Updates in regenerative endodontics for young general practitioners: a literature review


ABSTRACT

Improvements in science and technology have contributed immensely to every aspect of dental care. Regenerative endodontics (RE) aims to restore the vitality of the non-vital pulp; that is why regenerative therapy is considered as the future of dentistry. RE is anticipated to restore harmed or missing structures, including root, dentin, pulp-dentin complex, and living tissues identical to the exact origin that replaces the normal physiological functions of the pulp-dentin complex. The current review aimed to educate and update young general practitioners about RE by an overarching review of the available literature and give them a clear picture of the state of knowledge on the subject. An electronic search on PubMed and Google Scholar was undertaken to review articles on regenerative procedures and revascularization using specific keywords, such as “regenerative endodontics,” “revascularization,” “immature permanent teeth,” “stem cells,” “case report,” “growth factors,” and “scaffold,” to collect enough information and recommendations regarding the indications, medications, and methods of treatment currently practiced. This review article screened about 100 papers published from 1971 to 2020 in appropriate peer-reviewed journals. These articles were initially filtered based on the title and abstract. All the pieces were inspected thoroughly and then chosen as per the inclusion criteria. For additional information, relevant literature for RE in textbooks was also reviewed. Regenerative dental techniques are emerging as a worthy, advanced dentistry field, creating a paradigm shift in many dental specialties, including endodontics. Conventional endodontic therapy aims to preserve or restore the health of periarticular tissues by preventing or healing apical periodontitis. However, the goals of regenerative procedure extend beyond the purposes of conventional endodontic therapy and include continued root development and reestablishment of pulpal vitality.

Keywords: Regenerative, immature permanent teeth, apexification, case report.

Introduction

Statement of problem

Non-surgical conventional root canal treatments depend on techniques designed to accomplish the chemomechanical root canals preparation to eliminate infections [1]. However, because of the thin dentin walls and lack of apical constriction against which the obturation material can be placed, it is considered a significant challenge to handle or control permanent necrotic teeth with open apices [2]. Necrosis of an immature permanent tooth disrupts further development and leaves the tooth with narrow, fragile walls prone to fracture [3]. The science and technology improvements have contributed immensely to every aspect of dental care. Regenerative therapy is the future of dentistry as regenerative endodontics (RE) aims to restore the vitality of the non-vital pulp [4]. RE is anticipated to restore harmed or

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Received: 18 October 2021 | Accepted: 11 December 2021
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Aims and objective of regenerative treatment

The purpose of regenerative endodontic procedures (REPs) is to maintain the tooth structure to preserve optimal function and conserve the vitality of teeth damaged by trauma or dental caries [1]. The main advantage of REPs is strengthening the root by reinforcing the dentinal walls and further root development. A long-term follow-up is essential, and the intra-canal medicament, usually the calcium hydroxide, should be replaced every 3 months. The long-term usage of the calcium hydroxide raises the probability of developing deformities in the root walls and root fracture due to its porous characteristics. In contrast, absolute root development can result from a RE technique after short-term treatment. As a result, RE treatment increases the root length and thickness [1]. RE protocols have recently been considered a biologically based alternative approach, introducing the stem cells in the canal by tearing the periapical tissue [7]. The primary goal of REPs is to make up new tissue with a similar role and structure as dentin and pulp. Even though animal and human case reports have shown the development of additional tissues, they are frequently different from dentin and pulp parenchymal tissue.

Methodology of Literature Search

An electronic search on PubMed and Google Scholar was undertaken to review articles related to regenerative procedures and revascularization using specific keywords as “regenerative endodontics,” “revascularization,” “immature permanent teeth,” “stem cells,” “growth factors,” and “scaffold,” to collect enough information and recommendations regarding the indications, medications, and methods of treatment currently practiced. This review article screened about 100 papers published between 1971 and 2020 in appropriate peer-reviewed journals. These articles were initially filtered based on the title and abstract. All the pieces were inspected thoroughly and then chosen as per the inclusion criteria. Relevant literature for RE in textbooks was also reviewed for additional information.

Literature review

The continuing process of interaction between growth factors and stem cells in the attending of the bioactive scaffold. The clinical considerations for RE protocols include debridement of the root canals, infusion of the staging by laceration of the periapical tissue to induce blood clot and stimulate the stem cells in the root canal, and a sufficient coronal seal to prevent reinfection.

Regenerative procedures depend primarily on the chemical disinfection of the root canal system with the slightest to no instrumentation. The canal is further disinfected with an intracanal medicament succeeded by the induction of bleeding from the apical tissues into the channel [8].

