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Regenerative Endodontics- Clinical Guidelines and Protocol - Review

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Abstract

Tissue engineering has arisen as a paradigm for repair and regeneration of dentistry. With the principle of tissue engineering, including stem cells, scaffolds and signalling molecules, it is expected that actual pulp regeneration is an achievable aim Non-vital infected teeth with mature or immature root apex have long been treated with root canal therapy and apexification. The goal of regenerative endodontic procedures is to restore the pulp and dentin of the non vital immature teeth. Regeneration of the dental pulp can be performed by cell based and cell homing techniques based on observations of human and animal studies. The present article discusses the pulp revascularization technique and offers an overview of its methods with possible future prospects for pulp regeneration as a standard dental procedure.

Keywords: Regenerative endodontics, pulp revascularization, revitalization, pulp regeneration.

Introduction

Tissue engineering is an emerging concept in regenerative medicine. 'Tissue Engineering' was described by Langer and Vacanti(1993) as "an interdisciplinary field that applies the principles of engineering and life sciences towards the development of biological substitutes that restores, maintain or improve tissue functions."[1] Regenerative medicine has the potential to repair organs and tissues that are damaged by injury, accident, cancer, or congenital deformity. [2]

Regenerative Endodontics

'Regenerative Endodontics', a branch of regenerative medicine is defined as "biological procedures designed to replace weakened, diseased, or missing dental structures, including dentine and root as well as pulp-dentine

complex cells, with living, viable tissues, ideally of the same origin, that restore the normal physiological functions of the pulp dentin complex."[2]

Several strategies have emerged in recent years in endodontics including root canal revascularization, postnatal stem cell therapy, scaffold implantation, injectable scaffold delivery, pulp implantation, 3D- cell printing, and gene therapy.[2] Though these techniques are under research, the only clinically feasible procedure presently is pulp revascularization.[3] So far, five types of human dental stem cells have been isolated and characterized. This includes dental pulp stem cells (DPSCs), stem cells from exfoliated deciduous teeth (SHED), stem cells from apical papilla (SCAP), dental follicle progenitor stem cells (DFPCs), and periodontal ligament stem cells (PDLSCs).[4]

Cell-based approach

Tissue engineering techniques generally require the use of stem cells to facilitate sufficient cell differentiation and tissue formation by providing scaffolds and the supply of morphogenic signals.[2] Based on this triad, much of the research is designing cell-based therapies, i.e. the transplantation of autologous DPSCs, the use of injectable scaffolds, and the delivery of environmental signaling molecules either through dentine treatment or by integrating growth factors into scaffold delivery systems.[5] The first pulp tissue engineering research models were performed in animal models with the tooth slice/scaffold model. It has been observed that after stem cell transplantation in tooth slice, dentine cylinders, and whole tooth roots using a tissue-engineering concept, regeneration of pulp-like tissue as possible. Quite recently, in the clinical trial of teeth with irreversible pulpitis, a cell-based approach to pulp regeneration has been undertaken. Though the efficacy has not been demonstrated, it reflects the feasibility and safety of pulp regeneration with a stem cell-based approach.[5,6]

Nevertheless, many limitations and difficulties have to be overcome in clinical practice including availability, isolation, storage, expansion, and culture of autologous stem cells, handling and preventing contamination of stem cells, better facilities for manufacturing, regulatory policies by government, and skill of the clinician. Though the results are unpredictable and expensive with the cell-based approach, regenerative endodontics have the ability for root maturation.[5,6]

Cell homing approach

Stem cell homing is defined as "the recruitment of endogenous stem cells by mobilization factors to an injured site to induce repair or replace the damaged cells or tissues".[5] For the migration, proliferation, and differentiation of stem/progenitor cells, the cell homing strategy for pulp regeneration depends on signaling molecules.[6] Compared to the cell-based approach, this approach would be more clinically easily transferable since there is no requirement for cell isolation and expansion procedures and prior Food and Drug Administration (FDA) approval exist for the use of growth factors in the oral setting.[7]

In vivo studies, various mobilization factors including vascular endothelial growth factor and stromal-cell derived growth factor-1α (SDF-1α) have been shown to facilitate vascular formation (neovascularization) from DPSCs and PDLSCs for promoting angiogenesis in the root canal system, while SDF-1α controls the maintenance and survival of immature Bone marrow stromal stem cells(BMSSCs). SDF-1 also promotes SCAP cell migration, suggesting that SDF-1 α could be useful in a cell-homing-based regenerative therapy strategy for angiogenesis, fibroblast, and odontoblast development from SCAPs.[5]

