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Growth Factors and Cell Homing in Dental Tissue Regeneration

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Abstract

Purpose of Review—To summarize current views on the role and therapeutic potential of growth factors (GFs) within endodontic cell homing.

Recent Findings—Cell homing/revitalization techniques aim to regenerate dentin and pulp using endogenous cells. Clinically, revitalization has successfully created new vital tissue in necrotic permanent teeth with an open apex; however, there is no evidence of new odontoblasts, pulp tissue, or predictable extension in root length. Although the response is reparative rather than regenerative, exciting opportunities to improve these biologically-based strategies remain by (1) efficiently sequestering dentin-matrix-components (DMCs) using irrigants and dental materials (2) designing next-generation GF-releasing scaffold materials and (3) utilizing other sources of GF such as cells and plasma-rich plasma and plasma-rich fibrin.

Summary—GFs can promote reparative-dentinogenesis and pulp-like tissue formation. The future development and clinical approval of GF-functionalized-scaffolds is a priority; however, current focus should be to harness DMCs and target the interaction of stem cells and GFs.

Keywords

Cell homing; Dental pulp stem cell; Regenerative endodontics; Dentin-pulp complex; Growth factors; Functionalized scaffolds

Introduction

If dental caries progresses without remedial treatment, the microbial infection will advance and bacteria will invade the pulp tissue; this challenge will lead to irreversible pulpitis, pulp necrosis, and subsequent apical periodontitis [1]. Management of pulp necrosis is root canal treatment (RCT) in mature teeth; however, if the root formation is not complete, a combination of thin dentin walls and open apices make completion of conventional RCT challenging [2]. Immature pulpless teeth are also more vulnerable to injury, losing the ability

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to sense environmental change and are more prone to root fracture [3, 4]. Traditional treatment of immature teeth is apexification, which involves placing a tri-calcium silicate matrix at the apex [5], or inducing a barrier using calcium hydroxide; however, these techniques are not designed to induce extension of root length or width [6, 7] and generally have poor long-term prognosis [8]. Therefore, strategies to either maintain pulpal health or stimulate the development of new biological tissue are paramount, not only to promote minimally invasive solutions but also to retain or reinstate the capacity of the pulp to generate tertiary dentin and respond to injurious stimuli [9•].

Developing biological strategies to replace vital tissue in the root canal space has recently attracted significant attention under the banner of “Regenerative Endodontics” [10, 11••]. These pulp revitalization procedures [12] are not new, with successful revascularization in immature pulpless teeth reported in both monkeys and humans in the 1970s [13, 14]. Although this work demonstrated the potential for a pulp revascularization procedure to induce apical closure and continuous root formation in immature human teeth, the interest in this area diminished at that time [14]. Thirty years later, however, there is renewed interest in revitalization after human studies reported that connective tissues, blood vessels, dentin, and cementum-like tissues filled the root canal space after pulp revitalization procedures [15••, 16]. These data now suggest that this procedure has the potential to regenerate biological tissue if normal periapical tissues containing Hertwig’s epithelial root sheath and the apical papilla remain in a healthy state prior to a tissue engineering approach.

Growth factors (GFs) are polypeptides, which stimulate cell proliferation and are major growth-regulatory molecules for cells in culture and in vivo [17]. GFs and other morphogens form one of the three essential components of a tissue engineering approach in combination with an appropriate scaffold and progenitor or stem cell (SC) population [18]. Revitalization techniques in Endodontics do not rely on an expanded SC population being transplanted into the root canal, but rather on the use of mobilization factors, including GFs, chemotactic agents, and other signaling factors, to “home” the cells into the root canal system from the periapical vasculature to the site of injury. SC homing is defined as the recruitment of endogenous SCs from bone marrow and other niches by signaling “mobilization” factors to the site of injury to induce repair [19]. Several key mobilization factors have been identified, including granulocyte colony stimulating factor (G-CSF) [20], cytokines such as inter-leukin (IL)-8 [21] and Fms-like tyrosine kinase-3 (Flt-3) ligand [22], chemokines including stromal cell-derived factor-1 (SDF-1) [23, 24], as well the GFs, vascular endothelial growth factor (VEGF) [25], angiopoietin-1 (ANG-1) [26], and macrophage inflammatory protein-2 (MIP-2) [27]. GFs in particular are critical to the success of cell homing and can be sourced endogenously from the dentin matrix [28••], SCs or other cell populations [29], as well as platelet-rich-plasma (PRP) and platelet-rich-fibrin (PRF) [30], or exogenously within a functionalized-scaffold containing one or several GFs [31, 32] (abbreviations—Table 1). A range of GFs are considered important within pulp repair/regeneration (Table 2), including those targeted at cell differentiation from the transforming growth factor (TGF) superfamily to others aimed at cellular processes including angiogenesis, neurogenesis and cell migration [28••, 34].

The attraction of a cell-homing strategy in clinical Endodontics in comparison with a cell-based therapy is that there is no requirement for exogenous SCs to be isolated and supplied [35]. Pulp regeneration using SC transplantation has several inherent problems, including difficulties in obtaining regulatory approval, SC isolation and processing, the relatively high-cost associated with storage (cell cryopreservation), expansion, and a biological risk of immune-rejection, infection, and tumorigenesis [36]. The predictability of pulp revitalization using cell-homing therapy is limited by a lack of cells; however, recent reports demonstrated pulp-like regeneration with a cell-free technique employing GF cocktails [37]. Therefore, the clinical method of pulp revascularization may be modified in the future, for example, with use of selected GFs, morphogen cocktails, and functionalized scaffolds to regulate the formation of the vascular system as well as the hard tissues in the tooth. The focus of this review is to discuss current views on the source, role, and interaction of GFs within regenerative endodontic cell-homing procedures.

Review

How Important Are Growth Factors Within Regenerative Endodontics?

The American Association of Endodontists (AAE) describes Regenerative Endodontics as a concept of tissue engineering to restore the root canals to a healthy state, allowing for continued development of the root and surrounding tissue [10]. This description appears to exclude not only vital pulp techniques such as pulp capping or pulpotomy, but also experimental SC-based pulp regeneration therapies. In clinical reality, the AAE statement only currently includes the cell-homing “revitalization” technique, which is a cell-free technique attracting the host endogenous cells by GFs and other signaling molecules, rather than SC delivery [34].

During the initial revitalization visit, there is minimal instrumentation, profuse chemical disinfection, and placement of an inter-appointment medicament, while at the second appointment; mechanical agitation creates bleeding (to transplant endogenous SCs and GFs) from the periapical region and fibrin clot formation in the root canal, prior to coronal restoration [12]. Initial reports of the procedure offered the exciting prospect of regeneration of a vital biological tissue within the root canal system and continuing root development [38]. As a result, the aim of revitalization was established to resolve signs and symptoms, and promote further root length, thickness, and maturation as well as regenerating a vital responsive tissue in the root canal system [18]. Unfortunately, although a regenerative response seems possible when the epithelial root sheath of Hertwig, apical papilla, and vital tissue remain [15••], it is unlikely after pulp necrosis [39••]. Indeed, using current techniques, it appears that revitalization will at best stimulate repair, not regeneration with minimal gain in root length over traditional apexification techniques [7, 35]. As a result, specific attention was directed to developing methods, which harness the fossilized bioactive dentin matrix components, including GFs and other signaling molecules, with irrigants, medicaments, and materials [40–43]. Additionally, new functionalized scaffolds are being investigated as a mechanism for controlled delivery of angiogenic and other exogenous GFs to support and improve the regenerative response [31, 44].

