Journal of Advances in Medicine and Medical Research

32(7): 83-98, 2020; Article no.JAMMR.57036
ISSN: 2456-8899
(Past name: British Journal of Medicine and Medical Research, Past ISSN: 2231-0614, NLM ID: 101570965)

Regenerative Endodontics – Looking Inward

Shivani Mallishery[1*] and Tanvi Shah[1]

[1]Nair Hospital Dental College, Mumbai, India.

Authors' contributions

This work was carried out in collaboration between both authors. Author SM designed the study and wrote the protocol. Author TS managed the literature searches. The first draft of the manuscript was written and revised by both the authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMMR/2020/v32i730454
Editor(s):
(1) Dr. Chan-Min Liu, Xuzhou Normal University, China.
Reviewers:
(1) Patrícia Diogo, University of Coimbra, Portugal.
(2) D. N. Nirupama, India.
(3) Jorge Paredes Vieyra, Universidad Autonoma de Baja California Tijuana BC, Mexico.
Complete Peer review History: http://www.sdiarticle4.com/review-history/57036

Review Article

Received 17 March 2020
Accepted 24 May 2020
Published 30 May 2020

ABSTRACT

Regenerative endodontics has become a revolutionizing tissue engineering concept in the treatment of immature permanent teeth for over two decades. It has been described as a 'paradigm shift' in the treatment of immature teeth, since it fosters continued root maturation. An immature necrotic permanent tooth is usually a result of trauma or infection due to which the tooth becomes non-vital before completing root development. In such cases, the root walls are left thin and weak with an open apex. Traditional apexification procedures may resolve pathology but have not been able to prove tooth survival due to absence of continued root development and risk of root fracture. A successful regenerative endodontic procedure (REP) results in resolution of signs and symptoms of pathology, radiographic signs of healing, proof of continued root development as well as presence of pulp vitality due to the regeneration of pulp tissue in the root canal. Various stem cells, growth factors, scaffolds and suitable environment form the tetrad of elements necessary to induce regeneration of dental pulp. While there has been some success in isolating dental pulp cells with *in-vitro* experiments, it has been proven to be rather difficult to implement the same in a practical perspective *ex vivo*. Although there has been clinical success related to REP, histologically they seem to undergo guided endodontic repair rather than true regeneration of physiologic pulp tissue. This review provides an overview of components of tissue engineering, clinical protocol and predictable outcomes for REP and recent advances in regenerative dentistry.

**Corresponding author: E-mail: sshivani96@gmail.com;*

Keywords: Regenerative endodontics; tissue engineering; stem cells; clinical outcomes; immature teeth; open apex.

ABBREVIATIONS

BMP : Bone Morphogenic Protein
BMSSC: Bone Marrow Stem Cells
DFPC : Dental Follicle Progenitor Cells
DPSC : Dental Pulp Stem Cells
PDLSC : Periodontal Ligament Stem Cells
PRP : Platelet Rich Plasma
PRF : Platelet Rich Fibrin
REP : Regenerative Endodontic Procedures
SCAP : Stem Cells from Apical Papilla
SHED : Stem Cells from Human Exfoliated Deciduous Teeth

1. INTRODUCTION

One of the greatest challenges in endodontics is the management of an immature permanent tooth with a non-vital pulp. Incorporation of traditional endodontic treatment like apexification in a non-vital immature permanent tooth results in cessation of root development which renders the formation of thin and weak roots that are highly prone to root fractures [1-2]. Hence, alternative approaches that allow quantitative and/or qualitative increase in length and thickness of the root should be pursued.

According to American Association of Endodontists Glossary of Endodontic Terms, regenerative endodontic procedures are biologically based procedures designed to physiologically replace damaged tooth structures, including dentin and root structures, as well as cells of the pulp-dentin complex [3-4]. The primary function of pulp is to produce and maintain the vitality of dentin. Therefore, regenerative pulp should be capable of forming dentin to replace and repair the lost tissues. The term regenerative endodontics procedures (REP) refers to all the events that aim to regenerate and repair the pulp-dentin complex.

The first evidence of regeneration of dental tissues was in 1932 by G. L. Feldman, who showed evidence of regeneration of dental pulp under certain optimal biological conditions [5]. In 1971, a pioneer study in regenerative endodontics conducted by Nygaard-Ostby concluded that bleeding induced within a vital or necrotic canal led to resolution of signs and symptoms of necrotic cases and in certain cases, apical closure [6]. In the year 2000, Gronthos et al. identified and isolated odontogenic progenitor cells in an adult dental pulp, which proved to be a breakthrough in the regeneration of dental tissues [7].

Tissue engineering is the field of functional restoration of tissue structure and physiology for impaired or damaged tissues because of cancer, diseases and trauma [8]. Regenerative endodontics, a type of tissue engineering, has two common terminologies associated with it- revascularization and revitalization. Revasculari-zation refers to the re-establishment of vascularity in the pulp space post-injury to the original vascularity of the pulp of a traumatized immature tooth. Revitalization, on the other hand, describes non-specific vital tissues rather than just blood vessels [9]. For immature teeth with non-vital pulp, such revascularization/ revitalization treatment induces physiological root formation (apexogenesis), which thus results in tissue regeneration. By restoring root development and reinforcing dentinal walls, the strength of the root, and hence, long term retention of the tooth increases. Such treatment modality can prove to be an efficient alternative to conventional apexification procedures [10].