A combination of Granulocyte-Colony Stimulating Factor and Transforming Growth Factor-β1 can facilitate the processes of cell mineralization, including the formation of cement and bone, so that they can be used in immature adult teeth with open apex for root formation and elongation of apical regions. Irrigation with 17% ethylenediaminetetraacetic acid (EDTA) could liberate TGF-β from the dentine extracellular matrix. In the future, this measure can be embraced in cell-homing techniques in dental tissues to assist in dentine wall thickening. Improvement of the cell-homing approach will gradually replace existing techniques of revascularization of dentine-pulp, which cannot replicate the organization of natural pulp tissue.[5]

In a study, the root canals of extracted human single root teeth were filled with collagen scaffolds and combinations of growth factors such as basic fibroblast growth factors, platelet-derived growth factors, vascular endothelial growth factors, nerve growth factors, and bone morphogenetic proteins after pulp extirpation and sterilization. The teeth were inserted for 3 weeks into the subcutaneous tissue of rats. The histological analysis and enzyme-linked immunosorbent assay of the root canal space of the harvested teeth showed the development of vascularized and reinnervated tissues with dentine-like hard tissue.[8] In another animal study, related results were documented using an orthotopic infection model. Conversely, no odontoblast-like cells were observed along with the regenerated mineralized tissue in the histological examination.[9] Since there is no need for exogenous stem cells for conducting pulp regeneration clinically, cell-homing technique is theoretically better than a cell-based therapy (cell- transplantation). An example of cell-homing technique is pulp revascularization, but it is limited to the regeneration or repair of tissues in the pulp space and this cannot control the specific type of tissue regeneration in pulp space.[5,10]

The Current Dental Terminology of the 2011-2012 American Dental Association (ADA) accepted pulp regeneration as endodontic treatment and assigned it code (D3354).[11]

ADA codes for pulpal regeneration procedures [4]

1. "First Phase of Treatment (D3351): debridement and antibacterial medication
2. Interim Phase (D3352): interim medication replacement
3. Final Phase (D3354): Completion of regenerative treatment in an immature permanent tooth with a necrotic pulp. It does not include final restoration."

Background

Immature permanent teeth with necrotic pulp / apical periodontitis have historically been treated with apexification procedures before root canal filling using calcium hydroxide or apical MTA plugs.[11] There are some drawbacks to the calcium hydroxide used for creating an apical barrier in apexification, such as it needs several visits over an extended period, and repeated exposure to calcium hydroxide raises the possibility of root fracture. Treatment time can be reduced by apexification with MTA. Nevertheless, both calcium hydroxide and MTA cannot repair the weakened tissue, i.e., root canal wall thickening and apical closing with necrotic pulp / apical periodontitis in immature permanent teeth.[12] A novel concept of revascularization of immature nonvital, infected teeth has been developed in recent times.[2]

The regenerative endodontics procedure is not a new idea. It has a long history dating back to 1952 when a case study on the use of calcium hydroxide in a critical pulp amputation was published by Dr. BW Hermann.[13] In 1961, Nygaard-Ostby was the researcher who attempted to

explore the potential of tissue regeneration in endodontically treated teeth in which he partly filled the canal space by inducing periapical bleeding in dogs and humans. The histological analysis of the tissue that formed in the canal spaces was found to be not pulp-like tissue but fibrous connective tissue and cellular cement.[14]

Clinically, the first team to apply the principle of revascularization to the treatment of apical periodontitis and sinus tract in immature permanent teeth was Iwaya et al in 2001.[15] Their method was based on observations obtained with a mixture of antibiotics, ciprofloxacin, and metronidazole from the revascularization of prematurely reimplanted and auto transplanted dog teeth and root canal disinfection.[16,17] Their treatment resulted in the reduction of clinical symptoms/signs and apical periodontitis, as well as the development of canal wall thickening and the apical closure of the root of the immature permanent tooth.[15]

The revascularisation protocol was suggested by Banchs & Trope,[18] based on studies observed from

- Revascularization of reimplanted teeth,[19]
- Disinfection of root canal [16]
- Blood clot induction in the canal [20]
- Minocycline antibiotic was added to the composition used by Iwaya et al [15] (triple antibiotic paste).