What Is the Interaction Between Stem Cells and Growth Factors?

SCs form an essential component of tissue engineering and cell-homing procedures, being self-renewable and having the ability to differentiate into multiple tissue lineages [45, 46]. SC populations in dental and central niches are potentially important in contributing to revitalization procedures including dental pulp stem cells (DPSCs), stem cells for the apical papilla (SCAPs), human periodontal ligament stem cells (PDLSCs) as well as centrally residing SC populations such as human bone marrow stromal stem cells (BMSSCs), and hematopoietic stem cells (HSCs) [47]. SC behavior can be modulated by producing GFs themselves [29] and by GFs released from dentin [48], other cells [49], or scaffold materials [31].

DPSCs are located in the central region of the pulp space [50] and have the ability to migrate, proliferate, and differentiate into odontoblast-like cells after primary odontoblast death in reparative tertiary dentinogenesis [51]. Although they are unlikely to represent a significant SC niche after pulp necrosis, DPSCs can survive and differentiate down osteogenic/dentinogenic lineages in irreversible pulpitis and early necrosis when at least some vital tissue may persist apically [52]. Furthermore, GFs such as stromal cell-derived factor-1 (SDF-1) and basic fibroblast growth factor (bFGF) enhance DPSC migration in 3D collagen gels, while another GF, bone morphogenic protein (BMP)-7, induces osteogenic differentiation but not cell migration [53]. These data highlight the complimentary and varying role of individual GFs in dental regenerative processes. Furthermore, SDF-1 in combination with a collagen matrix enhances micro-vessel formation in DPSCs with involvement of autophagy in vivo [54]. DPSCs also indirectly promote cell migration and proliferation of neuronal progenitors, indicating that mobilization factors produced by DPSCs can recruit progenitors of neural cells and stimulate neuronal maturation and neuritogenesis [55].

SCAPs and DPSCs share similarities and differences. CD24 is present on the SCAP surface, but not on DPSCs [56], while SCAPs and DPSCs are likely to be the cell source for primary root formation [57]. After pulp necrosis, the recruitment of SCAPs or SCs migrating from the blood system becomes more important and more complex than recruitment of DPSCs due to a loss of pulpal blood supply [58]. During apical periodontitis, SCAPs retain vitality and stemness, and can undergo osteogenic and angiogenic differentiation under the influence of GFs [59]. Chemotactic GFs including SDF-1, TGF- β 1, platelet-derived growth factor (PDGF), G-CSF, and bFGF stimulate migration of SCAPs, while a combining GCSF and TGF- β 1 significantly enhanced mineralization responses [48]. From a translational perspective, SCAPs transplanted into the root canal formed a vascularized pulp-like tissue and new dentin-like tissue at the dentinal wall [60]. Furthermore, SCAPs in matrigel functionalized with the GFs, brain-derived neurotrophic factor (BDNF) and glial cell-derived neurotrophic factor (GDNF), promoted axonal outgrowth of trigeminal sensory neurons in vivo, indicating that SCAPs may provide a valuable resource for regeneration of neurites [61].

PDLSCs are located around the root, between the bone and cementum, participating in the formation of the cementum as well as bone formation [62]. During revascularization procedures, it is necessary to increase in the thickness and length of root dentin wall as well

as stimulating an apical closure with cementum and recruiting PDLSCs may contribute to this; however, it is unlikely that cells from the periodontal ligament will be able to differentiate into odontoblast-like cells in the pulp space [63].

As BMSSCs are capable of differentiation down the mesoderm lineage to osteoblasts, adipocytes, and chondrocytes, they are potentially a good cell source for pulp revitalization [64]. Indeed, stem cell factor (SCF) enhances cell migration, proliferation, and osteogenic differentiation of BMSSCs in vivo in a dental cell-homing model [65]. Angiogenesis and neovascularization are critical to HSC survival and migration with the potent angiogenic GF, VEGF, stimulating bone marrow-derived endothelial cells, and the recruitment of perivascular cells including CXCR4-positive cells via up-regulation of CXCL12 [66, 67]. Both in vitro and in vivo studies have demonstrated that human recombinant VEGF treatment strongly induced mobilization of endothelial precursor cells [68], highlighting the importance of dentally derived GFs to attract BMSCs to the injury site in the root canal.

How Important Is the Dentin Matrix as an Endogenous Source of Growth Factors?

During the procedure of cell homing within the root canal space the irrigant, dental restorative material and scaffold will not only contact migrating cells but will also interact with the dentin surface. Dentin is a reservoir for a broad range of bio-active dentin matrix components (DMCs), which are stored in the matrix during development. Several bioactive groups have been identified (Table 2) including BMPs, GFs, and tissue proteases [69–71], with the therapeutic harnessing of these molecules critical to the process of dentin-pulp repair [28••]. Indeed, the release of bioactive components, including angiogenic, metabolic, and chemotactic cytokines, is likely key to enabling regenerative processes following caries, dental trauma, or during revitalization procedures [70, 72, 73]. In support of this, the ability of ethylenediaminetetraacetic acid (EDTA) [42, 74], mineral trioxide aggregate (MTA) [41], calcium hydroxide [40], dental adhesives [75], ultrasonic activation [76], and epigenetic modifiers (histone deacetylase inhibitors) [33], to facilitate the release of DMCs and augment the regenerative response, has been demonstrated.

Irrigation, as part of chemical disinfection, has attracted particular attention as it has the potential to release a range of DMCs including GFs beneficial to cell migration, proliferation, and differentiation [42, 77]. Irrigating with 17% EDTA can release TGF- β family members from the extracellular matrix of dentin [40]. Sodium hypochlorite, however, may have a deleterious effect on SCAP cell survival and differentiation ability, leading to suggestions that the final rinse in cell-homing procedures should be with a 17% EDTA solution [78•], while others have highlighted improvements in SCAP cell adherence and viability using dexamethasone-releasing nanoparticles to condition dentin [79] (Fig. 1). An alternative disinfectant, chlorhexidine, in combination with 5 min of EDTA conditioning, also inhibited GF release from dentin compared with EDTA alone, but to a lesser extent than NaOCl [77]; notably, this inhibitory effect could be reversed if EDTA-conditioning was doubled to 10 min. The observed interaction could be due to substantivity of chlorhexidine interfering with GF release from dentin; however, it remains at present the most suitable alternative to NaOCl in revitalization cases. In terms of medicaments, calcium hydroxide is more effective in extracting DMCs than the most commonly used medicament in

revitalization procedures, triple-antibiotic paste (TAP) [12, 77], particularly in a water-based rather than oil-based formulation [40, 77]. Another advantage of EDTA and water-based calcium hydroxide medicaments is that they are relatively easy to remove compared with oil-based TAP and chlorhexidine solutions or gels. Furthermore, TAP formulations have been shown to inhibit SC survival [32], and a recent position statement has recommended that they now be superseded in revitalization by calcium hydroxide [11••].