The aim of the present review is to perform critical analysis and summarize the available evidence on components of tissue engineering, clinical protocol, predictable outcomes and limitations of REP. To perform this review, a web-based search on PubMed was done to find relevant literature on REP published in the last 10 years. Both abstracts and free full text articles of dental journals were reviewed. A combination of keywords were used as search terms. These include ‘regenerative endodontics’, ‘tissue engineering’, ‘outcomes’, ‘dental stem cells’, ‘challenges’, ‘dental scaffolds’, ‘growth factors’.

2. REGENERATIVE CAPACITY OF DENTAL TISSUES

The tooth is a complex organ that is formed by highly organized mineralized tissues encasing the dental pulp. Different mineralized tissues have different regenerative capabilities. Ameloblasts, derived from ectoderm, produce enamel after being stimulated by the odontoblasts. These cells have no regenerative capacity and undergo apoptosis after the formation of enamel matrix [11]. Odontoblasts and cementoblasts derived from ectomesen-

chyme lead to the formation of dentin and cementum respectively. These cells, unlike ameloblasts, have limited regenerative capacity. In response to stimuli causing mild injury at the pulp-dentin interface, progenitor cells are derived from pulp which produce tertiary dentin [12]. The dentin so produced helps in separating the damaged tooth structure from pulp, thus maintaining pulp vitality. Another example of regenerative capacity of dentin is dentin-bridge formation upon application of calcium hydroxide as a pulp capping agent [13]. Similar to dentin, cementum is also laid down throughout life in the form of cellular cementum at the root apex to compensate for passive eruption of the tooth [14]. Alveolar bone is derived from osteoblasts, which exhibit rapid turnover in response to mechanical stimulus [15]. Guided Tissue Regeneration (GTR) has been successfully incorporated into clinical practice to allow selective regeneration of functional periodontal ligament by using a barrier membrane [16].

3. ELEMENTS OF TISSUE ENGINEERING

The components of tissue engineering include stem cells, scaffolds and growth factors. The process involves incorporation of *ex vivo* expanded stem cells and growth factors incorporated within a 3-dimensional natural or synthetic polymer that provide an environment for cell proliferation and differentiation [17]. Although these three components are important, they cannot yield successful results without the fourth major component - a conducive environment. Tissue engineering approach involved in regenerative dentistry includes either *in vivo* implantation of an *in-vitro* cell culture with/without polymers or direct *in vivo* implantation of isolated cells and scaffolds [18].

3.1 Stem Cells

Stem cell biology is one of the fundamental components of regenerative medicine. The origin of any tissue can be traced back to its stem cells. Stem cells exhibit two properties- self renewal and plasticity. Self-renewal explains the property of these cells to divide thus producing more of themselves, while the potential of these cells to differentiate into different mature cell types is explained by the term plasticity [19].

These cells can be classified based on their origin as be embryonic stem cells (ES) or post-natal stem cells and based on their plasticity as pluripotent (capacity of maturing into cells belonging to any of the three germ layers) or multipotent (capacity to differentiate only into cells of the tissues from which they are derived) [20]. Although ES cells are more valuable in tissue engineering due to their pluripotency and greater plasticity, legal and ethical issues associated with their sourcing is a concern. Hence, multipotent post-natal stem cells are widely studied. Further, these cells could either be autogenous, allogeneic or xenogeneic.

A stem cell niche, identified in several connective tissues, is the microenvironment in which the stem cells reside and represents as little as 1% of total population. The identification of such niches is best performed after stimulation of injury to the tissues [20]. However, the source of these cells is still unclear. Most stem cells found in the orofacial region are mesenchymal stem cells. Stem cell population applied in REP include.

3.1.1 Dental pulp stem cells (DPSC)

These cells, isolated from human dental pulp, are capable of regenerating the odontoblasts with mineralized tubules and fibrous tissues with blood vessels; very similar to the pulp-dentin complex of a normal human tooth and have the unique ability to form mineralized tissues both *in-vitro* and *in-vivo* [7]. DPSCs share a similarity in gene expression with the precursors of osteoblasts, ie, Bone Marrow Stem Cells (BMSSCs): they exhibit the capacity of self-renewal following in-vivo transplantation and can develop into diverse phenotypes like adipocytes and neural precursors [21]. However, in contrast to BMSSCs, these cells have 30% higher proliferation rate and higher growth potential [22].

3.1.2 Stem cells from apical papilla (scap)

These unique post-natal stem cells are released in the root canal space from the apical papilla, when it is lacerated during the evoked-bleeding step of REP. They have greater capacity for dentin and tissue regeneration than DPSCs and high proliferative potential, reflected by higher telomerase activity [23]. These cells have high survival rate despite challenging conditions such as periapical infections, as they are equipped to receive nutrients and oxygen via diffusion from the apical surrounding tissues such as the vascularized granulomatous tissue present in

apical periodontitis and are highly significant in REP [20].

3.1.3 Periodontal ligament stem cells (pdlsc)

These are mesenchymal stem-cells isolated not just from human permanent teeth but also from deciduous and supernumerary teeth [24], which can differentiate into periodontal ligaments, cementum, alveolar bone, blood vessels and peripheral nerves. However, obtaining these cells could be difficult since they are collected from atraumatic extraction of healthy teeth, which is practiced only in the case of impacted teeth or for orthodontic purpose. It is now stated that PDLSC can also be isolated and expanded from inflamed PDL tissues, such as granulation tissue of periodontitis affected intra-bony pockets (i-PDLSC) [25]. However, i-PDLSCs have lesser osteogenic and cementum regeneration capacity compared to cells derived from healthy teeth [26]. Further, PDLSCs obtained from older adults have lesser regenerative capacity compared to younger donors [27].

