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Review Article

Regenerative Endodontic Procedures: A Tissue Engineering Perspective

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Regenerative Endodontic Procedures (REP) are biologically based procedures designed to replace the damaged dentin and root structures and cells of the pulp-dentin complex. The success of REP relies on incorporating three basic components: triad of tissue engineering (growth factors, scaffold, and stem cells), disinfection of root canal spaces, and coronal seal. The stem cells proliferate and differentiate into desired cell types and synthesize matrices of new tissue. The scaffold mimics the matrix to be formed and provides the appropriate environment for stem cells until a new matrix is laid down. The growth factors are biomolecules that direct the entire process of regeneration. Regeneration of tissue is only possible in disinfected canal spaces achieved through irrigants and intracanal medicaments. An effective coronal seal maintains the disinfected environment and prevents re-infection of the root canal space. This review discusses the current knowledge of regenerative endodontics from a tissue engineering perspective. It summarizes the effects of multiple factors' on the result of regenerative endodontic procedures to achieve more predictable results.

Introduction

Regenerative medicine is the branch of health sciences that develops methods to regrow, repair, or replace damaged or diseased cells, organs, or tissues [1]. Regenerative procedures in oral health sciences started in 1952 when Dr. B. W. Hermann reported vital pulp amputation using calcium hydroxide [2]. The past several decades have witnessed an advancement in the scope and clinical application of regenerative procedures in dentistry, including oral and maxillofacial surgery, periodontics, and endodontics. The Regenerative Endodontic Procedure (REP) is aimed toward the predictable replacement of damaged, diseased, or missing structures, including dentin and root structures, as well as cells of the pulp-dentin complex with viable live tissues, preferably of the same origin, that restores the normal physiologic functions of the pulp–dentin complex [3].

The concept of regeneration in endodontics was pioneered by Nygaard-Østby, who observed that blood clot formed during root canal filling aided in healing of the periapical lesion [4]. However, Iwaya et al. in 2001 first used the concept of re-vascularization to treat the immature permanent tooth with apical periodontitis [5]. This concept was based on the potential for revascularization and continued root development of replanted and auto-transplanted teeth [6,7]. The protocol for the revascularization procedure was proposed by Banchs et al [8]. The use of REP started in immature necrotic teeth intending to attain continued root development; however, it has now been successfully used in the treatment of mature necrotic teeth [9-11] and retreatment cases [12].

Conventionally, the non-surgical root canal therapy treats a tooth with irreversibly inflamed and necrotic pulp secondary to exposure to the bacteria and/ or its by-products. Though the outcome of root canal therapy is relatively predictable, it involves reshaping and filling the root canal space with nonvital material. The REP offers a biological treatment option by filling the root canal space with vital tissue with immune defense mechanisms and sensory innervation.

The REPs are based on the concept of tissue engineering. The purpose is to use a biological-based procedure, which can halt the pathological process while favoring the repair or replacement of the damaged components of the pulp-dentin complex. The vital pulp therapies aim to preserve and maintain pulpal health in teeth

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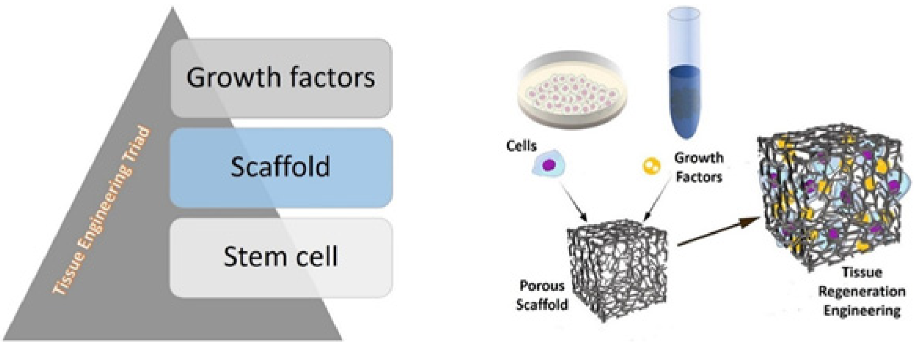

Figure 1: The key elements for tissue regeneration and engineering. Reprinted with permission from [13], Peng Z, et al. Nanotechnol. Rev. 9(1), (2020). ©2020, De Gruyter.

exposed to caries, trauma, restorative procedures, and anatomic anomalies. On the other hand, REP aims to reestablish vital new tissue, which replaces the dental pulp following liquefaction necrosis secondary to infection. The three key elements for tissue engineering are the stem cells, scaffold, and growth factors (figure 1). The combination of the porous scaffold with the growth factors, and extracellular matrix protein molecules tend to promote the cell attachment, its proliferation, and induces localized tissue regeneration. The stem cells proliferate and differentiate into desired cell types and synthesize matrices of new tissue. The scaffold mimics the matrix to be formed and provides the appropriate environment for stem cells until a new matrix is laid down. The growth factors are biomolecules that direct the entire process of regeneration. Therefore, tissue engineering offers a promising alternative for the biological reconstitution of lost or damaged organs and tissues rather than a replacement substitute.