The primary purpose of the regenerative endodontic procedure is the elimination of clinical symptoms/indications and the recovery of apical periodontitis, according to the main research paradigm for a regenerative technique of the American Association of Endodontists (AAE)]. The secondary goal is to thicken and/or initiate the root maturation of the canal walls.[21] Therefore, it can be argued that the primary goal of regenerative endodontic and traditional non-surgical root canal therapy is the same. The disparity between regenerative endodontic treatment and non-surgical root canal therapy is that the disinfected root canal region is filled with the natural tissue of the host in the preceding therapy and the channel area is filled with biocompatible foreign material in the latter.[22] This approach has been widely acknowledged in many subsequent literature analyses and the clinical considerations for Regenerative Therapy (AAE 2016).[23]

Revascularization / Revitalization/Regenerative Endodontics

The word 'revascularization' proposed by Iwaya et al [15] in 2001 refers to the reestablishment of pulp space vascularity following traumatic injuries that interrupt the blood supply of immature teeth to the pulp. The American Association of Endodontists adopted the term 'regenerative endodontics,' based on a tissue engineering concept, in 2007.[2] As the tissues regenerated in the canal space were not always blood vessels but both hard and soft tissues, revitalization instead of revascularization was eventually proposed as a more suitable term.[24] The European Society of Endodontology (ESE) (ESE 2016) used the word revitalization.' Revascularisation, revitalization, and regenerative endodontics are used in endodontic literature synonymously and interchangeably.[25]

Clinical Consideration For Regenerative Endodontic Procedure [4,23]

The clinical factors to be taken into consideration when conducting a regenerative technique are

- **A young patient with necrotic pulp and immature apex**

Pulp blood supply enables cellular and molecular elements of the innate and adaptive immune response system to be efficiently transported into the canal space of young immature permanent teeth with a wide-open apex. As a consequence, young immature permanent teeth should be more resistant to caries or traumatic injury than mature

permanent teeth. However, the presence of residual vital pulp in the canal space of immature permanent teeth with apical periodontitis can only be confirmed by histological analysis.[10]

Minimal or no instrumentation of the root canal system

The efficacy of the regenerative endodontic procedure (REP) disinfection has been doubted. Mechanical debridement of immature permanent teeth is not indicated for REP. In immature teeth, instrumentation can increase dentinal tubule fragility and damage the stem cells present in the apical region of the dentinal wall.[4] Dentin contains growth factors that have been trapped in dentinogenesis. Instrumentation can also remove the growth factor and other cells necessary for the process of regeneration.[4,5] According to the Cehreli et al. report, even though the number of cases is not adequate to be statistically important, it can be noted that after treatment, certain patients have recovered sensitivity to the tooth (vitality). This has been noted in cases where no instrumentation was carried out.[26]

Disinfection of the root canal system

Root canal system disinfection is vital to the effectiveness of REPs as inflammation hampers regeneration, repair, and activity of stem cells.[27] While infection/inflammation can result in homing of mesenchymal stem cells to the site of tissue injury by SDF-1 (stromal cell-derived factor) or other growth factors, and mesenchymal stem cells also have anti-inflammatory and immunoregulatory properties, pro-inflammatory cytokines, such as IL-1α, were shown to suppress stem cells to differentiate into tissue-committed somatic stem cells for regeneration or repair.[28] The chemical disinfection protocols of the root canal system rely not just on the bactericidal or bacteriostatic properties of the agents used, but rather on their effect on stem cell viability and proliferative action.[27]

A two-visit approach with the integrated use of irrigants and intracanal medicinal products is mentioned in the current clinical protocol since very few documented cases have been carried out in a single visit.[29,30] As suggested, the recommendations are:

- Copious irrigation using an irrigation system(EndoVAC, Closed-Ended Needles, or Side Venting Needles) that decreases the chance of extrusion of irrigants into the periapical area of about 20 ml of low concentration (1.5%) sodium hypochlorite(NaOCl) for 5 mins per canal.
- Then rinse using EDTA or saline (20 mL/canal, 5 min). To mitigate the extrusion of irrigants and damage to stem cells in the apical area, the irrigating needle should be located approximately 1 mm from the root end.[23]