3.1.4 Dental follicle progenitor cells (dfpcs)

DFPCs are isolated from the ectomesenchymal dental follicle or dental sac, which is responsible for the formation of periodontium [28-29]. Like other stem cells, they demonstrate adipogenic, neurogenic and osteogenic differentiation [30]. However, these cells are more proliferative than DPCS and SCAP [31] and display fibroblast-like morphology [28]. *In vitro*, they are capable of differentiating into PDL-like structures or calcified nodules with bone or cementum-like attributes. These calcified nodules resemble calcifications seen in calcifying epithelial odontogenic tumors or cemento-ossifying fibroma [32]. Further, after *in-vivo* implantation, STRO-1 positive dental follicle stem cells can form cementum and immortalized dental follicle stem cells are capable of forming new PDL [33].

3.1.5 Stem cells from human exfoliated deciduous teeth (shed)

These cells were isolated and expanded *ex vivo* by Miura et al. from the remnants of living, normal pulp tissue in exfoliated deciduous crown [34]. SHED develop at the 6th week of embryonic development and share common molecular characteristics with neural crest cells. Unlike other dental stem cells, SHED are easily accessible since they are sourced from naturally occurring tooth exfoliation phenomena. Compared to their adult counterparts (DPSCs), SHED have higher proliferative capacity and are less mature, hence exhibit greater potential of multi potential differentiation [35]. Due to their higher neurogenic potential, SHED have been extensively studied for the treatment of neural tissue injury or degenerative diseases, like Parkinson's Disease [36]. Researchers believe these cells can be stored (SHED banking) and can be used as a successful stem cell therapy for the treatment of various medical conditions [37].

3.2 Growth Factors/Morphogens

Growth factors are extracellularly secreted signals that bind to specific receptors on cells and play a major role in regulation of endogenous cells or stem cell recruitment, migration, proliferation and differentiation. The growth factors may be released by blood clot, Platelet Rich Plasma (PRP), Platelet Rich Fibrin (PRF) or from dentin matrix upon demineralization (caries, acid etching etc). Growth factors used in regenerative endodontics are platelet-derived growth factor (PDGF), transforming growth factor (TGF-β), Bone Morphogenic Protein (BMP), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), Colony stimulating factor (CSF), platelet derived growth factor (PDGF), epidermal growth factor (EFG) and insulin-like growth factor (IGF) [38]. These can be used for-

- Proliferation and differentiation of stem cells- PDGF, BMP, CSF, EGF, FGF, IGF
- Modulation of humoral and cellular immune response- Interleukin 1-13
- Angiogenesis- VEGF
- Wound healing and tissue regeneration- TGF alpha and beta.

Amongst these growth factors, BMP and TGF are the most crucial in regeneration of dental tissues in adults. They can either be used *in vivo*, where they are directly applied to exposed pulp or *ex vivo*, where they are first isolated with dental pulp stem cells which stimulate the formation of odontoblasts and finally transplanted to regenerate dentin [39].

3.3 Scaffold

Scaffolds are porous, degradable structures that can be implanted alone or in a combination with stem cells and growth factors to provide a 3-dimensional framework (sheets, gels or highly complex structures) that can support cell growth

and differentiation. Ideal properties of a scaffold include scaffold porosity to facilitate diffusion, biocompatibility and biodegradability, effective transportation of oxygen and nutrients, ability to support cell growth and differentiation, non-toxicity and adequate physical and mechanical strength [40]. Scaffolds can either be natural or synthetic.

3.3.1 Natural scaffolds

Natural scaffolds can be autologous like PRP, PRF and blood clot or derived from natural substances like collagen or glycosaminoglycans. PRP and PRF are first- and second-generation platelet concentrates respectively that stimulate the proliferation of stem cells. Although PRP has a rich source of growth factors, PRF has higher concentration of cytokines and stimulates faster healing. Blood clot has fewer cytokines and growth factors than PRP or PRF [40]. Intentional periapical filing to induce blood clot formation or venous blood drawn from patients can cause discomfort to the patient. Hence, collagen and glycosaminoglycan are used, which provide excellent tensile strength to the tissues, allow easy placement of stem cells and growth factors and control over resorption rate by altering its density. However, it is observed that collagen may adversely affect pulp tissue regeneration since pulp cells in collagen undergo marked contraction [41].

3.3.2 Synthetic scaffolds

Polymers such as polyglycolic acid (PGA), polylactic acid (PLA), polylactic co-glycolic acid (PLGA), polycaprolactone (PCL) are commercially available synthetic scaffolds. They allow precise control of physiochemical features like degradation rate, microstructure, strength, porosity and undergo degeneration by hydrolysis [42]. Scaffolds containing inorganic compounds like hydroxyapatite and tricalcium phosphate can be used to enhance osteoconductivity. The major disadvantage associated with synthetic scaffolds is inflammation at the site of implantation [42].

3.4 Environment

Tissue engineering, or regeneration of tissues requires a tetrad of elements. A conducive environment is crucial for any stem cell to proliferate or differentiate *in vivo* or *in vitro*. These include- A well-sealed restoration *in vivo* to prevent contamination of pulp [43], disinfection of the root canal system using intracanal irrigants like Sodium hypochlorite or chlorhexidine and antibiotic pastes [2] and large-scale *in vitro* regeneration of tissues requires bioreactors that mimic the internal environment of the body and provide appropriate physiological stress to enhance the mechanical properties of regenerated tissues [44].