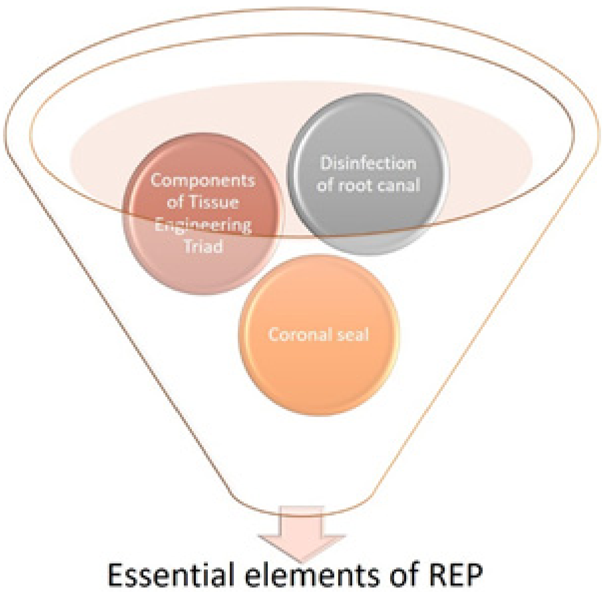

Figure 2: The key elements for regenerative endodontic procedure

Essential Elements for REP

The success of REP relies on incorporating the three basic components as shown in figure 2.

Tissue engineering components

Any tissue in the body consists of cells, matrix, and signaling systems. To simulate this, tissue engineering involves three components: stem cells, scaffold, and growth factors.

Stem cells are a self-renewing, undifferentiated cell type that can generate one or more differentiated cell types when exposed to the appropriate environmental stimuli [14]. Depending on their plasticity, stem cells are commonly subdivided into totipotent, pluripotent, and multipotent categories (figure 3). Depending on the source, stem cells can be classified as embryonic or postnatal [15]. *Embryonic stem cells* are during the early stages of embryo development and are totipotent cells [16]. On the other hand, *postnatal stem cells* are multipotent and can be obtained from individuals at any stage in life. Such tissues include bone marrow, brain, skin, muscle, and adipose tissue.

Sources of postnatal stem cells in the oral cavity

The oral cavity acts as a good source for postnatal stem cells, such cells can be derived from Stem Cells of the Apical Papilla (SCAP), inflammatory Periapical Progenitor Cells (iPAPCs), Dental Follicle Stem Cells (DFSCs), Dental Pulp Stem Cells (DPSCs), Periodontal

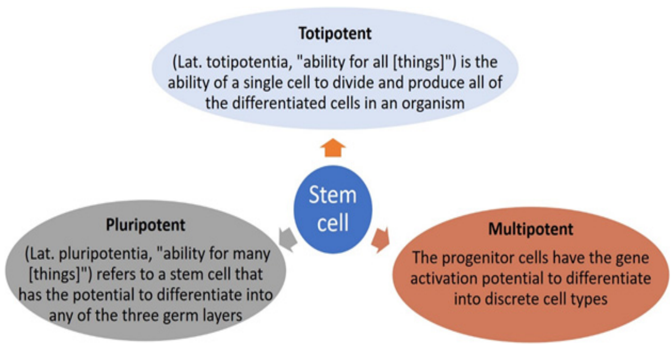

Figure 3: The subdivision of the stem cell

Table 1: Potential sources of intra-oral sources of stem cells and their notable features

Dental Pulp Stem Cells (DPSCs)	More commonly seen in the perivascular region and the cell-rich zone of Hohl adjacent to the odontoblastic layer [20,21]. Even healthy pulp coexists with inflamed tissue in a compartmentalized abscess, which can act as a source of endogenous stem cells [22]. It can be reprogrammed into multiple cell lineages such as odontoblast, osteoblast, chondrocyte, myocyte, neurocyte, adipocyte, corneal epithelial cell, and melanoma cell [23]. The components of the local microenvironment, such as growth factors and extracellular matrix protein, mainly dictate the differentiation of DPSC to a specific cell lineage. DPSCs can be induced into odontoblast lineage when treated with Transforming Growth Factor β1 (TGFβ1) alone or combined with Fibroblast Growth Factor (FGF2) [24]. Entire dentin-pulp-like complex regeneration with well-established vascularity has been observed in emptied root canal space by DPSC transplantation [25].
Stem Cells from Apical Papilla (SCAP)	The apical papilla's stem cells are the reservoir of undifferentiated Mesenchymal Stem Cells (MSCs) with great proliferative and odontogenic differentiation capacity [26]. Regulated by Hertwig's epithelial root sheath through a series of complex epithelial-mesenchymal interactions that dictate root development and shape [27]. Higher proliferation rates in vitro than DPSC and can form odontoblast-like cells [28]. Collateral circulation due to its proximity to periapical tissue [29] explains the survival of SCAP even during pulpal necrosis Hypoxic environments and bacterial endotoxins enhance dental stem cells' proliferation, survival, and angiogenic potential [30,31].
Inflammatory Periapical Progenitor Cells (iPAPCs)	It emerges in the newly formed granulation tissues. It comes from a local pool of resting stem cells in these tissue sites that expand in response to the inflammatory reaction caused by the presence of the foreign body [32]. These cells can potentially differentiate into mature osteoblastic cells [19].
Periodontal Ligament Stem Cells (PDLSCs)	Capable of differentiating along mesenchymal cell lineages to produce cementoblast-like cells, adipocytes, and connective tissue rich in collagen I [33]. They display cell surface marker characteristics and differentiation potential similar to Bone Marrow Stem Cells and DPSC [33].
Bone Marrow Stem cells (BMSCs)	They showed much better mineralization compared to the others [34]. In comparison, DPSCs presented more striking odontogenic capability than BMSCs [35].