Revascularization versus regeneration

Even though these techniques have traditionally been named revascularization or revitalization, we will refer to these as REPs. We assume that the latter term is more inclusive as this term encompasses all techniques that focus on achieving an organized repair of the dental pulp and does not exclude emerging new therapy patterns that may appear as the field of RE evolves [8]. Thus, there’s a clear distinction between revascularization and regeneration. Pulp revascularization means induction of angiogenesis in endodontically treated root canal. On the contrary, pulp regeneration means pulp revascularization and the restoration of functional odontoblasts and nerve fibers. An animal study revealed three types of tissues after treatment, including cementum-like tissue along the dentinal walls responsible for root wall thickening, bone-like tissue, and periodontal ligament-like tissue. Besides, partially lived pulp tissue and the existence of odontoblast cells lining the dentinal walls were noticeable in a case reported by. The study concluded that the tissue formed within the canal space does not function like pulp tissue, suggesting that revascularization is not a pulp regeneration but resembles a wound repair approach [6].

Principles of tissue engineering to the development of REPs

The research on the correct spatial assembly of definite stem cells, growth factors, and scaffolds to form a functional dentin-pulp complex.

Stem cells

Stem cells are undifferentiated embryonic or mature cells that persistently divide. They can create new stem cells and transform along a specified
molecular pathway. Totipotent embryonic stem cells can self-renew. On the contrary, stem cells can elect a differentiation program from only little prospective paths within an adult organ or tissue [9]. Mesenchymal stem cells exist in the apical papilla of immature teeth. These stem cells form the apical papilla (SCAP) can differentiate into odontoblast-like cells, making root dentin. An additional type of mesenchymal cells called dental pulp stem cells (DPSCs) was revealed and isolated previously. DPSCs can differentiate into odontoblast-like cells and form the dentin/pulp-like complex. SCAP and DPSCs show identical characteristics. It was observed that the bromodeoxyuridine uptake rate, in addition to the number of population doublings and tissue regeneration, is significantly higher in SCAP compared to DPSCs. These shreds of evidence indicate that SCAP obtained from a developing tissue may be an excellent cell source for tissue regeneration.

**Scaffolds**

Scaffolds are a three-dimensional extracellular matrix model that provides a biological and mechanical obligation to stem cells. An appropriate scaffold creates an environment that allows the cells to migrate, proliferate, and differentiate [10]. Scaffolds guide and support tissue regeneration and act as a transporter for particular cell types. There are two types of scaffolds: natural scaffolds, such as collagen, chitosan, silk, and fibrin, and synthetic scaffolds, such as polyglycolide and polyglycerol sebacate. Traditionally, the blood clot has been used as a scaffold in REPs. However, other 31 alternatives, including scaffolds with stem cells or growth factors, have lately gained attention as potential tools to improve regeneration treatment results [11]. The most frequently used venous blood derivatives are platelet-rich plasma (PRP). It is a mass of autologous plasma with a high platelet accumulation, which acts as a scaffold for its wealth of growth factors.

Platelet-rich fibrin (PRF) usage as a scaffold with growth factors has shown only minor advancements in periapical healing, dentinal wall thickening, root lengthening, and apical closure [11].

**Differences between PRP & PRF**

The classical PRP production protocol presupposes blood collection with two-step centrifugation: anticoagulant followed by artificial polymerization of the platelet concentrate using bovine thrombin and calcium chloride. PRF preparation excludes the redundant process of adding anticoagulants or bovine thrombin, similar to centrifuged natural blood. PRF promotes the proliferation of various cell types, encourages cellular differentiation, and supplements angiogenesis. The presence of cytokines, leucocytes, and few amounts of lymphocytes in PRF can play a significant role in the self-regulation of infectious and inflammatory phenomena [12].

**Growth factors**

Growth factors act as signals to enhance cellular proliferation or differentiation. Key growth factors involved in dental regenerative therapies include bone morphogenetic protein, transforming growth factor-beta, fibroblast growth factor, platelet-derived growth factor, insulin-like growth factor, and growth factors found in dentin. The major downside of growth factors is that various growth factors are required to encourage stem cells from multiple sources to obtain specific differentiation. Along with this, the safety, quantity, and delivery period of the growth factors pose a significant challenge. Another downside is that applying elevated loading levels of growth factors to reimburse for their physiologic solubility can result in undesirable side effects and confined spatial control.