4. PROCEDURE

REP is the mainly considered for a permanent necrotic tooth with an open apex in a compliant patient with no allergy to medications and antibiotics used in the procedure. Moreover, since the pulp canal space is involved in the procedure, it should be ensured that this canal space is not required for other restorative purpose [45].

Based on Cvek's classification of root development, immature necrotic permanent teeth suitable for REP include Stage 1 (less than one-half of root formation with open apex), stage 2 (one-half of root formation) and stage 3 (two-third of root development with open apex), due to short root, thin canals and wide-open apex. However, teeth at stage 4 of root development (nearly completed root with an open apex) can be treated by both REP and apexification, since the root has developed enough to withstand apexification [46].

Because of the encouraging results of regenerative procedures in young immature permanent teeth it has been tried for use in adult teeth with closed apices.

4.1 Traditional REP for Permanent Teeth with an Open Apex

Traditional REP is performed by inducing bleeding into the canal by over-instrumentation in immature permanent teeth.

4.2 REP Using Platelet Rich Plasma for Permanent Teeth with an Open Apex

Liu et al. showed that PRP stimulates cell proliferation and differentiation of the dentin-pulp complex, which suggested that PRP could be used as a scaffold for pulp capping [47].

4.3 Rep for Adult Mature Permanent Teeth

This procedure includes dental pulp and dentin regeneration in mature permanent teeth in adults. Broadly, there are 2 distinctive strategies for dental pulp and/or dentin regeneration in infected or traumatized mature permanent teeth

in adults. These include cell transplantation of *ex vivo* cultivated stem/progenitor cells and cell homing by molecules that recruit the patient's resident cells [49].

In a series of case reports, Paryani K et al. and Saoud et al. used modified regenerative endodontic procedures to enhance the probability of pulp revascularization in mature necrotic teeth. The resolution of apical radiolucency and regression of clinical signs and symptoms along with ingrowth of new vital tissue into the chemo-mechanically debrided canals were observed at recall appointments [50-51].

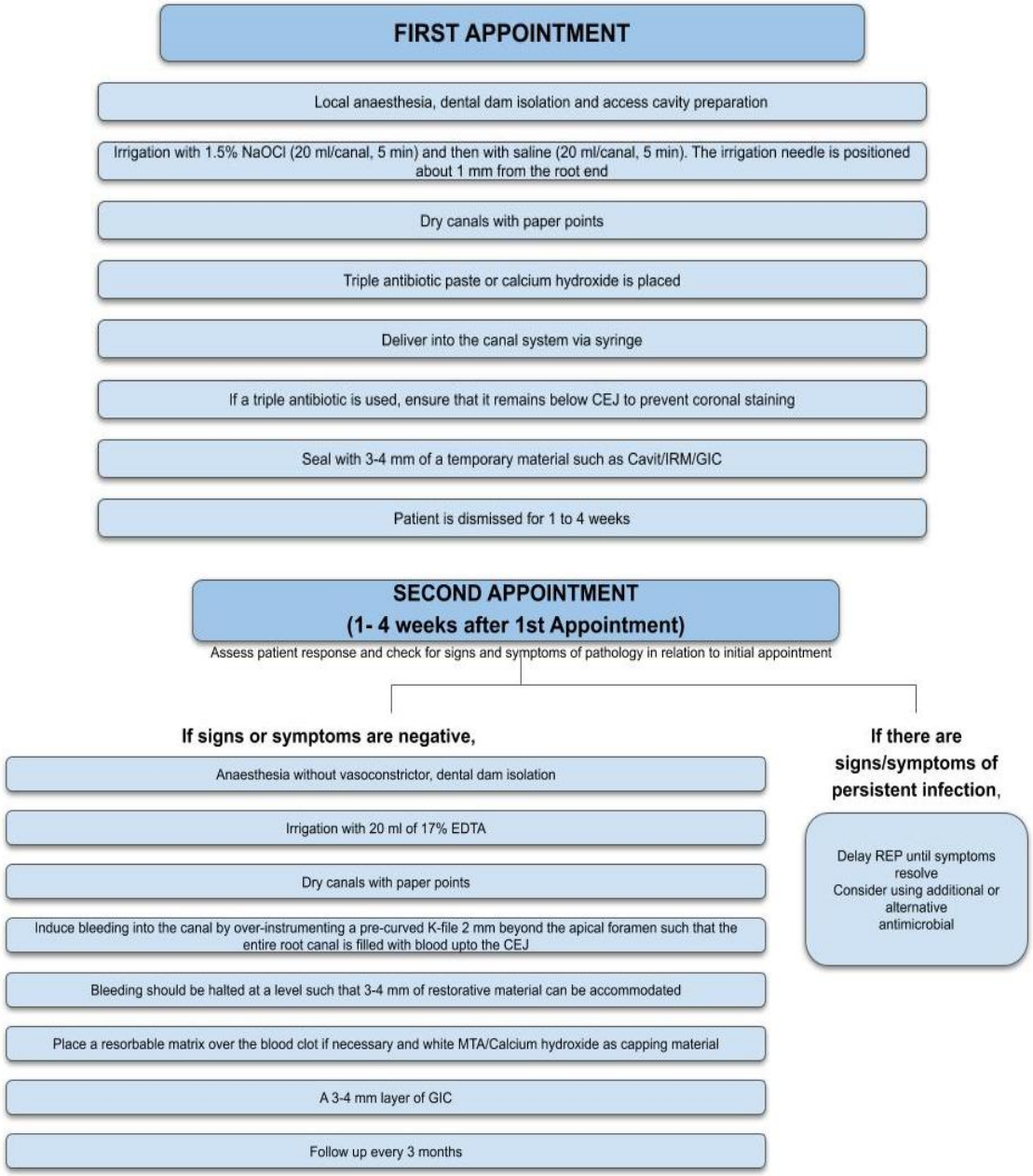

Fig. 1. Traditional regenerative endodontic procedure for a permanent tooth with an open apex as outlined by American Association of Endodontics (AAE) [45]

