs) and significantly reduce the expression of dentin sialophosphorprotein (DSPP)<sup>71</sup>. These authors suggested using lower concentrations like 1.5% and using 17% EDTA after NaOCl irrigation reduced the NaOCl negative effects, and increased the survival rate of SCAPs and

expression of DSPP<sup>71</sup>). Trevino *et al.* also acclaimed that irrigation with NaOCl can lower the survival rate of SCAPs, while inclusion of EDTA to the irrigation protocol was beneficial and increased the number of viable cells<sup>72</sup>).

### Chlorhexidine gluconate

The other disinfecting endodontic solution used in regenerative procedures is 2% chlorhexidine gluconate (CHX)<sup>73</sup>). The effect of CHX on DPSCs was measured and no viable cell was detected in samples irrigated with 2% CHX<sup>72</sup>). Ring *et al.* reported that 2% CHX, as well as 6% NaOCl, showed cytotoxic effects on DPSCs due to negative influence on their attachment to root canal wall surface<sup>74</sup>).

### EDTA

EDTA is an endodontic irrigating solution with chelating activity, which is suggested to be added to canal irrigation protocol as a final rinse for smear layer removal or in combination with NaOCl and CHX solutions<sup>72</sup>). Ring *et al.* demonstrated that the absence or presence of smear layer is not as effective on activity of DPSCs<sup>74</sup>). However, the addition of EDTA to other rinsing solutions can increase the viability of DPSCs<sup>72</sup>), and positively affect stem cell's attachment to root canal wall<sup>74</sup>). Authors showed that the time of irrigation with EDTA should be 1 min, while after 3 min the microhardness of dentin can significantly reduce<sup>75</sup>).

Pang *et al.* also noted that EDTA can induce DPSCs cell attachment and odontoblastic/osteoblastic differentiation<sup>76</sup>). Similar to other investigators Galler *et al.* recommend the usage of EDTA for canal irrigation<sup>77</sup>). In addition to favorable effect of EDTA on other solutions, it was indicated that EDTA can stimulate the release of pro-angiogenic growth factors in dentin matrix including TGF- $\beta$ , VEGF, FGF-2, PDGF, and BMP-2<sup>33,78,79</sup>).

### Triple antibiotic paste (TAP)

TAP is a disinfecting regimen containing three antibiotic pastes including: ciprofloxacin, metronidazole, and minocycline used for complete elimination of microorganisms inside necrotic root canal in regenerative procedures<sup>80</sup>). Bottino *et al.* indicated that the scaffolds containing 5% wt ciprofloxacin or 5 and 25% wt metronidazole were safe for hDPSCs, and only 25% wt ciprofloxacin had cytotoxic effects on pulp stem cells<sup>81</sup>). Bezwada *et al.* evaluated the intrinsic cytotoxicity of five fluoroquinolone antibiotics including: ciprofloxacin, levofloxacin, ofloxacin, moxifloxacin, and gatifloxacin on human corneal endothelial cells. These authors reported that among these antibiotics, ciprofloxacin showed the highest concentration- and time-dependent cytotoxicity, while levofloxacin had the lowest value<sup>82</sup>). Galley *et al.* indicated that ciprofloxacin can alter the inflammatory responses in endothelial cells. It was suggested that ciprofloxacin could decrease the expression of inflammatory cytokine IL-6, and increase the expression of the IL-8, a pro-angiogenic cytokine<sup>83</sup>). Michalska *et al.* indicated that metronidazole plus clindamycin had anti-angiogenic activity and could strongly interact with pro-angiogenic factors like FGF-2 and fibrin concentration and viscosity<sup>84</sup>).



Jung *et al.* demonstrated that minocycline has anti-angiogenic activity due to suppression of the hypoxia-induced vascular endothelial growth factor (VEGF) expression<sup>85</sup>). These authors also reported that minocycline can inhibit the activity of matrix metalloproteinase (MMPs)<sup>85</sup>). Li *et al.* showed that minocycline can accelerate breakdown of hypoxia-inducible factor-1 (HIF-1) and inhibit hypoxia-induced neovascularization<sup>86</sup>). Yao *et al.* reported that minocycline inhibits the migration of human aortic smooth muscle cell (HASMCs) by down-regulating PI3K/Akt pathway<sup>87</sup>). Although Arslan *et al.* recommended the passive ultrasonic irrigation (PUI) with 1% NaOCl to be useful for removal of the triple antibiotic paste<sup>88</sup>), but the complete removal may not be achieved.