DMCs alone can induce odontoblast differentiation [80], with two GFs present in dentin extracts, bFGF and/or VEGF, sufficient to form a pulp-like tissue by cell homing [37]. Others, however, have highlighted that the angiogenic GFs present in dentin were insufficient to induce endothelial differentiation in vivo [81]. The apparent differences evident in these studies are likely due to differences in experimental design, with the first study being an ex vivo culture-based analysis of dissected mouse tooth germs [37], while the second is an in vivo tooth slice/SC model transplanted subcutaneously into the dorsum of an immunocompromised mouse [81]. This highlights an important clinical consideration that although tissue engineering is often successful with in vitro modeling, in vivo when pulp tissue needs to be grown into an empty root canal space, critical processes such as angiogenesis may need GF supplements in order to develop effectively.

Are Cells Also an Endogenous Source of Growth Factors?

Much recent focus in regenerative endodontics has considered the role of dentin-bound GFs to chemotactically attract and orchestrate SC behavior [28••]. It is worth taking notice of the role of not just SCs, but other cells such as fibroblasts and fibrocytes in contributing to the expression of GFs and the regenerative response [49, 82]. Fibroblasts are the most common cell of the pulp and can produce GFs, including bFGF, VEGF, and PDGF aimed at mineralization, angiogenesis, and neurogenesis [49]. DPSCs have also been demonstrated to have a paracrine effect secreting GFs such as BDNF, nerve growth factor (NGF) VEGF, and GDNF [83•], while hypoxic conditions also increased the expression of VEGF and bFGF in DPSCs and fibroblasts [84]. In cases of pulp necrosis, the pulp cells will not contribute to the healing response; however, a range of bioactive molecules will also be released by the cells in the periapical region, albeit long-term bioavailability will be naturally compromised by high vascularity and rapid turnover in that area [28••].

Are There Any Other Endogenous Sources of Growth Factors?

Regenerative techniques employing PRP have become increasingly popular in oral surgery to augment wound healing [85], in periodontology to augment guided tissue regeneration procedures [86] and within endodontics as a supplement to promote cell-homing techniques [30]. PRP is essentially a concentrate of blood products containing a rich source of GFs, which forms a natural fibrin gel acting like a scaffold to stimulate bone and soft-tissue healing [87]. GFs, which have been sourced from PRP, include PDGF, platelet-derived epidermal growth factor (PDEGF), TGF- β , insulin growth factor (IGF)-1, VEGF, epidermal growth factor (EGF), epithelial cell growth factor (ECGF) [88–90], and a plethora of other signaling molecules. Although representing an attractive locally sourced supply of GFs, a disadvantage of using PRP clinically is the need for special “chairside” equipment (e.g., centrifuge), reagents for preparing PRP, as well as phlebotomy, which increases the cost and

invasiveness of the treatment [91]. A further technical problem is controlling the placement and release of GFs from the concentrate, which may lead to a rapid rather than sustained delivery of bioactive GFs. In order to improve its physical properties, it has been suggested that PRP can be mixed with collagen, hydrogels, alginates, or other scaffolds to solidify the structure and modulate its degradation speed [87].

Another similar product, PRF, is a fibrin matrix including platelets, cytokines, and GFs, which acts as a biodegradable scaffold [92]. In comparison to PRP, which requires the use of anticoagulant agents, PRF does not require additives and forms an improved fibrin structure protecting the developing tissues and controlling the gradual release of GFs as the membrane degrades [93]. Some claim that PRF accelerates tissue growth compared with PRP [94], while conversely, others have shown no improvement in outcome compared with a blood clot alone [95]. A recent systematic review demonstrated no difference between PRP, PRF, and blood clot alone when comparing the outcome measures of increased wall thickening and root closure [96]. Notably, small numbers and short follow-up limit available clinical studies in this area.

Do We Need to Use Exogenous Sources of Growth Factors?

Artificial support systems incorporating GFs, so-called functionalized or “doped” scaffolds, are necessary to assist tissue regeneration when transplanting SCs into the root canal space and could be useful as an adjunct to cell-homing techniques for improved outcomes [31]. Although it has been reported that DMCs alone can induce differentiation of DPSCs into odontoblasts both in vitro [80] and in vivo [97], it is challenging within the confines of the necrotic root canal space to supply sufficient nutrients and oxygen to the recruited cells, to maintain cell survival and functional activity. Angiogenesis and neovascularization is a critical step in tissue regeneration process, and ready supply of angiogenic GFs is essential. Notably, DMCs alone could not induce endothelial differentiation in vivo [31] despite the presence of angiogenic GFs in dentin [98]. Practically, the current methods of extracting DMCs by the use of etchants or irrigants are limited by lack of control, specificity, and likely of short duration of action of the released GFs [33]. The gold standard endodontic irrigant sodium hypochlorite is likely to both inactivate the extracted DMCs as well as reduce the survival and differentiation capabilities of apical SC populations so that the biological response is significantly diminished [78•, 99]. One solution is to optimize the irrigation protocol to maximize GF survival; however, this may compromise disinfection leading to further infection. Another solution is to develop biomaterials, which incorporate a mechanism to control and sustain the release of GFs to aid SC migration, attachment, and rapid vascularization of the pulp space. These functionalized scaffolds could be injected into the root canal space and incorporate GFs such as bFGF, TGF- β 1, PDGF, and VEGF [100–102], and other morphogens incorporated into the structure. Although seemingly a physiologically attractive solution, the use of exogenous GFs embedded in scaffolds is complicated by GF instability and short-half life, expense, safety, ethical issues, and the need for extensive clinical trials in various phases before approval. Perhaps, judicious GF selection or the functionalized scaffold is the best way forward, as it may be that pro-angiogenic GFs are more important than others are, with one clinical study reporting no

advantage after 18 months of an injectable scaffold with bFGF over a traditional revitalization technique [103].

What Is the Currently the Optimal Way to Stimulate GF Release in Cell-Homing?