Sodium hypochlorite

Even though higher concentrations are potentially toxic to periapical tissue, Trevino et al observed that the survival rate of human stem cells of the apical papilla (SCAP) when exposed to 6% NaOCl, followed by 17% EDTA and then 6% NaOCl again, was 74%. NaOCl concentrations of 1.5 percent have been shown to have mild cytotoxic effects on SCAP and are therefore recommended.[21]

EDTA (Ethylenediaminetetraacetic acid)

To improve SCAP longevity as well as partly reverse the adverse effects of NaOCl, the use of 17% EDTA was suggested. EDTA operates by demineralizing the dentine and exposing the dentine matrix, and this in turn allows growth factors to be released from the dentine matrix.[32] EDTA dentin conditioning has facilitated adhesion, migration, and differentiation of dental pulp stem cells. Therefore, a final EDTA rinse is recommended before a blood clot is formed.[33]

Placement of intracanal medicaments

Triple antibiotic paste: Hoshino et al and Sato et al used triple antibiotic paste to sterilize in vitro infected root canals.[16,17] The antimicrobial mixture is thought to avoid polymicrobial infection and to have a synergistic impact. A double antibiotic paste including metronidazole and ciprofloxacin or Triple Antibiotic Paste (TAP) with 1:1:1 ciprofloxacin, metronidazole, and minocycline have indeed been commonly used in REPs. They have been shown to effectively disinfect infected root canals with the diffusion of the drugs throughout the entire root dentine. A major drawback, however, is the discoloration observed in teeth after minocycline use.[10] Many other antibiotics, including amoxicillin, cefaclor, and rokitamycin, have been used together with ciprofloxacin and metronidazole. However, some have proposed that one antibiotic, Augmentin, could be as efficient as a triple antibiotic in REP.[34]

The AAE protocol advises TAPs at concentrations not exceeding 0.1 mg / mL. At this concentration, TAP contributes to the survival and proliferation of stem cells and is also successful in eliminating microorganisms within the root canal.[21]

Calcium hydroxide

AAE guidelines have suggested calcium hydroxide in REP due to its strong antimicrobial effects.[23] Calcium hydroxide is beneficial to SCAP survival and proliferation. Besides, the analysis found that SCAPs had the maximum survival when cultured on dentine exposed to calcium hydroxide compared to dentine exposed to 1 mg / mL or higher TAP concentrations. Clinically, the analysis of case studies using calcium hydroxide as an intracanal medicament revealed further root maturation.[35] It is recommended that if Ca(OH)2 paste is used, it should be applied in the coronal half of the root to enable the dentinal walls to thicken.[40] Latest experiments have shown that the binding of human apical cells to root dentine was stronger when treated with calcium hydroxide than with TAP in vitro.[35] It has been hypothesized that long-term treatment with calcium hydroxide as an intracanal dressing can increase the risk of root fracture. The authors suggested that the root fracture following the formation of calcium hydroxide could be more related to the root development stage than to the long-term application of calcium hydroxide.[10]

- **Provision of blood clot or scaffold in the canal**

The use of a blood clot to regenerate dental pulp tissues had first been practiced by Ostby and succeeded in the formation of granulation tissues, fibrous tissues, or cement-like tissues in the root canals.[14] In 1974, Myers and Fountain were able to produce 0.1-1.0 mm of soft connective tissue in the root canal using blood clots. The blood clot consists of a fibrin matrix that traps the cells required for tissue regeneration.[37] The abundant amount of growth factors helps the blood clot to play a crucial role in cell differentiation and thereby facilitate tissue regeneration. At the second appointment, after the canal disinfection is complete and the symptoms continue to resolve, the REPs progress to the stage of creating scaffold. It generally occurs 2 to 4 weeks after the start of treatment. Induction of intra-canal bleeding in REP is deliberately induced from the periapical tissue.[21]

The initiation of periapical bleeding into the canal space was not always possible. This may be attributed to the extreme destruction of the periapical tissues. If periapical bleeding cannot be stimulated during the treatment visit, the treatment can be delayed until the periapical tissues recover from the major injury.[38] This requires the insertion of a sterile # 20 pre-curved K file 2 mm beyond the apical foramen to initiate bleeding. This allows the entire root canal to be filled with blood to the CEJ level.[23] The procedure has its limits on the unpredictable nature of

the cell concentration and composition entrapped in the clot for restoring the function.[2]

Platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) are being used as a scaffold rather than a blood clot since PRP and PRF are rich in growth factors that could serve to enhance the regeneration of the pulp-dentine complex.[39,40]

Effective coronal seal

A coronal barrier is placed after the creation of a blood clot in the root canal. The reinfection and failure of the treatment are prevented by this cervical barrier which seals the canal and prevents microleakage.[36] MTA is the material of choice for coronal seal in regenerative procedures, being a bioceramic material, it sets in the presence of blood clot and is highly resistant to bacterial penetration. A 3mm layer of white MTA is placed over the blood clot, followed by 3-4 mm of GIC. Biodentine and calcium-enriched mixture can be used as an alternative to MTA. Composite restoration is placed over the GIC. [21,23]

Size of apical diameter

The apical diameter of 0.5–1.0 mm has been shown to have the best clinical success rate following regenerative endodontic procedure and was found to be ideal for patients aged 9–18 years. Typical sizes of human cells vary from 10 to 100 micrometers. As a consequence, osteoblasts, cementoblasts, periodontal ligament cells, and endothelial cells can quickly invade the canal space by apical foramen of less than 0.5 mm in diameter.[10]

Mechanism of Revascularization: [42]

1. A few pulp cells may remain vital at the apical end of the root canal and may proliferate and differentiate into odontoblasts in blood clot/matrix under the organizing influence of Hertwig's epithelial root sheath cells, which are very immune to inflammation and infection.
2. Dental pulp stem cells that are multipotent may migrate from the apical end and get implanted in the intact dentinal walls. They may differentiate into odontoblasts and further, deposit tertiary or atubular dentin.
3. Stem cells in the periodontal ligament- can proliferate and expand from the apical end to the root canal. The differentiated cells deposit hard tissue at the apical end as well as on the lateral wall.
4. Stem cells from the apical papilla (SCAP) or the bone marrow- bleeding induced by instrumentation beyond the apical foramen can transfer mesenchymal stem cells into the canal lumen from the bone, which has the extensive proliferative ability.
5. Blood clots being a rich source of growth factors could play an important role in regeneration. These include platelet-derived growth factor, vascular endothelial growth factor (VEGF), platelet-derived epithelial growth factor, and tissue growth factor and could stimulate differentiation, growth, and maturation of fibroblasts, odontoblasts, cementoblasts from the immature, undifferentiated mesenchymal cells in the newly formed tissue matrix.
6. As a rich source of growth factors, blood clots could play a major role in regeneration. These include a platelet-derived growth factor, a vascular endothelial growth factor (VEGF), a platelet-derived epithelial growth factor, and a tissue growth factor that may promote undifferentiated mesenchymal cells differentiation, proliferation, and maturation of fibroblasts, odontoblasts and cementoblasts in the newly developed tissue matrix.[42]

Limitations

The revascularized tooth is still susceptible to pulpal disease. Sometimes the entire canal may get calcified and compromise esthetics. All these can probably increase the

complexity of retreatment in the future. The concentration and composition of cells in the clot might make this revascularisation procedure unpredictable. Revascularisation might not be the choice of treatment if post and core are required in the final restoration.[42]

Guidelines for revascularization procedure: [21, 23, 42]

First visit

- Obtaining Informed consent
- Local anesthesia & Rubber dam isolation.
- Access opening
- Minimal or no instrumentation.
- Copious, gentle Irrigation using 20ml of 1.5% NaOCl and 17% EDTA solution using an irrigation system.
- Dry canals with paper points.
- Triple antibiotic paste/calcium hydroxide for 3-4 weeks.
- Seal with 3-4 mm of temporary material.

Second visit(1-4 weeks after 1st visit)

Assess response to initial treatment. If signs/symptoms of persistent infection are evident, consider additional treatment with antimicrobial, or alternative antimicrobial.

- Local anesthesia without vasoconstrictor & isolation with rubber dam.
- Copious, gentle irrigation using 20 ml of 17% EDTA.
- Dry with paper points.
- Scaffold creation with the initiation of bleeding by over instrumentation.
- Place a resorbable matrix such as CollaPlug, Collacote, CollaTape, or other material over the blood clot if necessary and white MTA for the cervical barrier.
- Double seal using 3-4 mm of Composite layer of glass ionomer
- Follow up done at 3, 6, 9, 12, 24, and 48 months.