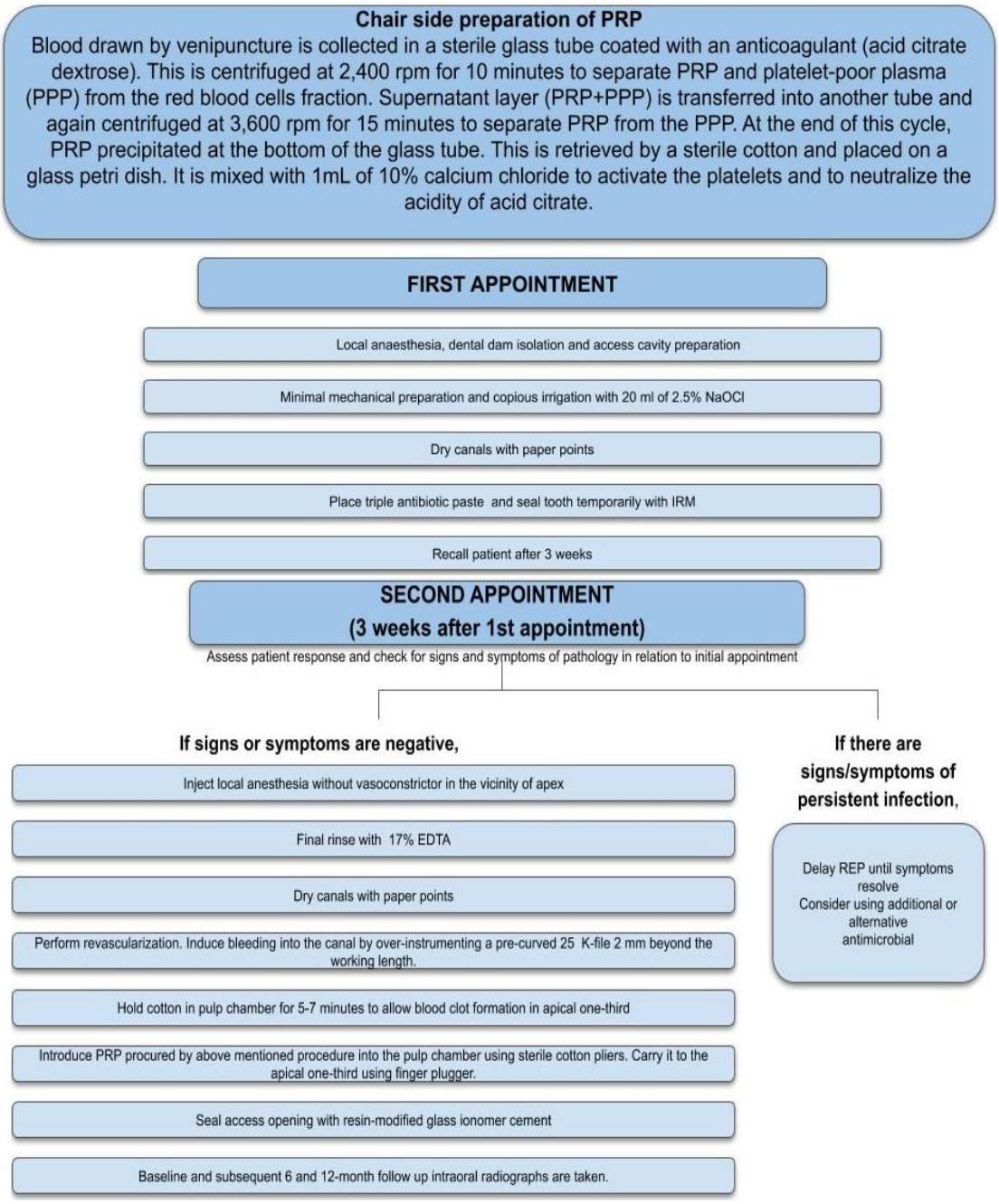

Fig. 2. Regenerative endodontic procedure using Platelet Rich Plasma (PRP) for a permanent tooth with an open apex [48]

The size of the apical foramen has been much stressed upon when attempting regeneration in a permanent tooth with closed apex. Cells such as fibroblasts, osteoblasts, cementoblasts and endothelial cells migrate through the apical foramen into the canal to produce PDL, bone, cementum, blood vessels in the canal space. Based on the size range of these cells (10-100 microns), it is believed that they can easily migrate through an apical foramen of diameter lesser than 0.5 mm [46]. However, Fang et al. concluded that the highest success rate of REP is attained in apical diameters of 0.5-1 mm [52]. Further research is needed in this area to

determine if the size of the apical foramen actually influences the outcome of REP in teeth with closed apex.

5. OUTCOME OF REGENERATIVE ENDODONTIC PROCEDURES

For any clinical procedure, the challenge starts when we try to define what a successful outcome is. When results for revascularization procedures are considered, evidence of root formation and reestablishment of pulpal function is vital to measure the success of treatment [3].

Reports demonstrate that regenerative endodontics is a viable treatment option which has been described as a 'paradigm shift' that allows for continued root development, a return of vitality and health in formerly necrotic immature teeth [53]. Successful outcome of REP could mean different things in patient, clinician and researcher-centered perspective [54].