## CONCLUSIONS

The evaluation of angiogenesis events in regenerative endodontic field is quite a new approach in this era. The present review focused on possible influences of dental treatments and materials on angiogenesis during regenerative endodontic procedures, and the following outcomes can be drawn.

- In dentin-pulp complex regeneration, the local angiogenesis events occur at the site of necrotic or injured tissues. Two distinct roles can be considered for pulpal local angiogenesis: 1) the blood supplying the inflammatory phase which should bring the immune cells and components, and the nutrition required for both inflammatory and regeneration phases; 2) the recruitment of perivascular stem cells which in addition to local stem cells can be differentiated into odontoblast-like cells for the production of reparative dentin.
- The dentin matrix might play an important role as a reservoir of intrinsic growth factors. These factors can be released due to changes imposed on dentin structure and also in response to the treatments and materials used on dentin surfaces. In light of this, the pro-or anti-angiogenic properties of dental materials used for dentin-pulp complex regeneration should be considered as one of the important characteristics for selecting an ideal dental pulp capping material. This issue was also raised by Tziafas *et al.*<sup>27</sup>) besides the traditional criteria such as easy handling, infection control, good sealing ability, and exploiting endogenous signaling for dentinal bridge formation. These authors also mentioned the use of exogenous signaling molecules in new pulp capping strategies<sup>9</sup>).
- In dental pulp regeneration procedures, most of the materials including irrigating solutions or antibiotic pastes have anti-angiogenic effects. However, EDTA solution has pro-angiogenic activity among other solutions. The residual antibiotic paste inside the canal can delay or jeopardize the angiogenesis process. It has been suggested that in future studies, other more biocompatible agents such as levofloxacin, instead of ciprofloxacin, to be evaluated for regenerative procedures.
- Future studies regarding the angiogenic properties of dental materials will lead to a better understanding of their mechanism of action in

angiogenesis events at applied area. In addition, the enrichment of these materials with pro-or anti-angiogenic factors can be considered as target goal for regulation and establishment of balanced angiogenesis events. This balanced neoangiogenesis can promote the healing process of dentin-pulp complex on one side and prevent pulp tissue necrosis on the other. The additional biomolecules can enhance the biocompatibility of dental materials as well as their treatment outcomes in regeneration procedures.

Concerning the regenerative endodontics, it should be mentioned that the trend of restoring lost tissue with biological tissues, rather than replacing with synthetic materials, is appreciable and promising. However, the similarity of the structure and functions of restored tissues to the primary tissue must be taken into consideration.