Ligament Stem Cells (PDLSCs), Bone Marrow Stem Cells (BMSCs), Tooth Germ Progenitor Cells (TGPCs), Salivary Gland Stem Cells (SGSCs), Stem Cells from Human Exfoliated Deciduous teeth (SHED), Oral Epithelial Stem Cells (OESCs), Gingival-derived Mesenchymal Stem Cells (GMSCs), and Periosteal derived Stem Cells (PSCs) [17,18]. However, the stem cells more likely to be involved in REP are localized around the periapical region. These include SCAP, iPAPCs [19], PDLSCs, BMSCs, and DPSCs (if the vital pulp is still present apically) (table 1) (figure 4).

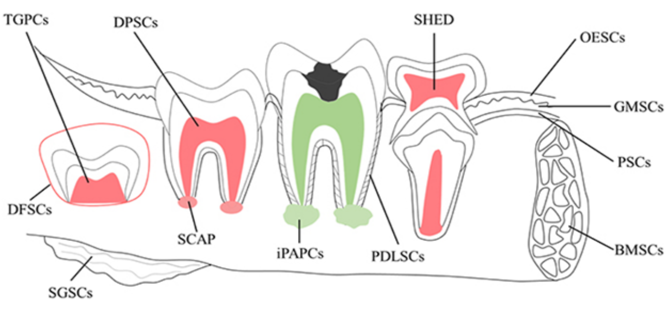

Figure 4: Various potential sources of postnatal stem cells in the oral cavity. Reprinted with permission from [36], Yang B, et al. Front. Physiol. 8, (2017). ©2017, Frontiers

Cell transplantation and cell homing

The knowledge of the source of stem cells is critical for developing the protocol for REP. The incorporation of stem cells for regenerative endodontics involves two approaches [37]:

Cell transplantation

Cell transplantation involves isolating stem cells from the host, followed by ex-vivo cultivation of stem/progenitor cells and transplantation of the cultivated cells into the tooth's root canal (figure 5). The cell-based approach resulted in pulpal regeneration by Dental Pulpal Stem Cell transplantation in animal models [38]. Many challenges are present in clinical practice, such as isolation, culture, and storage of autologous stem cells, high cost, regulatory hurdles for approval, and the clinician's skill.

Cell homing

Cell homing represents a much easier approach by utilizing already existing stem cells (figure 5). Cell homing involves two distinctive cellular processes: cell recruitment and cell differentiation. Cell recruitment refers to the directional migration of stem/progenitor cells to the site of tissue defects. Cell differentiation refers to transforming stem/progenitor cells into mature cells [37]. In the context of dental pulp and dentin regeneration, stem/progenitor cells differentiate into odontoblasts and pulp fibroblasts and need to induce the sprouting of neural fibrils and endothelial cells along with other angiogenesis-related cells.

The entire process of cell homing relies on growth factors to recruit stem cells into root canal space. In animal studies, dental pulp-like tissue was found in canal space on subcutaneous implantation of extracted and sterilized teeth after infusion with suitable scaffold and growth factors [39]. Studies have shown that bleeding evoked from periapical tissue during REP causes a significant accumulation of undifferentiated stem cells into canal space and a significant accumulation of transcripts for the stem cell markers CD73 and CD105 (up to 600-fold) compared with levels found in the systemic blood [40]. Even in fully mature teeth, one study has demonstrated that inducing bleeding into canal space by over-instrumentation into the periradicular tissues results in a robust influx of cells with mesenchymal stem cell markers, similar to that seen in immature teeth [41]. This can be justified because the size of apical foramen, even in mature teeth, is much larger than the size of stem cells and does not affect the outcome of regenerative procedures [42]. Thus, it appears that MSCs from the apical region can be delivered into the root canal spaces in both immature and mature teeth. However, some evidence suggests that MSCs have decreased proliferative and differentiation potential with aging [43].

Scaffold is a three-dimensional structure that supports cell organization and vascularization. The principal objective of scaffold design is to mimic the physical and biochemical microenvironment of the root canal and simulate the native extracellular matrix (ECM)

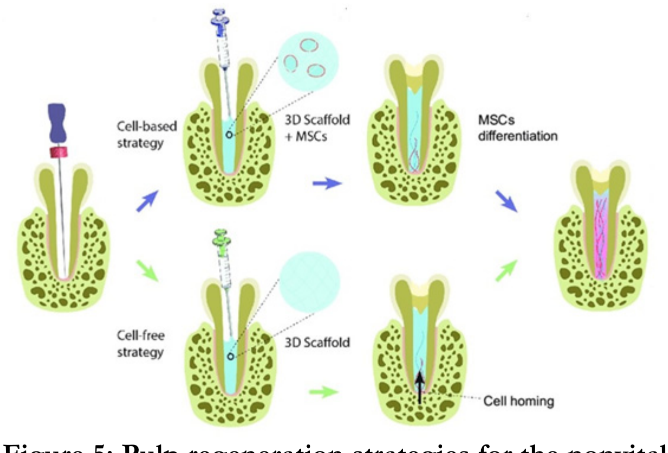

Figure 5: Pulp regeneration strategies for the nonvital permanent tooth, the cell homing and cell transplantation. Reprinted with permission from [44], Ducret M, et al. Eur. Cells Mater. 41, (2021). ©2021, Forum Multimedia Publishing

until cells seeded within the scaffold and/or derived from the host tissue can synthesize a new, natural matrix [45]. This extracellular matrix promotes SCAP adhesion and migration, thus serving as a template for tissue regeneration [46]. Scaffolds incorporating growth factors, such as TGF-â1 (Transforming Growth Factor-â1), BMP-2 (Bone Morphogenic Protein), VEGF (Vascular Endothelial Growth Factor), and PDGF (Platelet-Derived Growth Factor), further support odontogenic differentiation and drive pulpal revascularization [47].