5.1 Patient-based Outcomes

Criteria of treatment success for clinicians and researchers may not always meet the needs and desires of the patients. Outcomes need to focus more on patient satisfaction as they are the primary contributors for their own health and we need to respect patient autonomy. The following are patient-centered outcomes of REP.

5.1.1 Resolution of symptoms

When standardized treatment protocols for REPs and apexification procedures (MTA and Calcium hydroxide) were compared, REP and MTA plug apexification procedures were equally effective in resolving signs and symptoms of disease and survival over 18 months in 100% and 95% of all patients, respectively, whereas apexification procedures using calcium hydroxide were significantly less effective (77%) [10]. However, Alobiad et al. found REPs to promote healing in 79% of patients treated, whereas apexification procedures promoted healing in 100% of the patients [55].

5.1.2 Survival of tooth

From a patient's perspective, an ideal treatment should result in increased functional life of the tooth. This is especially important when survival of an immature permanent tooth is considered, since early loss of a permanent tooth will not only cause malocclusion, but also impair craniofacial development. As mentioned earlier, REP has shown greater survival rate than MTA [10].

5.1.3 Esthetics

An important patient-centered outcome is preservation or restoration of esthetics. However, coronal staining may occur when TAP or MTA is used as an intracanal medicament. This staining is believed to be caused by minocycline, a constituent of TAP [56-58]. However, this coronal staining can be prevented by either using a dentinal adhesive to block dentinal tubules or by substituting minocycline with cefuroxime or Arestin [59].

5.2 Clinician-based Outcomes

Clinicians base the success of treatment based on clinical and radiographic exam such as no pain, soft tissue swelling or sinus tract (between first and second appointments), resolution of apical radiolucency (6-12 months after treatment) and positive response to pulp sensitivity tests.

5.2.1 Radiographic signs

One of the primary clinician-centered outcomes is radiographic signs of resolution of apical lesion and continued root development. Increased width of root walls is generally observed before apparent increase in root length and often occurs 12-24 months after treatment. In comparison to apexification, a significant increase in root canal length and width was seen in teeth treated with REP as compared to the teeth treated for MTA or Calcium Hydroxide Apexification [60-61]. Several studies have concluded that although REPs predictably promote healing of apical periodontitis in more than 90% of the cases, radiographic root development is far less predictable [23].

5.2.2 Vitality response

Positive response to pulp sensitivity test is considered to be a sign of pulp vitality [62]. Re-establishment of pain perception indicates the presence of vital, vascularized tissues with normal physiological response. Clinicians have noted positive response to cold or electric pulp sensitivity testing in 60% of published cases [63].

5.3 Scientist/researcher-based Outcomes

Scientist-based outcomes are the ones that are not directly related to clinical success of REP, but address difficulties in the procedure through

research, to promote substantial future advances in regenerative endodontics.

5.3.1 Animal studies

Several animal studies were conducted to determine the histological outcome of regenerative endodontic procedures. The effect of different combinations of stem cells, growth factors and scaffolds has been studied extensively on dog and ferret models [64-67]. All these studies showed formation of hard tissues at the apex and apical closure, however none of the studies showed true regeneration of the pulp-dentin complex. It has been observed that the newly formed hard tissue appears cellular, atubular, hence resembling cementum [68].

5.3.2 Histology

According to a study conducted by Torabinejad et al. and Nosrat et al. on human immature permanent teeth using PRP, the tissues that grow into the root canals after regenerative endodontic procedures resemble periodontium, that is, fibrotic PDL, collagen fibres and cementum-like hard tissues [67,69]. However, no evidence of odontoblasts could be seen histologically. Nosrat et al. compared the histological sections of human immature permanent teeth using a novel hydroxyapatite scaffold and blood clot. All the specimens histologically showed dentin, dentin associated newly formed mineralized tissue, newly formed connective tissues and the PDL. However, none of the specimens showed pulp-like tissues [70].

6. FUTURE OF REGENERATIVE ENDODONTICS

Regenerative procedures require more research as they have a potential to replace conventional root canal therapy in the future, provided a predictable, feasible and economical protocol can be developed for the same [10,71-73]. Since its onset over 40 years ago, several research advancements have been made in the field. These mainly include components of cell-based regenerative therapy and tissue engineering. Potential areas of research in the development of regenerative endodontics include.

6.1 3-D Bioprinting

Synthetic 3D scaffolds can be generated using a bioink, which is composed of blending printable alginate hydrogel with soluble and insoluble fractions of the dentin matrix [74]. It involves precise placement of cells into a soft scaffold as directed by a computer-aided design. The soluble dentin molecules of the matrix significantly enhance odontogenic differentiation of the mesenchymal stem cells encapsulated in these bioprinted hydrogels. Such bioprinting allows for precise and reproducible positioning of cellularized scaffolds, which can be useful to study cell interactions and the effect of cell organization on cell growth and function [74].

6.1.1 Injectable scaffold

This is a type of 3-D scaffold, composed of injectable hydrogel, which is placed in the root canal to provide a substrate for organized cell proliferation. To promote bone regeneration, calcium phosphate cement scaffolds have been used along with osteoinductive growth factors [75].