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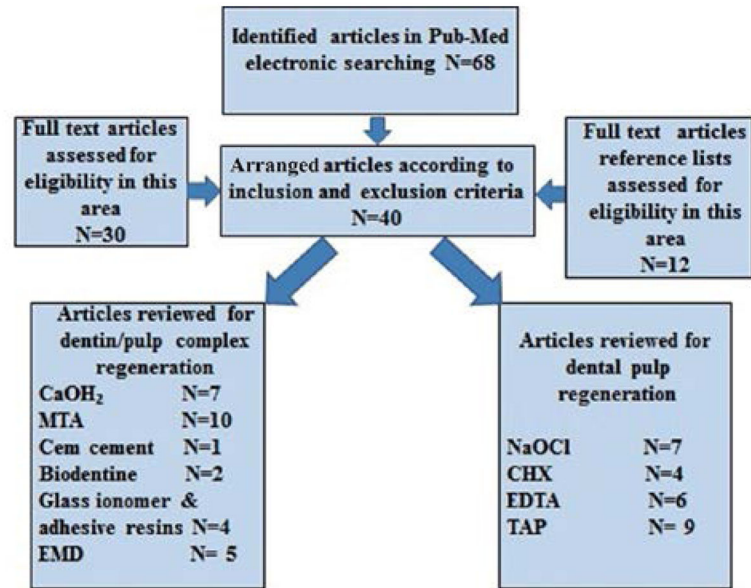
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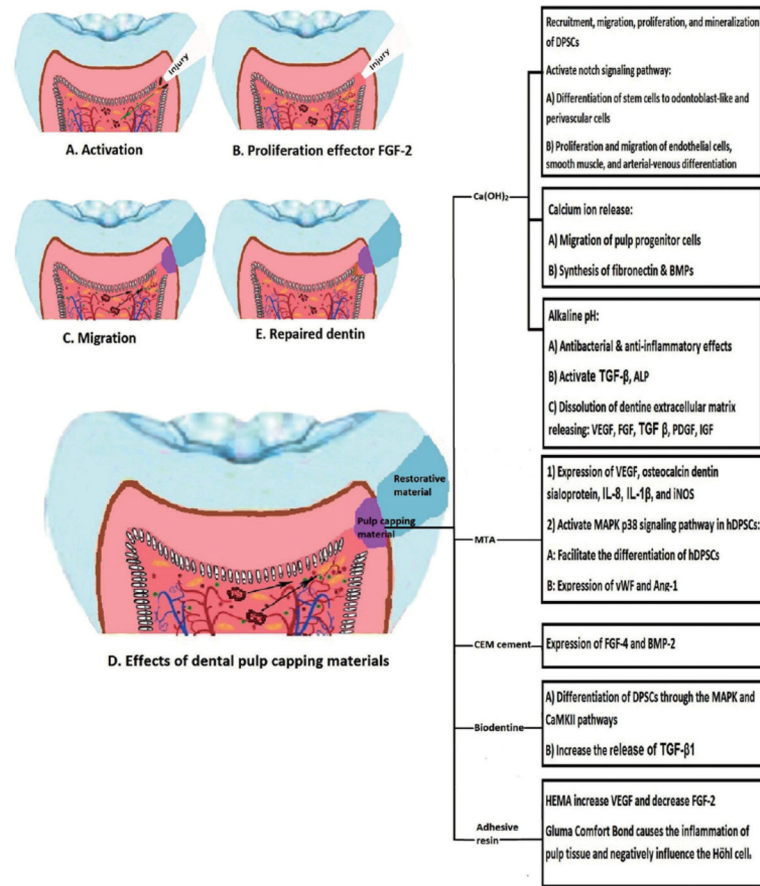
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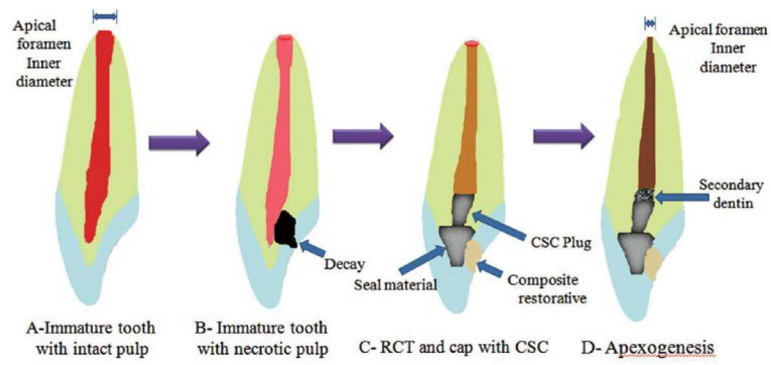


**Fig. 1.**  
A flowchart of search strategy to identify English articles from January 2005–April 2014 based on inclusion and exclusion criteria in this review study.



**Fig. 2. Schematic presentation of dentin-pulp complex regeneration**

A: deep injury imposed to the pulp tissue, which activates regenerative processes; B: Recruitment of pulp stem cells and expression of angiogenic factors such as FGF-2, which are responsible for proliferation of endothelial cells; C: Migration of proliferated endothelial cells to the injured area; D: The angiogenic effects of different pulp capping materials in the regenerative processes; E: Differentiation of stem cells to odontoblast-like cells and secretion of reparative dentin. Please note the vascular structure returning to its normal architecture at the end of the regeneration process.



**Fig. 3. Schematic presentation of dental pulp regeneration processes in an immature tooth**

A: Immature tooth with intact pulp; B: Necrotic dental pulp and accumulation of stem cells of the apical papilla (SCAP) in periapical area; C: Mechanical provocation of bleeding from the periapical site to transport the SCAPs into the empty canal space and establishment of a coronal seal by MTA cement and composite resin materials; D: Formation of pulp-like tissue in apical and mid third of root, which contributes to the maturation of apical third and apical foramen closure by the formation of a cementum-like tissue.