The ideal characteristics of a scaffold include biocompatibility, promoting cellular activities such as cell adhesion, biodegradability and highly porous structure with an interconnected network of microscopic spaces, favorable mechanical properties, and highly reproducible and adaptable fabrication process for different shapes or sizes [45]. Scaffolds may be in preformed or injectable varieties. Preformed scaffolds offer the advantage of definite conformations, while the compliant nature of injectable scaffolds allows them to adapt to the unique anatomy of the scaffold's destination [48]. Scaffolds can be host-derived, naturally derived, and synthetic material.

Host-derived

Host-derived scaffolds can be blood clots or platelet concentrates.

Blood clot

Induction of bleeding into canal space by over-instrumentation into the periapical area followed by the natural mechanism of clotting results in forming a blood clot scaffold into canal space [49,50]. It offers the advantage of delivery of stem cells from periapical tissues into root canal space through the apical foramen [40]. It also allows endogenous hemostatic factors to enter the canal space along with stem cells, which then guide the process of cellular differentiation and proliferation, vascularization, and tissue regeneration. Further, blood forms cross-linked fibrin networks on clotting, which act as an autologous scaffold, thus providing all three important components for tissue engineering. The main advantage of the blood clot is that it is autologous and, therefore, does not induce any foreign body reaction. Also, low cost, clinical simplicity, and short procedural time make it the most common scaffold choice in the REP. The major challenge includes difficulty in inducing the bleeding, as sufficient bleeding cannot be induced in all cases. Moreover, the number of growth factors and stem cells in induced blood clots cannot be regulated, and thus, it will promote healing rather than controlled pulpal regeneration [51]. These limitations of blood clots as scaffolds led to research for more consistent, effective scaffolds.

Platelet concentrates play a pivotal role in tissue regeneration by releasing growth factors (GFs) and cytokines such as PDGF and VEGF, which stimulate cell proliferation, matrix remodeling, and angiogenesis. Platelet concentrate utilizes the same source, i.e., the Patient's blood, as a scaffold and source of growth factors. Moreover, its use does not cause laceration of the apical papilla, thus minimizing the disruption in periapical tissue, which is the key source of stem cells in cell-homing-based regenerative procedures. Platelet Rich Plasma (PRP) and Platelet Rich Fibrin (PRF) are the most commonly used platelet concentrates.

Platelet-rich plasma (PRP)

It is an autologous first-generation platelet concentrate. Various PRP preparation techniques are available, but they have some points in common. Blood is collected in a test tube containing anticoagulant trisodium citrate and processed by centrifugation. First

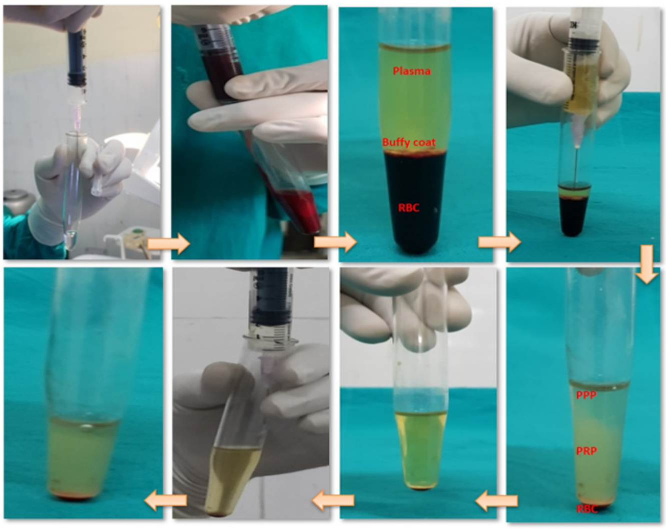

Figure 6: Sequential stages of platelet rich plasma (PRP) preparation

centrifugation at 300g for 5 min results in three separate layers, red blood cells (RBCs) at the bottom, acellular plasma or platelet poor plasma (PPP) as supernatant, and a 'buffy coat' layer appears in between. Depending on the preparation of Leukocyte-and Platelet-Rich Plasma (L-PRP) or Pure Platelet-Rich Plasma (P-PRP), PPP is removed with or without a buffy coat, respectively (figure 6). The second centrifugation at 700g for 17 min results in obtaining platelet concentrate, which triggers platelet activation and fibrin polymerization on mixing with thrombin or calcium chloride [52,53].

Platelet Rich Fibrin (PRF)

PRF is a second-generation platelet concentrate that is purely autologous, named Choukroun's PRF after its inventor [54]. Blood is collected without anticoagulant and centrifuged at 3000 rpm (app 400 G) for 10 min. After centrifugation, three layers are formed: the RBC base layer, acellular plasma top layer, and a PRF clot in the middle (figure 7). This technique forms a leucocyte- and platelet-rich fibrin (L-PRF) clot, which is based on a natural coagulation process and does not require biochemical modification of the blood [55]. The advantage of PRF is slow fibrin polymerization and slow destruction by remodeling, allowing more availability of cytokines, thus affecting the healing process [55-57].