**Indications of RE**

(AAE, 2018)

- Pulp necrosis with an immature apex.
- When post and core are not needed.
- Non-vital traumatized tooth.
- Compliant patients and parents.
- Patients who are not allergic to antibiotics and medicaments must complete the procedure.

**Irrigation solutions**

In order to get a successful regenerative procedure, irrigation solutions have to be chosen based on their capability to enhance the stem cells’ survival and proliferative capacity and their bacteriostatic and bactericidal characteristics [13]. The disinfection step depends mostly on the irrigant’s effectiveness. The most commonly used irrigant is sodium hypochlorite, which possesses antimicrobial characteristics against most endodontics pathogens. Sodium hypochlorite used in various concentration such as 5.25%, 2.5%, and 0.5%. Higher concentrated solutions are preferred to gain clinical success [11]. The second type of irrigant is chlorhexidine (CHX). Despite its favorable antimicrobial properties, CHX is not biocompatible, limiting pulpal stem cells’ survival and adherence to dentinal walls. Moreover, ethylenediaminetetraacetic acid (EDTA) is the most commonly used chelating agent and has been shown to enhance the release of growth factors that stick in dentin. Citric acid and mixture of a tetracycline isomer an acid and a detergent are other chelating agents used to remove the smear layer [14].

The use of EDTA enhances the survival and the intimate adhesion of stem cells. In addition, outcomes showed that EDTA induces osteoblastic differentiation and cell attachment, which was discovered solely in the group in which the differentiation of DPSCs was placed in direct contact with the EDTA-treated dentin surfaces. These findings demonstrate that EDTA helps obtain successful results in RE [15].

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Intracanal medicament

The most commonly intracanal medicament used in RE is the triple antibiotic paste, a mixture of metronidazole, ciprofloxacin, and minocycline [14]. This mix is very effective against bacteria commonly found in infected root canals in \textit{in vivo} and \textit{in vitro} studies. Even though the mixture of amoxicillin and clavulanic acid has not yet been used in a published case, it has been proven efficacious against 100\% of endodontic bacteria. Also, it may be a significant medicament substitutional for patients without a history of a penicillin-like drug allergy [16]. The concentration of antibiotic paste medicaments that leads to the death of 50\% of cells was in the range of 1 to 6 mg/ml. Furthermore, triple antibiotic paste (TAP), double antibiotic paste (DAP), modified triple antibiotic paste (mTAP), and Augmentin resulted in comparable survival rates when tested at the concentration nearest to the LC50 of TAP (1 mg/ml) [16]. Conversely, the usage of calcium hydroxide had no destructive effect on SCAP survival at any of the concentrations tested [16].

Cumulatively, the data showed that elevated concentrations of antibiotic mixtures hurt the survival of SCAP. In contrast, lower concentrations and Ca(OH)2 at all concentrations are favorable to SCAP survival and proliferation. These results highlighted the significant point that intracanal medicaments have to be used at bactericidal concentrations while having minimal effects on the viability of stem cells [16]. For the success of revascularization, the procedure requires a disinfected, bacteria-free root canal system [17]. The intra-canal dressing, calcium hydroxide, and triple antibiotic paste composed of 250 mg Ciprofloxacin, 400 mg Metronidazole, and 50 mg Minocycline, manipulated in propylene glycol vehicle, are the most widely used medicaments [17].

Under a published review, triple antibiotic pastes and calcium hydroxide were placed as intracanal medicaments in 86\% and 13.5\% of studies, respectively. Other authors have applied formocresol as an inter-appointment medicament. However, it showed that formocresol improves root thickness and length. Even though triple antibiotic paste has been demonstrated to be more efficacious in eliminating bacteria, it has the possibility for tooth discoloration due to the contact between minocycline and the canal walls through the procedure. Removing minocycline or replacing minocycline with amoxicillin, clindamycin, tetracycline, or doxycycline has been reported to solve tooth discoloration [11]. In conventional root canal treatment and apexification procedures, calcium hydroxide is usually used as an intracanal medication due to its antimicrobial characteristics [11].

Coronal seal

Mineral trioxide aggregate (MTA) is a biocompatible material that can resist bacterial infection due to its bioactive characteristics [7]. It is considered as the material of choice in regenerative procedures and vital pulp therapy because it enhances new complex tissue formation and survivability of undifferentiated DPSC after their exposure to MTA. Biodentine is a calcium silicate material that does not wash out easily, does not cause crown discoloration, and is simple to handle [11].