The outcome of regenerative procedures

In its publication, the American Association of Endodontists describes the goal of REP by three measures: [23]

• Primary goal (essential): Elimination of symptoms and evidence of periapical healing.

• Secondary goal (desirable): The increased thickness of root wall and/or increased length of the root.

• Tertiary goal: A positive response to vitality testing.

The Outcomes/Results of Regenerative Endodontic procedures can be evaluated at four levels:

1) Clinical outcome

Clinically success of periapical healing is assessed from [15,36]

- Absence of sensitivity to percussion/ palpation.
- Absence of sinus tracts or swelling.

2) Radiographic outcome

Ideally, this entails complete osseous healing of the periapical lesion, an improvement in the thickness and length of the root, and radiographic apex formation. Chen., et al. have outlined 5 styles of potential teeth responses handled with REPs: [43]

Type 1 – with increased thickening of the canal walls and continued root maturation.

• Type 2- with no significant continuation of root development with the root apex remaining blunt and closed.

• Type 3- with continued root development with the apical foramen remaining open.

• Type 4 - with severe calcification (obliteration) of the canal space.

• Type 5- with hard tissue barrier formed between the coronal MTA plug and the root apex in the canal.

3) Pulp vitality testing

The positive response suggests a high degree of success, i.e. reinnervation of the root canal, independent of the type

of tissue developed inside the canal.[21] The existence of a dense layer of MTA (3-4 mm), as well as layers of GIC cement and restorative materials such as composite resin, applied over the plug, could contribute to unfavorable responses to vitality testing.[36]

4) Histological evidence of dentin–pulp regeneration

After REPs, histological analysis of the tissues developed within root canal spaces in human teeth and animals revealed bone regeneration and root growth with cementum deposition, but none of the experiments showed regeneration of dentin-pulp complex inside the root canal space.[44]

Unpredictable outcome

Recently, after a regenerative endodontic procedure conducted using platelet-rich plasma (PRP) as a scaffold, Torabinejad and Faras presented clinical, radiographic, and histologic results exhibiting "pulp-like vital connective tissue."[39]

In addition to this, literature also documents many cases in which pulp regeneration and root growth failed while adopting proper REP procedure. After treatment of an immature maxillary central incisor with necrotic pulp, Lenzi and Trope reported empty root canal space.[45] A case where root maturation took place in a maxillary central incisor was described by Nosrat et al., while a regenerative endodontic treatment resulted in an empty root canal space.[46] Even in immature teeth of animals and human with no history of pulpal and periapical history, revealed the only formation of bone and cementum like tissues in the disinfected pulp-dentin complex but no dentin-pulp complex.[44]

Cement-like hard tissue was accumulated on the root canal walls even after employing tissue engineering techniques, and bony islands were located in the root canals. Another unfavorable occurrence is the development of a hard-tissue barrier within the canal between the coronal MTA plug and the root apex.[43] Such results cannot be regarded as "clinical failures," but they indicate that the outcome of the new pulp regeneration procedure may be uncertain.

Future Perspective

Quite recently, to treat mature permanent teeth with necrotic pulps and apical periodontitis, regenerative endodontic treatment has been used. Full mechanical debridement is necessary to help prevent root canal inflammation and eradicate necrotic tissue, the main difference in regenerative endodontic treatments for mature teeth with contaminated, necrotic pulps.

Regenerative endodontic treatment of mature permanent teeth with apical periodontitis may result in the reduction of clinical symptoms and the resolution of apical periodontitis, equivalent to conventional non-surgical root canal therapy.[48] Consequently, regenerative endodontic therapy provides another management choice for necrotic pulps of mature permanent teeth. 3D Bioprinting, injectable scaffold, bioengineered tooth and gene therapy are promising future research domains in the field of regenerative endodontics.[2, 5]

Recent research indicates that dental pulp tissue engineering could be a feasible alternative to necrotic teeth management,[49] but there are issues to the ideal root canal preparation method, disinfection, ideal stem cell source, ideal stem cell transplant scaffolding, ex-vivo stem cell handling technique, and the production of healthy, effective and within reasonable costs.

Therefore, regeneration of the dental pulp can be accomplished by cell-mediated and cell homing methods based on observations of human and animal regenerative endodontics.[5]

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