6.2 Gene Therapy

Gene therapy involves introducing specific genes into target cells, either to compensate for abnormal genetic material or to stimulate the natural biological process for regeneration of a desired tissue. These genes can be delivered using viral (adenovirus, lentivirus, herpes simplex virus) or non-viral (plasmids, peptides, DNA-ligand complexes, liposomes) vectors, either *ex vivo* or *in vitro* [76,3]. The viral vectors are modified such that they do not cause disease but are capable to stimulate an infection. Gene therapy can be applied in regenerative endodontics by delivering mineralizing genes into the vital pulp cells of necrotic or symptomatic teeth [77]. The *ex vivo* therapy is preferred for stimulation of reparative dentin [78]. However, except for a research done by Rutherford in 2001 using c-DNA encoded mouse BMP-7 [79], currently the knowledge based on this therapy is purely theoretical. Widespread clinical application of such therapy is yet to be developed considering the potential health hazard posed by the vectors in this therapy [80].

6.3 Vascular and Nervous Regeneration

Pulp hemostasis is primarily regulated by its nervous and vascular supply. As mentioned earlier, stem cells like DPSCs and SHED have neurogenic potential. It is also demonstrated that members of the BMP family have beneficial effects on nerve regeneration. Pulp vasculature

is vital for proliferation and differentiation of stem cells [8]. Angiogenesis can be accelerated in an engineered tissue by slowly releasing growth factors like VEGF from the scaffold. Utilizing gene therapy along with VEGF and BMP can be potentially used in regeneration of pulp-dentin complex [81].

6.4 Bioengineered Tooth

It is said that an organ can be bioengineered by reproducing the developmental processes during organogenesis [82]. Like tooth, several organs develop from interactions between epithelial and mesenchymal cells. Hence a bioengineered tooth can be developed *in vitro* by replicating the steps of development of a tooth germ. Ikeda et al. and Oshima et al. demonstrated in an adult mouse, that a fully functional bioengineered tooth can be achieved through the transplantation of a bioengineered tooth germ. This tooth, which was erupted and occluded, had the correct tooth and alveolar structure, hardness of mineralized tissues for mastication, and response to noxious stimulations in cooperation with tissues of oral and maxillofacial region [83-84]. Therefore, these bioengineered teeth can be used as a replacement for missing or lost teeth.

7. LIMITATIONS

7.1 Patient Co-operation

REP is usually indicated for relatively young patients. Such patients are often nervous, frightened, or impatient. Further, when PRP is utilized for such procedures, venipuncture is required. Hence, REP could be challenging to perform if patient cooperation is compromised.

7.2 Barriers in Stem Cell Transplantation

Stem cell transplantation is a common practice in medicine, especially in the treatment of haematopoeitic disorders. However, extensive *ex vivo* culturing of mesenchymal stem cells is required, while ensuring proper protocols of isolation and expansion are followed. Lack of such affordable facilities and dental stem cell banking system, and the risks of immune rejection restricts the use of these cell-based therapies in clinical practice [85,86].

7.3 Tooth Discolouration

As mentioned earlier, coronal staining may occur when antibiotics pastes containing minocycline and bismuth oxide are used as an intracanal medicament [56-58]. Additionally, these materials show greater color change when they come in contact with blood [87].

7.4 Unpredictable Outcome

The nature of tissue formed in the canal after REP remains unpredictable. According to studies conducted by Nosrat et al. and Torabinejad et al., the regenerated tissues resemble periodontium, rather than the pulp. However, Shimizu et al. showed the presence of pulp-like connective tissue after revascularization in more than one-half of the canal [88]. Further, clinical trials involving long-term prognosis and impact of REP beyond 18 months are limited, and hence, unknown [89].

7.5 Lack of expertise

Treatment of teeth with immature roots is challenging due to thin dentin walls, hence root fracture can easily occur during mechanical debridement. Lack of both endodontic expertise and facilities involved in the handling of stem cells for clinical application of cell-based REP makes it a challenging technique currently.

8. CONCLUSION

Regenerative endodontic procedures provide a successful alternative for the treatment of necrotic teeth with open apex, by permitting continued root development. Several studies have demonstrated root development with high rates of resolution of clinical and radiographic signs and symptoms upon treatment using REP. However, current clinical protocols of REP foster repair rather than regeneration. Moreover, the resemblance of the regenerated tissue to a healthy pulp-dentin complex is debatable.