GFs will likely have temporal and spatial expression, as well as specific roles during cell-homing procedures; however, the exact nature complex interaction remains to be fully elucidated. In order to better understand the clinical interaction of GFs, it is sensible to analyze this in a sequential fashion while highlighting the best current therapeutic strategy to optimize GF release during revitalization. Preoperatively, there is unlikely to be significant release of reparative GFs in the necrotic/infected canal; however, SCAP populations have been shown to be resilient to infection and inflammatory processes ready to be utilized after effective infection control [59]. After endodontic access, a combination of minimal instrumentation, disinfection with chlorhexidine, and a final rinse with 17% EDTA will stimulate the release of GFs (e.g., TGF β -1, VEGF) and other mobilization factors from dentin, which will promote SC chemotaxis from the apical papilla and beyond. The inward, migrating SCs will also release GFs, and in both, an autocrine and paracrine fashion promote [83•] proliferation, expanding their population and directing differentiation down several lineages such as angiogenesis, neurogenesis, and mineralization. These processes will be aided by continued GF release from dentin, induced by the calcium hydroxide intra-canal medicament after the first visit [40]. The calcium hydroxide dressing will be removed again with prolonged irrigation with EDTA (releasing further GFs including members of the TGF superfamily), and mechanical agitation of the periapical area will bring in both cells (SC and other), which will be embedded in and release GFs extracellularly from the developing fibrin clot [12, 58]. The placement of MTA in contact the clot will induce the release of DMCs in a prolonged fashion, which contribute GFs and other bioactive molecules to augment the ongoing regenerative processes in cell homing [41]. At this stage, there will be an effective, “GF cocktail,” harvested from both dentine and cellular sources. That said, determining the exact contribution between GFs released by cells and extracted from the dentin matrix is complex; however, it is suffice to comment that both are likely to contribute significantly in a symbiotic manner to cell proliferation and differentiation within revitalization procedures.

Although it is clear that although odontoblast-like differentiation is possible with GFs alone in vitro, it is not yet possible to regenerate the dentin-pulp complex in vivo in cases of pulp necrosis [32]. In order to develop new regenerative strategies, exogenous GFs or GF cocktails are needed to drive the biological response GFs in a functionalized tissue engineering scaffold. Although the focus of this report is GFs role within cell-homing procedures, if we extrapolate evidence from SC-based regenerative therapies, it is evident that the use of transplanted SCs and selected exogenous GFs including bFGF [104], or chemokines such as SDF-1, can regenerate pulp tissue in vitro and in vivo [105]. Although complicated by safety, expense, and ethical issues, novel research in SC-based therapies has progressed to the clinical trial stage in Japan, a trial in which a DPSC population was transplanted with the cytokine G-CSF in an atelocollagen scaffold [106, 107]. This exciting work also offers opportunities to extrapolate and consider the most effective way to optimize

the combination of migrating SCs, exogenous signaling molecules/GFs, and extracellular scaffolds [108] in revitalization procedures.

Conclusions

Revitalization has recently gained momentum as a biologically based alternative to traditional apexification techniques for teeth with open apices. Although regeneration of the damaged dentin-pulp complex is possible with revitalization, a reparative process with no increase in root length is more likely at present. Attempts to improve the response have focused on understanding the release and interaction of GFs, in order to improve regenerative endodontic treatments. GFs appear sufficient to “home” SCs into the canal space, promoting dentinogenesis and pulp tissue formation in vivo; however, optimizing angio-genesis and neurogenesis by supplementing and sustaining GF release is critical. GFs are available from endogenous sources including dentin, SCs, and concentrated blood products (e.g., PRP), which avoid the ethical, safety, and expense of developing delivery mechanisms for exogenous GF release. Future studies should aim to optimize the sequestration of bioactive DMC components as well as develop next-generation functionalized scaffolds and delivery platforms for GF application within revitalization and cell homing.

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References

Papers of particular interest, published recently, have been highlighted as:

• Of importance

•• Of major importance

1. Reeves R, Stanley HR. The relationship of bacterial penetration and pulpal pathosis in carious teeth. *Oral Surg Oral Med Oral Pathol.* 1966;22:59–65. [PubMed: 5220026]
2. Luder HU. Malformations of the tooth root in humans. *Front Physiol.* 2015;6:307. [PubMed: 26578979]
3. Andreasen JO, Farik B, Munksgaard EC. Long-term calcium hydroxide as a root canal dressing may increase risk of root fracture. *Dent Traumatol.* 2002;18:134–7. [PubMed: 12110105]
4. Caplan DJ, Cai J, Yin G, White BA. Root canal filled versus non-root canal filled teeth: a retrospective comparison of survival times. *J Public Health Dent.* 2005;65:90–6. [PubMed: 15929546]
5. Simon S, Rilliard F, Berdal A, Machtou P. The use of mineral trioxide aggregate in one-visit apexification treatment: a prospective study. *Int Endod J.* 2007;40:186–97. [PubMed: 17305695]
6. Jeeruphan T, Jantarat J, Yanpiset K, Suwannapan L, Khewsawai P, Hargreaves KM. Mahidol study 1: comparison of radiographic and survival outcomes of immature teeth treated with either regenerative endodontic or apexification methods: a retrospective study. *J Endod.* 2012;38:1330–6. [PubMed: 22980172]
7. Alobaid AS, Cortes LM, Lo J, Nguyen TT, Albert J, Abu-Melha AS, et al. Radiographic and clinical outcomes of the treatment of immature permanent teeth by revascularization or apexification: a pilot retrospective cohort study. *J Endod.* 2014;40:1063–70. [PubMed: 25069909]