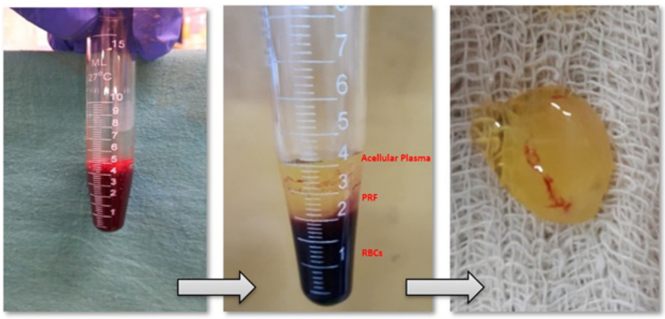

Figure 7: Sequential steps involved in the fabrication of platelet-rich fibrin (PRF)

Naturally-derived Polymeric Scaffolds [58,59]

The naturally-derived polymer scaffolds include alginate, hyaluronic acid and derivatives, chitin and chitosan derivatives, collagen and demineralized or native dentin matrix.

Alginate

Alginate is a natural polysaccharide, purified from the cell walls and intracellular spaces of brown seaweed and has been extensively used in biomaterial applications [60]. It offers rapid gelation feature as well as good mixing properties with other biopolymers however it has inadequate mechanical strength [59]. Also a study by Lambricht et al [61] determined that scaffolds containing only alginate may have limited potential in regenerative endodontic procedures with SCAP.

Hyaluronic acid and derivatives

Hyaluronic acid (HA) is one of the glycosaminoglycan in extracellular matrix and plays important roles by preserving the extracellular spaces [58]. HA is often available in the form of an injectable fluid and thus able to adapt to the morphology of the root canal and have a relatively fast setting time. It can interact with SCAP membrane receptors such as CD44, thus activating signaling pathways that tends to drive cellular migration which is consider to be critical to SCAP recruitment to the root canal space [61]. Also HA degradation products may include pro-angiogenic growth factors, which are instrumental in the revascularization of the regenerated dental tissues [62] however it degrades rapidly and lacks mechanical integrity in an aqueous environment. Also the SCAP viability with this scaffold is inconclusive [61,63].

Chitin and chitosan derivatives

Chitosan is produced commercially by deacetylation of chitin, which is the structural element in the exoskeleton of crustaceans and cell walls of fungi [64]. Chitosan has been prepared in the form of nanoparticles [65] which offers increased surface area for cell adhesion and biological activity. Advantages of chitosan include its broad-spectrum antibacterial properties [48,64,65]. Chitosan nanoparticles are mechanically strong, are resistant to degradation by bacterial enzymes, and have been shown to improve SCAP adhesion, viability, and differentiation, even in environments that have been exposed the powerful root canal antimicrobial agent, NaOCl [64]. Disadvantages include its complex gelation and degradation scheme limiting its use in its naturally-occurring form [48].

Collagen

Collagen is one of the most naturally occurring biomolecule. Type I collagen is the most abundantly used, and best promotes DPSC proliferation and mineralization capacity compared to other collagen types [48,66]. Collagen may also be processed into a variety of formats, including porous sponges, gels, and sheets, However, the difficulties encountered with use of collagen scaffold studies are its low mechanical strength, irregular biodegradation and the generation of tissues that resemble connective tissue [67].

Demineralized or native dentin matrix

A variety of collagenous and non-collagenous proteins are present in the organic matrix of dentin [68]. Demineralized dentin matrix releases bioactive molecules that signal associated dentinogenic events [69]. It shows direct induction of differentiating odontoblast like cells and indirect matrix synthesis [70]. It has osteoinductive and osteoconductive properties [71]. However the demineralization process is time consuming (2–6 days). Drawback of demineralization is that prolonged acid exposure may negatively