Apical barrier

Traditionally, the technique of REPs was apexification, which included promoting the formation of a calcified apical barrier to close the open apex and preventing the obturation material from being extruded beyond the apex [1]. The intracanal medication usually used is the non-setting calcium hydroxide [Ca(OH)2]. However, Ca(OH)2 has some disadvantages, such as the dressing should be replaced every 3 months needing for multiple visits, does not provide an appropriate environment for host cell proliferation, reduce the root strength that increases the susceptibility of developing defects in the walls and cervical root fracture due to its porous characteristics [12].

Case Report

A 12-year-old male patient was referred to the endodontic department at Riyadh Elm University Dental Hospital in Riyadh from the pediatric department. He presented with pain related to both upper left and right first molars in the clinic. The patient’s family and medical histories were noncontributory and didn’t reveal allergy to antibiotics and medicaments necessary to complete the procedure. For dental history, he previously had simple dental procedures. Clinical examination showed mild pain upon percussion and no response to cold test for both teeth. Periodontal examination confirmed probing depth within normal limits (i.e., < 3 mm) and normal physiologic mobility. Radiographic examination showed an immature apex (Figures 1 and 2). Hence, the diagnosis was necrotic teeth with acute periodontitis. Consultation with the restorative department was taken to ensure that a post would not be needed to restore both teeth in the future. After taking the history and clinical examination, both teeth were good candidates for a revascularization procedure. After that, informed consent was obtained from the patient’s parents. They were informed about the treatment period, which would be extended for more than two visits, and the potential adverse effects of antimicrobials, such as staining of crown or root, absence of response to treatment, and pain or infection. They were also informed about the alternative treatment as MTA apexification, extraction, or no cure. At the first appointment, 2% lidocaine with 1:100,000 epinephrine was given, then single rubber dam isolation was applied. After that, the access cavity was prepared. Profuse and gentle irrigation with a low concentration (1.5\%) of 20 ml sodium hypochlorite using a needle with a closed-end and side-vents to minimize the risk of extrusion of irrigant into the periapical space, then the canals were irrigated with saline, and 17\% EDTA with irrigating needle
positioned about 1 mm from root apex, to minimize stem cells cytotoxicity in the apical tissues.

Canals were gently dried with paper points. After that, calcium hydroxide was delivered into the channels, and the tooth was temporized with a cavit (3-4 mm) for 4 weeks as seen in Figures 3 and 5. The initial treatment response was assessed at the second visit, the patient had no signs or symptoms, and the tooth was not sensitive to percussion or palpation. The tooth was anesthetized with 3% mepivacaine without vasoconstrictor and isolated by a rubber dam. Cavit and calcium hydroxide were removed, and gentle irrigation with 17% EDTA was done. Canals were gently dried with paper points. The bleeding was created into the canals by an over-instrumenting technique by rotating a pre-curved K-file at 2 mm preceding the apical foramen until the entire canal was filled with blood to the level of cementoenamel junction (CEJ) that allows for 3-4 mm of restorative material. After forming a blood clot in all canals, white MTA was applied as capping material. A 3-4 mm layer of glass ionomer was applied gently over the capping material and cured for 40 seconds. After 3, 6, 12, and 15 months of the procedure, the patient was recalled for evaluating the results. Recall examination visits showed that the tooth had no symptoms and was functional. There were no signs of inflammation or infection as sinus tract or swelling. The tooth was not sensitive to percussion and palpation, and the pulp vitality test response was also positive. The radiograph examination revealed the healing of apical radiolucency, an increase in the width of root walls, and an increase in the root length as seen in Figures 4 and 6.
Figure 6. Healing of the apical radiolucency, an increase in the width of the root walls, and an increase in the root length.

Discussion

Disinfection of the canal system

Disinfection of the necrotic immature teeth with a combination of minocycline, ciprofloxacin, and metronidazole antibiotics has been a part of the revascularization treatment in different reported cases. Keswani and Pandey [18], in their clinical study, described the use of the TAP to be efficacious for the disinfection of infected necrotic pulps. Additionally, Nosrat et al. [6] reported successful and favorable outcomes for revascularization procedures by using TAP. Another in vitro study by Hoshino et al. [14] revealed that most bacteria were sensitive to concentrated metronidazole and the bacterial recovery decreased significantly to zero when the antibiotics were combined. The main drawbacks mentioned in Keswani and Pandey [18] and Hoshino et al.’s [14] reports include bacterial resistance and the possibility of crown discoloration by triple antibiotics that contain minocycline. To overcome the problem of discoloration, Keswani and Pandey [18] pointed to keeping the antibiotic paste below the CEJ.