8. Katebzadeh N, Dalton BC, Trope M. Strengthening immature teeth during and after apexification. *J Endod.* 1998;24:256–9. [PubMed: 9641130]
- 9•. Wolters WJ, Duncan HF, Tomson PL, Karim IE, McKenna G, Dorri M, et al. Minimally invasive endodontics: a new diagnostic system for assessing pulpitis and subsequent treatment needs. *Int Endod J.* 2017;50:825–9 [PubMed: 28776717] Highlighted problems with current classification of pulpitis and suggestive new classification. Linked new minimally invasive strategies to management.
10. Murray PE, Garcia-Godoy F, Hargreaves KM. Regenerative endodontics: a review of current status and a call for action. *J Endod.* 2007;33:377–90. [PubMed: 17368324]
- 11•. Galler KM, Krastl G, Simon S, Van Gorp G, Meschi N, Vahedi B, et al. European Society of Endodontology position statement: revitalization procedures. *Int Endod J.* 2016;49:717–23 [PubMed: 26990236] This position statement endorsed by the European Society of Endodontology (ESE) describes European views on revitalization.
12. Galler KM. Clinical procedures for revitalization: current knowledge and considerations. *Int Endod J.* 2016;49:926–36. [PubMed: 26715631]
13. Nevins AJ, Finkelstein F, Borden BG, Laporta R. Revitalization of pulpless open apex teeth in rhesus monkeys, using collagen-calcium phosphate gel. *J Endod.* 1976;2:159–65. [PubMed: 819611]
14. Nevins A, Wrobel W, Valachovic R, Finkelstein F. Hard tissue induction into pulpless open-apex teeth using collagen-calcium phosphate gel. *J Endod.* 1977;3:431–3. [PubMed: 275441]
- 15•. Shimizu E, Jong G, Partridge N, Rosenberg PA, Lin LM. Histologic observation of a human immature permanent tooth with irreversible pulpitis after revascularization/regeneration procedure. *J Endod.* 2012;38:1293–7 [PubMed: 22892754] Demonstrated histologically that regeneration of pulp-like tissue possible in vivo in human teeth if the epithelial root sheath of Hertwig and the apical papilla are intact.
16. Peng C, Zhao Y, Wang W, Yang Y, Qin M, Ge L. Histologic findings of a human immature revascularized/regenerated tooth with symptomatic irreversible pulpitis. *J Endod.* 2017;43:905–9. [PubMed: 28416306]
17. Goustin AS, Leof EB, Shipley GD, Moses HL. Growth factors and cancer. *Cancer Res.* 1986;46:1015–29. [PubMed: 3002607]
18. Kim SG, Malek M, Sigurdsson A, Lin LM, Kahler B. Regenerative endodontics: a comprehensive review. *Int Endod J.* 2018 10.1111/iej.12954.
19. Andreas K, Sittertinger M, Ringe J. Toward in situ tissue engineering: chemokine-guided stem cell recruitment. *Trends Biotechnol.* 2014;32:483–92. [PubMed: 25059433]
20. Petit I, Szyper-Kravitz M, Nagler A, Lahav M, Peled A, Habler L, et al. G-CSF induces stem cell mobilization by decreasing bone marrow SDF-1 and up-regulating CXCR4. *Nat Immunol.* 2002;3:687–94. [PubMed: 12068293]
21. Laterveer L, Lindley IJ, Hamilton MS, Willemze R, Fibbe WE. Interleukin-8 induces rapid mobilization of hematopoietic stem cells with radioprotective capacity and long-term myelolymphoid repopulating ability. *Blood.* 1995;85:2269–75. [PubMed: 7718900]
22. Solanilla A, Grosset C, Duchez P, Legembre P, Pitard V, Dupouy M, et al. Flt3-ligand induces adhesion of haematopoietic progenitor cells via a very late antigen (VLA)-4- and VLA-5-dependent mechanism. *Br J Haematol.* 2003;120:782–6. [PubMed: 12614209]
23. Aiuti A, Webb IJ, Bleul C, Springer T, Gutierrez-Ramos JC. The chemokine SDF-1 is a chemoattractant for human CD34+ hematopoietic progenitor cells and provides a new mechanism to explain the mobilization of CD34+ progenitors to peripheral blood. *J Ex Med.* 1997;185:111–20.
24. Hattori K, Heissig B, Rafii S. The regulation of hematopoietic stem cell and progenitor mobilization by chemokine SDF-1. *Leuk Lymphoma.* 2003;44:575–82. [PubMed: 12769333]
25. Rafii S, Heissig B, Hattori K. Efficient mobilization and recruitment of marrow-derived endothelial and hematopoietic stem cells by adenoviral vectors expressing angiogenic factors. *Gene Ther.* 2002;9:631–41. [PubMed: 12032709]

26. Arai F, Hirao A, Ohmura M, Sato H, Matsuoka S, Takubo K, et al. Tie2/angiopoietin-1 signaling regulates hematopoietic stem cell quiescence in the bone marrow niche. *Cell*. 2004;118:149–61. [PubMed: 15260986]
27. Wang J, Mukaida N, Zhang Y, Ito T, Nakao S, Matsushima K. Enhanced mobilization of hematopoietic progenitor cells by mouse MIP-2 and granulocyte colony-stimulating factor in mice. *J Leukoc Biol*. 1997;62:503–9. [PubMed: 9335321]
- 28••. Smith AJ, Duncan HF, Diogenes A, Simon S, Cooper PR. Exploiting the bioactive properties of the dentin-pulp complex in regenerative endodontics. *J Endod*. 2016;42:47–56 [PubMed: 26699924] State of the art review highlighting the role of dentin matrix components to contribute GFs and other bioactive factors to pulp repair and regeneration.
29. Zhang J, Lu X, Feng G, Gu Z, Sun Y, Bao G, et al. Chitosan scaffolds induce human dental pulp stem cells to neural differentiation: potential roles for spinal cord injury therapy. *Cell Tissue Res*. 2016;366:129–42. [PubMed: 27147262]
30. Bezgin T, Yilmaz AD, Celik BN, Kolsuz ME, Sonmez H. Efficacy of platelet-rich plasma as a scaffold in regenerative endodontic treatment. *J Endod*. 2015;41:36–44. [PubMed: 25459571]
31. Piva E, Silva AF, Nör JE. Functionalized scaffolds to control dental pulp stem cell fate. *J Endod*. 2014;40:S33–40. [PubMed: 24698691]
32. Bottino MC, Pankajakshan D, Nör JE. Advanced scaffolds for dental pulp and periodontal regeneration. *Dent Clin N Am*. 2017;61:689–711. [PubMed: 28886764]
33. Duncan HF, Smith AJ, Fleming GJ, Reid C, Smith G, Cooper PR. Release of bio-active dentine extracellular matrix components by histone deacetylase inhibitors (HDACi). *Int Endod J*. 2017;50:24–38. [PubMed: 26609946]
34. Kim SG, Zhou J, Solomon C, Zheng Y, Suzuki T, Chen M, et al. Effects of growth factors on dental stem/progenitor cells. *Dent Clin N Am*. 2012;56:563–75. [PubMed: 22835538]
35. Eramo S, Natali A, Pinna R, Milia E. Dental pulp regeneration via cell homing. *Int Endod J*. 2018;51:405–19. [PubMed: 29047120]
36. Kim SG, Zheng Y, Zhou J, Chen M, Embree MC, Song K, et al. Dentin and dental pulp regeneration by the patient's endogenous cells. *Endod Topics*. 2013;28:106–17. [PubMed: 24976816]
37. Kim JY, Xin X, Moioli EK, Chung J, Lee CH, Chen M, et al. Regeneration of dental-pulp-like tissue by chemotaxis-induced cell homing. *Tissue Eng Part A*. 2010;16:3023–31. [PubMed: 20486799]
38. Banchs F, Trope M. Revascularization of immature permanent teeth with apical periodontitis: new treatment protocol? *J Endod*. 2004;30:196–200. [PubMed: 15085044]
- 39••. Shimizu E, Ricucci D, Albert J, Alobaid AS, Gibbs JL, Huang GT, et al. Clinical, radiographic, and histological observation of a human immature permanent tooth with chronic apical abscess after revitalization treatment. *J Endod*. 2013;39:1078–83 [PubMed: 23880282] Demonstrated that although successful clinically, histo-logically there no pulp tissue, only bone-like fibrous connective tissue in case of pulp necrosis.
40. Graham L, Cooper PR, Cassidy N, Nor JE, Sloan AJ, Smith AJ. The effect of calcium hydroxide on solubilisation of bio-active dentine matrix components. *Biomaterials*. 2006;27:2865–73. [PubMed: 16427123]
41. Tomson PL, Grover LM, Lumley PJ, Sloan AJ, Smith AJ, Cooper PR. Dissolution of bio-active dentine matrix components by mineral trioxide aggregate. *J Dent*. 2007;35:636–42. [PubMed: 17566626]
42. Galler KM, Widbiller M, Buchalla W, Eidt A, Hiller KA, Hoffer PC, et al. EDTA conditioning of dentine promotes adhesion, migration and differentiation of dental pulp stem cells. *Int Endod J*. 2016;49:581–90. [PubMed: 26114662]
43. Alghilan MA, Windsor LJ, Palasuk J, Yassen GH. Attachment and proliferation of dental pulp stem cells on dentine treated with different regenerative endodontic protocols. *Int Endod J*. 2017;50:667–75. [PubMed: 27272393]
44. Niu Y, Li Q, Ding Y, Dong L, Wang C. Engineered delivery strategies for enhanced control of growth factor activities in wound healing. *Adv Drug Deliv Rev*. 2018 10.1016/j.addr.2018.06.002.