Table 2: Various growth factors and their effects on the target cells

Platelet-Derived Growth Factor (PDGF)	Released by platelets. Has potency in promoting angiogenesis and cell proliferation [92]. Enhances the proliferation of fibroblasts in the human dental pulp [93]. PDGF has 4 isoform homodimers: AA, BB, CC, and DD, in addition to a heterodimer, PDGF-AB [94]. Diverging effects of PDGFs on odontoblastic differentiation depend on the dimeric form [95]. PDGFs stimulate cell proliferation and dentin matrix protein synthesis but appear to inhibit alkaline phosphatase (ALP) activity in dental pulp cells in culture. DSP (Dentin Sialo Protein) expression {a unique marker of dentinogenesis} is inhibited by PDGF-AA but enhanced by PDGF-AB and PDGF-BB, although the mineralized tissue formation is inhibited [95,96]. PDGF-BB may increase VEGF expression in osteoblasts and promote angiogenesis at the site of dental pulp injury [97].
Transforming Growth Factor-β (TGF-β)	Released mainly from platelets, macrophages, and bone [98]. The three isoforms, TGFβ1, TGFβ2, and TGFβ3, are detected in human dentin [99]. Increase cell proliferation and production of the extracellular matrix in dental pulp tissue culture [100]. Important cellular signaling molecules for odontoblast differentiation and stimulation of dentin matrix secretion Secreted by odontoblasts and deposited within the dentin matrix [101]. Effect of TGFβ1 can be synergistically upregulated by Fibroblast Growth Factor-2 (FGF2), as evidenced by the increased ALP activity, the formation of mineralized nodules, and the expression of DSP (Dentin SialoProtein) and dentin matrix protein-1 [24]. Chemotactic on dental pulp cells in vitro. Plays an important role in the immune response during dental pulp injury [102,103].
Bone Morphogenetic Protein (BMP)	It comprises a subgroup of the TGFβ superfamily. BMP2, BMP4, BMP7, and BMP11 are clinically significant due to their role in inducing mineralization [104]. Recombinant human BMP2 stimulates the differentiation of adult pulp stem cells into an odontoblastoid morphology in culture [105]. Similar effects of TGF B1-3 and BMP7 have been demonstrated in cultured tooth slices [106,107].
Vascular Endothelial Growth Factor (VEGF)	has a specific affinity to endothelial cells and plays a key role in angiogenesis [108]. VEGF family includes VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placenta growth factor. Among these isoforms, VEGF-A is the most versatile in function [109]. VEGF appears to induce the differentiation of human dental pulp cells into endothelial cells [110]. The functions of VEGF involve the proliferation of endothelial cells and enhancing their survival, stimulating neovascularization in the area of injury [111]. Interestingly, VEGF increases the proliferation and osteogenic differentiation of dental pulp cells under osteogenic conditions, suggesting a possible stimulatory role of VEGF in osteogenesis [112].
Fibroblast Growth Factor (FGF)	Currently, 22 members have been identified in humans, of which FGF2 appears significant in regenerating the pulp-dentin complex [113]. It is a potent angiogenic factor stimulating new blood vessel formation. PDGF in the dental pulp, along with VEGF [114] and FGF2, induces the migration of dental pulp cells [115]. It stimulates the proliferation of dental pulp cells without differentiation, whereas FGF2 combined with TGFß1 induces differentiation of dental pulp cells into odontoblast-like cells and synergistically upregulates the effect of TGFß1 on odontoblast differentiation [24].
Insulin-like Growth Factor (IGF)	IGFs comprising IGF-1 and IGF-2 [116]. Of the two isoforms, IGF-1, also known as somatomedin C, has potency in the growth and differentiation of dental pulp cells [117]. IGF-1 with PDGF-BB has a synergistic effect on the proliferation of dental pulp cells in vitro [118].
Nerve Growth Factor (NGF)	Also known as neurotrophins. Promote the survival and maintenance of sympathetic and sensory neurons. Play a role in regulating tooth morphogenesis and innervation in rat tooth development [119]. Induce the differentiation of immortalized dental papilla cells into odontoblasts in vitro, suggesting that it acts as a stimulant for mineralization [120].

affect noncollagenous proteins involved in new bone formation [72,73].

Naturally-derived scaffolds have signaling molecules that aid in cell recognition and adhesion [48]. However, their use is limited due to the risk of pathogen transmission, foreign body response, poor mechanical properties, and product variability [46,48].

Synthetic Scaffolds [58,59,74]

Various synthetic scaffolds used in the REPs are polymers of Lactic Acid, Polysaccharide hydrogel (VitroGel 3D), Self-assembling peptide hydrogel and Calcium Polyphosphate/Calcium Phosphate Cement.

Polymers of lactic acid

This include Poly-L-Lactic acid (PLLA) and Poly-L-lactic-coglycolic acid (PLGA). They have slow degradation rate and mechanical properties can easily be modulated due to the its structure. PLLA scaffolds enhances the attachment, proliferation and differentiation of DPSCs cells [75] and bilayered PLGA scaffolds induce dentinogenic differentiation of DPSCs [76]. However the disadvantage being release of acidic residues due to degradation of these scaffolds which may affect local cell viability [59].

Polysaccharide hydrogel

VitroGel 3D, was evaluated as a potential injectable SCAP scaffold by Xiao et al. [77] in vitro and in vivo. The morphology and behavior of SCAP growth in the 3D hydrogel system is very similar to the natural growth state [78]. The speed of the hydrogel formation and its final strength could be modified by altering the mixing ratios of diluted hydrogel solution [77]. VitroGel 3D imposes practical issues such as the necessity to bank SCAP and associated cost [59].

Self-assembling peptide hydrogel

Puramatrix^{TM} is a synthetic, self-assembling peptide hydrogel. The hydrogel exists as an aqueous solution, however, polymerizes to solid gel instantly when exposed to physiologic salt conditions [79]. It enables DPSC viability and proliferation in vitro [80].

Calcium polyphosphate (CPP)/calcium phosphate cement (CPC)

CPP has similarity to naturally-occurring bone [81]. CPP scaffold has no cytotoxic effect on the DPSC, improved cell adhesion and migration [82]. On degradation, released calcium and phosphorous contributes to the formation of calcified tissues, such as dentin [83]. CPC has also been investigated as a scaffold for human DPSC [84]. Although it has relatively weak strength properties, the incorporation of chitosan has been shown to increase flexural strength of CPC based scaffolds [85]. CPC scaffold supports DPSC viability and proliferation[84].

Synthetic scaffolds avoid the risk of transmitting pathogens and can have consistent production processes that ensure properties such as mechanical strength, porosity, and rate of biodegradation are uniform [48,63]. However, these materials lack the intrinsic signaling abilities of naturally-derived scaffolds and have high costs resulting from their complex production [48].