Intra-canal medicaments

De Jesus Soares et al. [19] described a new suggestion for revascularization with mechanical decontamination and intracanal medication composed of calcium hydroxide and 2% CHX gel. This new proposal can be used to treat an immature tooth with necrotic canals. A study by Ruparel et al. [16] presumed that intracanal medicaments at high concentrations, such as calcium hydroxide and TAP or DAP, are harmful to SCAP. This study showed that medicaments at concentrations currently used in RE procedures, except for calcium hydroxide, harm SCAP survival.

Root canal irrigation

Trevino et al. [13] described that various root canal irrigation protocols modify SCAP survival. Irrigation protocols can influence stem cell survival adjacent to the walls of the root canals, supposedly by direct and indirect mechanisms. This outcome did not assess all the likely combinations of irrigants and their effects on cells. Subsequent studies must focus on evaluating various concentrations and periods of use for each irrigant.

New tissue formation

Zhu et al. [20] investigated the new tissue formation into the pulp space. The histological analysis of the experimental root canals showed newly formed tissue made up of complex tissues and blood vessels in a matrix of fibrous connective tissue.

In addition to cementum-like tissue covering the internal root canal walls, bone-like tissue in the canal space was observed [20]. Nygaard-Ostby and Hjortdal [21] explained the reconstruction processes in the canals after total pulp removal. The fibrous connective tissue formation took place in most teeth with vital pulp and accompanied accumulation of cellular cementum on the canal wall in some teeth.

Blood clot

The considerable differences in the results could be assigned to multiple variables, such as the presence or absence of a blood clot in the root canal and the length of the observation period. The outcomes did not give a clear answer to whether tissue formation depends on a blood clot. Recently, there have been reports of effective RE without intracanal blood clot formation [21]. On the contrary, Thibodeau et al. reported that roots containing a blood clot after disinfection had better results in a dog model than those with a blood clot in the canal apical part [3].

Clinical outcome and success rate

Necrotic teeth with open apices have been traditionally treated with the apexification procedure. Based on most current evidence, the success rate for apexification and REPs is similarly high (i.e., above 90%). Nonetheless, more extensive randomized clinical trials with adequate follow-up periods are warranted to provide greater insight into the long-term outcome for both apexification and regenerative procedures [11].

The American Association of Endodontists defined the degree of success of clinical considerations for RE techniques by:

The essential goal is the elimination of clinical signs and symptoms and the evidence of bony healing.

A desirable goal focuses on increasing the wall thickness and root length.

Finally, the tertiary goal is the positive response to the vitality test.
In other words, the aim of resolving the signs and symptoms of infection and healing the bone is generally achievable [22].

**Radiographic outcomes**

In all reported cases, the radiographs exhibit continued root development and root apex closure. Apical closure was observed at 12- and 15-month follow-ups. Other cases with periapical lesions demonstrated that the rate of healing was 80%-100% [20]. Saoud et al. [23] reported that 90% of the revascularization cases had the complete healing of periapical lesions after 12-month of follow-up. Additionally, all cases were clinically successful, without any signs or symptoms. Five types of responses for teeth treated with RE procedures were observed in the case series [22]:

Type 1: showed increased thickening of the canal walls and continued root developments.

Type 2: not many continuations of root development with blunt and closed root apex.

Type 3: apical foramen remains open with continued root development.

Type 4: severe obliteration of the canal space.

Type 5: rigid tissue barrier formed within the canal between the coronal MTA plug and the root apex.

**Follow-up**

Regarding follow-ups, long-term follow-up is essential to assure the long-term outcome [24]. Several clinical case series concerning revascularization and revitalization procedures have been published, with follow-up times varying from 6 months to 3.5 years. They recorded high clinical success (78%-100%) and interpretation of radiographic periapical pathology (80%-100%) [23].

**Limitations**

A study mentioned the typical limitations of RE: the inability to evoke bleeding, improper barrier placement (e.g., MTA), and coronal discoloration. The dentist should be familiar with these challenges to overcome them adequately [8].

**Conclusion**

RE procedures are emerging as a significant, evolving field of dental care, creating a paradigm shift in many dental specialties, including endodontics. The goal of conventional endodontic therapy is to preserve or repair the health of periradicular tissues by preventing or healing apical periodontitis. However, the goals of regenerative procedure extend beyond the goals of conventional endodontic therapy and include continued root development and reestablishment of pulpal vitality.