45. Fischbach GD, Fischbach RL. Stem cells: science, policy, and ethics. *J Clin Invest*. 2004;114:1364–70. [PubMed: 15545983]
46. Mason C, Dunnill P. A brief definition of regenerative medicine. *Regen Med*. 2008;3:1–5. [PubMed: 18154457]
47. Bojic S, Volarevic V, Ljubic B, Stojkovic M. Dental stem cells-characteristics and potential. *Histol Histopathol*. 2014;29:699–706. [PubMed: 24446280]
48. Fayazi S, Takimoto K, Diogenes A. Comparative evaluation of chemotactic factor effect on migration and differentiation of stem cells of the apical papilla. *J Endod*. 2017;43:1288–93. [PubMed: 28578888]
49. Jeanneau C, Lundy FT, El Karim IA, About I. 47. Potential therapeutic strategy of targeting pulp fibroblasts in dentin-pulp regeneration. *J Endod* 2017;43:S17–S24. [PubMed: 28778507]
50. Saito K, Oshima H. Differentiation capacity and maintenance of dental pulp stem/progenitor cells in the process of pulpal healing following tooth injuries. *Journal J Oral Biosci*. 2017;59:63–70.
51. Cooper PR, Takahashi Y, Graham LW, Simon S, Imazato S, Smith AJ. Inflammation-regeneration interplay in the dentine-pulp complex. *J Dent*. 2010;38:687–97. [PubMed: 20580768]
52. Alongi DJ, Yamaza T, Song Y, Fouad AF, Romberg EE, Shi S, et al. Stem/progenitor cells from inflamed human dental pulp retain tissue regeneration potential. *Regen Med*. 2010;5:617–31. [PubMed: 20465527]
53. Suzuki T, Lee CH, Chen M, Zhao W, Fu SY, Qi JJ, et al. Induced migration of dental pulp stem cells for in vivo pulp regeneration. *J Dent Res*. 2011;90:1013–8. [PubMed: 21586666]
54. Yang JW, Zhang YF, Wan CY, Sun ZY, Nie S, Jian SJ, et al. Autophagy in SDF-1 α -mediated DPSC migration and pulp regeneration. *Biomaterials*. 2015;44:11–23. [PubMed: 25617122]
55. Gervois P, Wolfs E, Dillen Y, Hilken P, Ratajczak J, Driesen RB, et al. Paracrine maturation and migration of SH-SY5Y cells by dental pulp stem cells. *J Dent Res*. 2017;96:654–62. [PubMed: 28141971]
56. Sonoyama W, Liu Y, Fang D, Yamaza T, Seo BM, Zhang C, et al. Mesenchymal stem cell-mediated functional tooth regeneration in swine. *PLoS One*. 2006;1:e79. [PubMed: 17183711]
57. Sonoyama W, Liu Y, Yamaza T, Tuan RS, Wang S, Shi S, et al. Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: a pilot study. *J Endod*. 2008;34:166–71. [PubMed: 18215674]
58. Galler KM, Eidt A, Schmalz G. Cell-free approaches for dental pulp tissue engineering. *J Endod*. 2014;40:S41–5. [PubMed: 24698692]
59. Chrepa V, Pitcher B, Henry MA, Diogenes A. Survival of the apical papilla and its resident stem cells in a case of advanced pulpal necrosis and apical periodontitis. *J Endod*. 2017;43:561–7. [PubMed: 28190588]
60. Huang GT. Pulp and dentin tissue engineering and regeneration: current progress. *Regen Med*. 2009;4:697–707. [PubMed: 19761395]
61. de Almeida JF, Chen P, Henry MA, Diogenes A. Stem cells of the apical papilla regulate trigeminal neurite outgrowth and targeting through a BDNF-dependent mechanism. *Tissue Eng Part A*. 2014;20:3089–100. [PubMed: 24837134]
62. Isaka J, Ohazama A, Kobayashi M, Nagashima C, Takiguchi T, Kawasaki H, et al. Participation of periodontal ligament cells with regeneration of alveolar bone. *J Periodontol*. 2001;72:314–23. [PubMed: 11327058]
63. Huang GT, Gronthos S, Shi S. 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. [PubMed: 19767575]
64. Abdallah BM, Kassem M. New factors controlling the balance between osteoblastogenesis and adipogenesis. *Bone*. 2012;50: 540–5. [PubMed: 21745614]
65. Ruangsawasdi N, Zehnder M, Patcas R, Ghayor C, Siegenthaler B, Gjoksi B, et al. Effects of stem cell factor on cell homing during functional pulp regeneration in human immature teeth. *Tissue Eng Part A*. 2017;23:115–23. [PubMed: 27762658]
66. Hattori K, Dias S, Heissig B, Hackett NR, Lyden D, Tateno M, et al. Vascular endothelial growth factor and angiopoietin-1 stimulate postnatal hematopoiesis by recruitment of vasculogenic and hematopoietic stem cells. *J Exp Med*. 2001;193:1005–14. [PubMed: 11342585]