Growth Factors

(GFs) act as signaling molecules. They are polypeptides or proteins and bind to specific receptors on the surface of target cells [86]. The GFs tend to act locally on the target cells. Growth factors mediate various cellular functions, including migration, proliferation, differentiation of stem cells into odontoblasts, interstitial fibroblasts, vascular endothelial cells, and sprouting nerve fibers during dentin-pulp regeneration. Cell homing instead of cell transplantation for dentin pulp tissue regeneration relies mainly on delivering growth factors into canal space.

The REP relies mainly on two sources of growth factors. One source is an external scaffold, for example, blood clots or platelet concentrates. The main source of these growth factors are platelets, including PDGF and VEGF. These have mostly angiogenic and proliferative effects [87] and thus attract stem cells to the site and increase their proliferation. However, these growth factors do not significantly affect stem cells' differentiation into odontoblast-like

Table 3: Most commonly used intra-canal irrigants and their properties

Sodium hypochlorite (NaOCl)	Choice of irrigants for disinfection not only in RCT but also in REP.
	Proven excellent bactericidal efficacy [123] and tissue dissolution capacity [124].
	Various concentrations have been used in several REP studies
	Decreases both SCAP survival and differentiation in a concentration-dependent manner.
	Concentration of 1.5% of NaOCl has minimal effects on the survival and differentiation of stem cells.
	Maximum clinically used concentration results in greatly diminished stem cell survival and loss of odontoblast-like cell differentiation [125].
	Also, in high concentrations, denature dentin-derived growth factors [126].
	Has both direct and indirect effects on stem cell toxicity [127].
Ethylene diamine tetra acetic acid (EDTA)	It is a chelating agent used to remove the smear layer in conventional root canal therapy [128].
	It withdraws calcium ions from the hydroxyapatite crystal lattice, leading to superficial demineralization and releasing growth factors.
	Dentin conditioning with 17% EDTA promote greater survival of SCAP, expression of Dentin Sialo Phospho Protein (DSPP), and formation of pulp-like tissue with blood vessels and polarized cell [90].
	However, the presence or absence of the smear layer had little influence on DPSC activity [129]. However, the use of EDTA after 6% NaOCl attenuates its undesirable effects [130].

cells [88]. Growth factors such as TGF beta1, BMP-2, or FGF-2 are required for this differentiation. These growth factors are found entombed in the extracellular matrix of dentin [89] and can be utilized by conditioning the dentin surface [90]. These growth factors can be sufficient for odontoblastic differentiation of mesenchymal stem cells of dental origin, especially BMP2, which is crucial in regulating the odontogenic differentiation of stem cells and stimulation of mineralization of newly formed matrix [91] (Table 2).

Nerve growth factor (NGF)

Also known as neurotrophins. Promote the survival and maintenance of sympathetic and sensory neurons. Play a role in regulating tooth morphogenesis and innervation in rat tooth development [119]. Induce the differentiation of immortalized dental papilla cells into odontoblasts in vitro, suggesting that it acts as a stimulant for mineralization [120].

Disinfection

The main objective of endodontic treatment is to cure or prevent periradicular periodontitis [121]. The most crucial step to achieve this goal is the disinfection of the root canal system. The same principle applies in REP; studies have shown intraarticular infection should be controlled for possible pulp tissue regeneration to occur in REP [122]. In immature teeth, mechanical cleaning procedures are avoided to prevent further thinning of dentinal walls. Thus, disinfection relies solely on chemical means, such as irrigants and intra-canal medicaments. Moreover, the choice of disinfectant in REP is determined by its bactericidal or bacteriostatic properties and its effect on stem cells.

Irrigants used in endodontics serve two purposes: disinfection of the root canal system. Another important advantage is the release of entrapped growth factors from dentin by demineralization caused by the use of an irrigant. The most commonly used irrigants in REP are Sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA) (Table 3).

To avoid the extrusion of irrigants into the periapical space and to minimize toxicity to stem cells in apical tissues, a closed-ended or side-vented irrigating needle should be used by positioning it about 1mm from the root end. Recent studies have recommended a final rinse with normal saline for improving cell viability on the dentin surface [131,132].

Intra canal medicament

Most commonly used intra-canal medicament in regenerative endodontic procedures is summarized in table 4.

Whether to use TAP or Calcium Hydroxide has been a topic of debate. TAP exhibits a concentration-dependent toxic effect on the survival of SCAPs in vitro. At concentrations of pastel-like consistency, there was similar toxicity for all other combinations of antibiotics, such as double antibiotic paste, amoxicillin with clavulanic acid, and modified TAP. However, toxicity is greatly diminished at 0.1 mg/mL or 0.01 mg/mL concentrations. In contrast, a commercial preparation of calcium hydroxide had no detrimental effect on SCAP survival [145]. One study reported that despite using different techniques with copious irrigation, <20% of TAP could be removed, with the residual 80% remaining tightly bound to dentin to a depth >350 mm. Conversely, >85% of $Ca(OH)_2$ could be removed with irrigation alone, and the residual labeled $Ca(OH)_2$ was found in dentinal depths < 350mm [146]. Moreover, residual antibiotics remaining on dentin walls can directly interact with stem cells brought into the root canal system [135]. Studies have shown that no viable SCAPs were found when cultured in contact with dentin conditioned with the highest concentration of TAP tested of 1000 mg/mL (equivalent to a pastelike consistency). However, SCAPs cultured on dentin conditioned with 1 mg/mL TAP had survival equivalent to SCAPs cultured on dentin