67. Tamma R, Ribatti D. Bone niches, hematopoietic stem cells, and vessel formation. *Int J Mol Sci.* 2017;18.
68. Asahara T, Takahashi T, Masuda H, Kalka C, Chen D, Iwaguro H, et al. VEGF contributes to postnatal neovascularization by mobilizing bone marrow-derived endothelial progenitor cells. *EMBO J.* 1999;18:3964–72. [PubMed: 10406801]
69. Cassidy N, Fahey M, Prime SS, Smith AJ. Comparative analysis of transforming growth factor- β isoforms 1–3 in human and rabbit dentine matrices. *Arch Oral Biol.* 1997;42:219–23. [PubMed: 9188992]
70. Smith AJ. Vitality of the dentin-pulp complex in health and disease: growth factors as key mediators. *J Dent Educ.* 2003;67:678–89. [PubMed: 12856968]
71. Mazzoni A, Tjäderhane L, Checchi V, Di Lenarda R, Salo T, Tay FR, et al. Role of dentin MMPs in caries progression and bond stability. *J Dent Res.* 2015;94:241–51. [PubMed: 25535202]
72. Dung SZ, Gregory RL, Li Y, Stookey GK. Effect of lactic acid and proteolytic enzymes on the release of organic matrix components from human root dentin. *Caries Res.* 1995;29:483–9. [PubMed: 8556753]
73. Charadram H, Farahani RM, Harty D, Rathsam C, Swain MV, Hunter N. Regulation of reactionary dentin formation by odonto-blasts in response to polymicrobial invasion of dentin matrix. *Bone.* 2012;2012(50):265–75.
74. Smith AJ, Tobias RS, Cassidy N, Plant CG, Browne RM, Begue-Kirn C, et al. Odontoblast stimulation in ferrets by dentine matrix components. *Arch Oral Biol.* 1994;39:13–22. [PubMed: 8179504]
75. Ferracane JL, Cooper PR, Smith AJ. Dentine matrix component solubilization by solutions of pH relevant to self-etching dental adhesives. *J Adhes Dent.* 2013;15:407–12. [PubMed: 23560260]
76. Widbiller M, Eidt A, Hiller KA, Buchalla W, Schmalz G, Galler KM. Ultrasonic activation of irrigants increases growth factor release from human dentine. *Clin Oral Investig.* 2017;21:879–88.
77. Galler KM, Buchalla W, Hiller KA, Federlin M, Eidt A, Schiefersteiner M, et al. Influence of root canal disinfectants on growth factor release from dentin. *J Endod.* 2015;41:363–8. [PubMed: 25595468]
78. Martin DE, De Almeida JF, Henry MA, Khaing ZZ, Schmidt CE, Teixeira FB, et al. Concentration-dependent effect of sodium hypochlorite on stem cells of apical papilla survival and differentiation. *J Endod.* 2014;40:51–5 [PubMed: 24331991] Ex vivo study highlighting the effects of increasing sodium hypochlorite concentration on the biological response of SCAP.
79. Shrestha S, Torneck CD, Kishen A. Dentine conditioning with bioactive molecule releasing nanoparticle system enhances adherence, viability, and differentiation of stem cells from apical papilla. *J Endod.* 2016;42:717–23. [PubMed: 26960576]
80. Bègue-Kirn C, Smith AJ, Ruch JV, Wozney JM, Purchio A, Hartmann D, et al. Effects of dentin proteins, transforming growth factor beta 1 (TGF beta 1) and bone morphogenetic protein 2 (BMP2) on the differentiation of odontoblast in vitro. *Int J Dev Biol.* 1992;36:491–503. [PubMed: 1295560]
81. Bento LW, Zhang Z, Imai A, Nör F, Dong Z, Shi S, et al. Endothelial differentiation of SHED requires MEK1/ERK signaling. *J Dent Res.* 2013;92:51–7. [PubMed: 23114032]
82. Yoshida N, Edanami N, Tohma A, Takeuchi R, Ohkura N, Hosoya A, et al. Detection of bone marrow-derived fibrocytes in human dental pulp repair. *Int Endod J.* 2018 10.1111/iej.12940.
83. Lambrichts I, Driesen RB, Dillen Y, Gervois P, Ratajczak J, Vanganswinkel T, et al. Dental pulp stem cells: their potential in reinnervation and angiogenesis by using scaffolds. *J Endod.* 2017;43:S12–6 [PubMed: 28781091] Comprehensive review highlighting the role of DPSCs and GFs in angiogenesis and neurogenesis.
84. Aranha AM, Zhang Z, Neiva KG, Costa CA, Hebling J, Nör JE. Hypoxia enhances the angiogenic potential of human dental pulp cells. *J Endod.* 2010;36:1633–7. [PubMed: 20850667]
85. Albanese MEL, Polizzi B, Campisi G. Platelet-rich plasma (PRP) in dental and oral surgery: from the wound healing to bone regeneration. *Immun Ageing.* 2013;10:23. [PubMed: 23763951]
86. Roselló-Camps À, Monje A, Lin GH, Khoshkam V, Chávez-Gatty M, Wang HL, et al. Platelet-rich plasma for periodontal regeneration in the treatment of intrabony defects: a meta-analysis on

- prospective clinical trials. *Oral Surg Oral Med Oral Pathol Oral Radiol.* 2015;120:562–74. [PubMed: 26453383]
87. Rodriguez IA, Growney Kalaf EA, Bowlin GL, Sell SA. Platelet-rich plasma in bone regeneration: engineering the delivery for improved clinical efficacy. *Biomed Res Int.* 2014;392398. [PubMed: 25050347]
 88. Volpi P, Quaglia A, Schoenhuber H, Melegati G, Corsi MM, Banfi G, et al. Growth factors in the management of sport-induced tendinopathies: results after 24 months from treatment. A pilot study. *J Sports Med Phys Fitness.* 2010;50:494–500. [PubMed: 21178936]
 89. Lyras DN, Kazakos K, Georgiadis G, Mazis G, Middleton R, Richards S, et al. Does a single application of PRP alter the expression of IGF-I in the early phase of tendon healing? *J Foot Ankle Surg.* 2011;50:276–82. [PubMed: 21440462]
 90. Alsousou J, Ali A, Willett K, Harrison P. The role of platelet-rich plasma in tissue regeneration. *Platelets.* 2013;24:173–82. [PubMed: 22647081]
 91. Jadhav G, Shah N, Logani A. Revascularization with and without platelet-rich plasma in nonvital, immature, anterior teeth: a pilot clinical study. *J Endod.* 2012;38:1581–7. [PubMed: 23146641]
 92. Hotwani K, Sharma K. Platelet rich fibrin—a novel acumen into regenerative endodontic therapy. *Rest Dent Endod.* 2014;39:1–6.
 93. Simonpieri A, Del Corso M, Vervelle A, Jimbo R, Inchingolo F, Sammartino G, et al. Current knowledge and perspectives for the use of platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) in oral and maxillofacial surgery part 2: bone graft, implant and reconstructive surgery. *Curr Pharm Biotechnol.* 2012;13:1231–56. [PubMed: 21740370]
 94. Narang I, Mittal N, Mishra N. A comparative evaluation of the blood clot, platelet-rich plasma, and platelet-rich fibrin in regeneration of necrotic immature permanent teeth: a clinical study. *Contemp Clin Dent.* 2015;6:63–8. [PubMed: 25684914]
 95. Zhou R, Wang Y, Chen Y, Chen S, Lyu H, Cai Z, et al. Radiographic, histologic, and biomechanical evaluation of combined application of platelet-rich fibrin with blood clot in regenerative endodontics. *J Endod.* 2017;43:2034–40. [PubMed: 29032818]
 96. Lolato A, Bucchi C, Taschieri S, Kabbaney AE, Del Fabbro M. Platelet concentrations for revitalization of immature necrotic teeth: a systematic review of the clinical studies. *Platelets.* (5): 383–92.
 97. Tziafas D, Alvanou A, Panagiotakopoulos N, Smith AJ, Lesot H, Komnenou A, et al. Induction of odontoblast-like cell differentiation in dog dental pulps after in vivo implantation of dentine matrix components. *Arch Oral Biol.* 1995;40:883–93. [PubMed: 8526798]
 98. Roberts-Clark DJ, Smith AJ. Angiogenic growth factors in human dentine matrix. *Arch Oral Biol.* 2000;45:1013–6. [PubMed: 11000388]
 99. Galler KM, D'Souza RN, Federlin M, Cavender AC, Hartgerink JD, Hecker S, et al. Dentin conditioning codetermines cell fate in regenerative endodontics. *J Endod.* 2011;37:1536–41. [PubMed: 22000458]
 100. Zieris A, Prokoph S, Levental KR, Welzel PB, Grimmer M, Freudenberg U, et al. FGF-2 and VEGF functionalization of starPEG-heparin hydrogels to modulate biomolecular and physical cues of angiogenesis. *Biomaterials.* 2010;31:7985–94. [PubMed: 20674970]
 101. Galler KM, Hartgerink JD, Cavender AC, Schmalz G, D'Souza RN. A customized self-assembling peptide hydrogel for dental pulp tissue engineering. *Tissue Eng Part A.* 2012;18:176–84. [PubMed: 21827280]
 102. Silva CR, Babo PS, Gulino M, Costa L, Oliveira JM, Silva-Correia J, et al. Injectable and tunable hyaluronic acid hydrogels releasing chemotactic and angiogenic growth factors for endodontic regeneration. *Acta Biomater.* 2018 10.1016/j.actbio.2018.07.035.
 103. Nagy MM, Tawfik HE, Hashem AA, Abu-Seida AM. Regenerative potential of immature permanent teeth with necrotic pulps after different regenerative protocols. *J Endod.* 2014;40:192–8. [PubMed: 24461403]
 104. Takeuchi N, Hayashi Y, Murakami M, Alvarez FJ, Horibe H, Iohara K, et al. Similar in vitro effects and pulp regeneration in ectopic tooth transplantation by basic fibroblast growth factor and granulocyte-colony stimulating factor. *Oral Dis.* 2015;21:113–22. [PubMed: 24495211]

105. Iohara K, Imabayashi K, Ishizaka R, Watanabe A, Nabekura J, Ito M, et al. Complete pulp regeneration after pulpectomy by transplantation of CD105⁺ stem cells with stromal cell-derived factor-1. *Tissue Eng Part A*. 2011;17:1911–20. [PubMed: 21417716]
106. Nakashima M, Iohara K, Murakami M, Nakamura H, Sato Y, Aiji Y, et al. Pulp regeneration by transplantation of dental pulp stem cells in pulpitis: a pilot clinical study. *Stem Cell Res Ther*. 2017;8:61. [PubMed: 28279187]
107. Nakashima M, Iohara K. Recent progress in translation from bench to a pilot clinical study on total pulp regeneration. *J Endod*. 2017;43:S82–6. [PubMed: 28778509]
108. Kawamura R, Hayashi Y, Murakami H, Nakashima M. EDTA soluble chemical components and the conditioned medium from mobilized dental pulp stem cells contain an inductive microenvironment, promoting cell proliferation, migration, and odontoblastic differentiation. *Stem Cell Res Ther*. 2016;7:77. [PubMed: 27387974]

Fig. 1. Schematic drawing representing the influence of dentin matrix components (DMCs) extracted by a range of etchants, irrigants, dental materials, and epigenetic modifying agents, (histone deacetylase inhibitors [HDACi]) on the promotion cell migration, angiogenesis, neurogenesis, mineralization, and regenerative events

Table 1

A list of abbreviations and definitions used in the text and figure

Abbreviation	Definition
ANG-1	Angiopoietin-1
AR	Amphiregulin
bFGF	Basic fibroblast growth factor
BMP	Bone morphogenetic protein
BDNF	Brain-derived neurotrophic factor
DPSC	Dental pulp stem cell
EGF	Epidermal growth factor
ECGF	Epithelial cell growth factor
EDTA	Ethylenediaminetetraacetic acid
EG-VEGF (PK)	Prokineticin-1
Flt-3	Fms-like tyrosine kinase 3
GDF-15	Growth/differentiation factor 15
GDNF	Glial cell-derived neurotrophic factor
G-CSF	Granulocyte colony stimulating factor
GF	Growth factor
HB-EGF	Proheparin EGF-like growth factor
HSC	Hematopoietic stem cell
HGF	Hepatocyte growth factor
BMSSC	Human bone marrow stromal stem cell
INS	Insulin
IGF	Insulin growth factor
IL	Interleukin
MIP-2	Macrophage inflammatory protein-2
MTA	Mineral trioxide aggregate
NGF	Nerve growth factor
NT	Neurotrophin
PDLSC	Periodontal ligament stem cell
PDEGF	Platelet-derived epidermal growth factor
PDGF	Platelet-derived growth factor
PIGF	Placenta growth factor
PRF	Platelet-rich fibrin
PRP	Platelet-rich plasma
SC	Stem cell
SCAP	Stem cells of the apical papilla
SCF	Stem cell factor
SDF-1	Stromal cell-derived factor-1
TGF	Transforming growth factor
VEGF	Vascular endothelial growth factor

Table 2

A list of abbreviations for the 28 GFs analyzed in the commercial antibody array (RayBio)

Abbreviation	Protein definition	Present in dentine extracts
AR	Amphiregulin	No: Not detected or below threshold
BDNF	Brian-derived neurotrophic factor	Yes: Expressed
bFGF	Basic fibroblast growth factor	Yes: Expressed
BMP-4	Bone morphogenetic protein 4	No: Not detected or below threshold
BMP-5	Bone morphogenetic protein 5	No: Not detected or below threshold
BMP-7	Bone morphogenetic protein 7	Yes: Expressed
β -NGF	Beta-nerve growth factor	No: Not detected or below threshold
EGF	Pro-epidermal growth factor	No: Not detected or below threshold
EG-VEGF (PK)	Prokineticin-1	No: Not detected or below threshold
FGF-4	Fibroblast growth factor 4	Yes: Expressed
FGF-7 (KGF)	Fibroblast growth factor 7	Yes: Expressed
GDF-15	Growth/differentiation factor 15	Yes: Expressed
GDNF	Glial cell-line-derived neurotrophic factor	Yes: Expressed
GH	Growth hormone	No: Not detected or below threshold
HB-EGF	Proheparin EGF-like growth factor	No: Not detected or below threshold
HGF	Hepatocyte growth factor	Yes: Expressed
IGF-1	Insulin growth factor	Yes: Expressed
INS	Insulin	Yes: Expressed
NT-3	Neurotrophin 3	No: Not detected or below threshold
NT-4	Neurotrophin 4	No: Not detected or below threshold
PDGF-AA	Platelet-derived growth factor subunit A	Yes: Expressed
PIGF	Placenta growth factor	Yes: Expressed
SCF	Stem cell factor	No: Not detected or below threshold
TGF- α	Prot transforming growth factor alpha	No: Not detected or below threshold
TGF- β -1	Transforming growth factor beta 1	Yes: High expression
TGF- β -3	Transforming growth factor beta 3	No: Not detected or below threshold
VEGF-A	Vascular endothelial growth factor A	Yes: Expression
VEGF-D	Vascular endothelial growth factor D	No: Not detected or below threshold

Dentine matrix components were extracted over 14 days with 10% EDTA or three epigenetic modifying agents (histone deacetylase inhibitors). Combination of unpublished and modified published data highlights the range of GFs present in dentine [33]

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