Table 4: Various intra canal medicaments used in REPs with their description

Triple Antibiotic Paste (TAP)	An antibiotic mixture composed of ciprofloxacin, metronidazole and minocycline. Hoshino et al. first used this combination dissolved in a macrogol/propylene glycol vehicle to disinfect infected deciduous teeth by placing it at the orifice followed by diffusion into the canal space and associated tissues, a procedure they called "Lesion Sterilization and Tissue Repair Therapy" [133,134]. TAP was used in a Regenerative Endodontic Procedure for the first time in 2004 [8]. Safe therapeutic circulating levels of these antibiotics in humans are at concentrations of about 0.001-0.01mg/mL, whereas "pastelike" formulations (previously used for placing into root canal) are at levels of about 1000 mg/mL, which is much higher as compared to desired concentration [135]. Another major concern with the use of TAP was discoloration caused by minocycline. Different combinations of drugs were tried to overcome this limitation like double antibiotic paste (ciprofloxacin, metronidazole) [136], a modified triple antibiotic paste (ciprofloxacin, metronidazole, cefaclor) [137], and Augmentin (Amoxicillin and Clavulonic acid). The European Society of Endodontics (ESE) position statement suggests that in the absence of strong evidence to support the use of antibiotics in Regenerative Endodontic Procedures, their use should be avoided (ESE 2018) [138]. American Association of Endodontists (AAE) recommends a concentration of 1-5 mg/ml in REP (AAE 2021) to avoid damage to stem cells from the apical papilla [139].
Calcium Hydroxide [$Ca(OH)_2$]	It is recommended as an intracanal medication in REP because of its good antimicrobial properties and high pH of 12.5-12.8 [140]. Some literature studies reported that prolonged treatment with $Ca(OH)_2$ formulations, with high pH levels (~12), alters dentinal composition and results in increased susceptibility to fractures [141,142]. However, a recent study reported that root fracture after $Ca(OH)_2$ dressing might be more related to the stage of root development than to the long-term use of $Ca(OH)_2$ [143]. Removal of Calcium hydroxide can be done using 17% EDTA [144].

exposed to saline (control). Interestingly, SCAPs had the highest survival when cultured on dentin previously exposed to $Ca(OH)_2$ [147].

Thus, calcium hydroxide can be considered the first choice for intracanal medication in REPs. Alternatively, antibiotic pastes such as TAP can be used after calcium hydroxide if the treatment dictates more aggressive disinfection. Still, the concentration of the drug should remain within the therapeutic range (1-5 mg/mL) [133, 145]. A recent study confirmed the effect of irrigants and medicament on growth factor release [147]. Nanobubble-enhanced antimicrobial agents are being researched to improve the efficiency of disinfecting agents [148]. However, additional mechanical cleaning procedures should be used in mature teeth to achieve better disinfection of the root canal system [149]. Previous Studies on mature permanent teeth with necrotic pulps and apical periodontitis reported mechanical preparation to small sizes like #30 K-file [9] or to #35 K-file [10]; however, some authors reported larger preparation till size #60 K-file [11].

It has been proven that teeth with apical diameters <1.0 mm achieved clinical success after regenerative endodontic treatment [150]. In one of the animal studies, even an apical foramen of 0.32 mm did not prevent the ingrowth of new tissue in two-thirds of the pulp canal 90 days after transplantation [151]. The average size of cells in the human body ranges from 10-100 microns, much smaller than the average size of the apical foramen of human teeth (0.2–0.3 mm) [42]. Thus, mechanical cleaning in mature teeth should be done judiciously to achieve sufficient disinfection, avoiding over-preparation to gain larger apical foramen size.

Coronal Seal

After disinfecting the canal and providing essential elements for tissue engineering, another important challenge is maintaining that microenvironment by providing a tight coronal seal. This demands biocompatible sealing material, providing a tight bacterial seal and enhancing cell proliferation and differentiation [152]. The Mineral trioxide aggregate (MTA) has been used as a capping material with a minimum 3 mm thickness over the scaffold in REP [153]. However, discoloration was seen with the use of grey MTA. To overcome this, white MTA or other tricalcium silicate material was suggested. Biodentine has similar physical properties to MTA but lacks heavy metals; thus, color stability is maintained with time [154,155]. Moreover, it has a setting time of 12 min, much less than MTA [156]. The Biodentine® (Biodentine, Septodont, Saint-Maur-des-Fossés, France) has been shown to induce the osteogenic differentiation and mineralization of dental pulp and mesenchymal stem cells [157]. Moreover, coronal plug thickness does not influence the fracture strength of immature teeth [153]. Limiting the extent of capping material use of collaplug/collatape should be considered, especially in blood clot cases or injectable scaffolds. This collagen plug will resorb over time. Capping material is then covered by a layer of Glass ionomer, followed by composite restoration.

Conclusion

Regenerative Endodontic Procedures based on tissue engineering principles allow growth factor-mediated cell homing, proliferation, and differentiation, leading to organized tissue in disinfected pulp spaces. The successful implementation of regenerative procedures requires clinicians to understand the biological basis and how these procedural steps of the therapeutic intervention influence cell survival, migration, angiogenesis, proliferation, and differentiation. Choices of irrigants and medicaments must be made based on their antimicrobial efficacy and their effect on stem cells and growth factors in the microenvironment. Future research aiming at a better understanding of the interplay of different factors in the regeneration process will lead to the outcome of a regenerated pulp-dentin complex.

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