No matter how theoretically correct these clinical attempts are, their outcome will be variable due to multifactorial reasons like practitioner skill and variation, severity of the disease, patient's intrinsic response, and case selection. Although regenerative endodontics has a promising potential to be an effective biological approach to restore the vitality of teeth, additional research and clinical trials are required to develop clinical applications and outline predictable outcomes for the procedure. Upon significant research, over time, regenerative endodontics may open doors to a wide range of possibilities of dental tissue regeneration.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Cvek M. Prognosis of luxated non-vital maxillary incisors treated with calcium hydroxide and filled with gutta-percha. A retrospective clinical study. Dental Traumatology. 1992;8(2):45-55.
   Available:https://doi.org/10.1111/j.1600-9657.1992.tb00228.x
2. Huang GJ. Apexification: The beginning of its end. International Endodontic Journal. 2009;42(10):855-66.
   Available:https://doi.org/10.1111/j.1365-2591.2009.01577.x
3. Murray PE, Garcia-Godoy F, Hargreaves KM. Regenerative endodontics: A review of current status and a call for action. Journal of Endodontics. 2007;33(4):377-90.
   Available:https://doi.org/10.1016/j.joen.2006.09.013
4. Available:https://www.aae.org/specialty/clinical-resources/glossary-endodontic-terms/. [Accessed on 14/5/2020]
5. Bansal R, Jain A. Current overview on dental stem cells applications in regenerative dentistry. Journal of Natural Science, Biology, and Medicine. 2015;6(1):29.
   DOI: 10.4103/0976-9668.149074
6. Nygaard-Östby B, Hjortdal O. Tissue formation in the root canal following pulp removal. European Journal of Oral Sciences. 1971;79:333-349.
   DOI: 10.1111/j.1600-0722.1971.tb02019.x
7. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) *in vitro* and *in vivo*. Proceedings of the National Academy of Sciences. 2000;97(25):13625-30.
   Available:https://doi.org/10.1073/pnas.240309797
8. Nakashima M, Akamine A. The application of tissue engineering to regeneration of pulp and dentin in endodontics. Journal of Endodontics. 2005;31(10):711-8.
   Available:https://doi.org/10.1097/01.don.0000164138.49923.e5
9. Lin LM, Kahler B. A review of regenerative endodontics: current protocols and future directions. Journal of Istanbul University Faculty of Dentistry. 2017;51(3 Suppl 1):S41.
   DOI: 10.17096/jiufd.53911
10. Jeeruphan T, Jantarat J, Yanpiset K, Suwannapan L, Khewsawai P, Hargreaves KM. Mahidol study 1: Comparison of radiographic and survival outcomes of immature teeth treated with either regenerative endodontic or apexification methods: A retrospective study. Journal of endodontics. 2012;38:1330-1336.
    Available:https://doi.org/10.1016/j.joen.2012.06.028
11. Thesleff I, Tummers M. Tooth organogenesis and regeneration. In Stem Book [Internet]. Harvard Stem Cell Institute; 2009.
    [PMID: 20614625]
12. Smith AJ, Patel M, Graham L, Sloan AJ, Cooper PR. Dentine regeneration: Key roles for stem cells and molecular signalling. Oral Biosci Med. 2005;2(2/3):127-32.
13. Swarup SJ, Rao A, Boaz K, Srikant N, Shenoy R. Pulpal response to nano hydroxyapatite, mineral trioxide aggregate and calcium hydroxide when used as a direct pulp capping agent: An *in vivo* study. Journal of Clinical Pediatric Dentistry. 2014;38(3):201-6.
    Available:https://doi.org/10.17796/jcpd.38.3.83121661121g6773
14. Gottlieb B. Continuous deposition of cementum. The Journal of the American Dental Association. 1943;30(11):842-7.
    Available:https://doi.org/10.14219/jada.archive.1943.0172
15. King GJ, Keeling SD, Wronski TJ. Histomor-phometric study of alveolar bone turnover in orthodontic tooth movement. Bone. 1991;12(6):401-9.
    Available:https://doi.org/10.1016/8756-3282(91)90029-I
16. Takata T, Wang HL, Miyauchi M. Attachment, proliferation and differentiation of periodontal ligament cells on various guided tissue regeneration membranes. Journal of Periodontal Research. 2001;36(5):322-7.

Available:https://doi.org/10.1034/j.1600-0765.2001.360508.x

17. Neel EA, Chrzanowski W, Salih VM, Kim HW, Knowles JC. Tissue engineering in dentistry. Journal of dentistry. 2014;42(8): 915-28.
[PMCID: PMC5090995 PMID: 27857762]
18. Huang GT. Dental pulp and dentin tissue engineering and regeneration-advancement and challenge. Frontiers in bioscience (Elite edition). 2011;3:788-800.
DOI: 10.2741/e286
[PMCID: PMC3289134]
[NIHMSID: NIHMS331027]
[PMID: 21196351]
19. Zipori D. The stem state: Plasticity is essential, whereas self-renewal and hierarchy are optional. Stem cells. 2005; 23(6):719-26.
Available:https://doi.org/10.1634/stemcells.2005-0030
20. Diogenes A, Henry MA, Teixeira FB, Hargreaves KM. An update on clinical regenerative endodontics. Endodontic Topics. 2013;28(1):2-3.
Available:https://doi.org/10.1111/etp.12040
21. Gronthos S, Brahim J, Li W, Fisher LW, Cherman N, Boyde A, DenBesten P, Robey PG, Shi S. Stem cell properties of human dental pulp stem cells. Journal of dental research. 2002;81(8):531-5.
Available:https://doi.org/10.1177/154405910208100806
22. Shi S, Bartold PM, Miura M, Seo BM, Robey PG, Gronthos S. The efficacy of mesenchymal stem cells to regenerate and repair dental structures. Orthodontics & craniofacial research. 2005;8(3):191-9.
Available:https://doi.org/10.1111/j.1601-6343.2005.00331.x
23. Sonoyama W, Liu Y, Fang D, Yamaza T, Seo BM, et al. mesenchymal stem cell-mediated functional tooth regeneration in swine. Plos One. 2006;1(1):e79.
DOI: 10.1371/journal.pone. 0000079
24. Seo BM, Miura M, Gronthos S, et al. Investigation of multipotent postnatal stem cells from human periodontal ligament. The Lancet. 2004;364:149-155.
Available:https://doi.org/10.1016/S0140-6736(04)16627-0
25. Park JC, Kim JM, Jung IH, Kim JC, Choi SH, Cho KS, Kim CS. Isolation and characterization of human periodontal ligament (PDL) stem cells (PDLSCs) from the inflamed PDL tissue: *In vitro* and *in vivo* evaluations. Journal of Clinical Periodontology. 2011;38(8):721-31.
Available:https://doi.org/10.1111/j.1600-051X.2011.01716.x
26. Zhu W, Liang M. Periodontal ligament stem cells: current status, concerns, and future prospects. Stem Cells International. 2015;2015.
Available:https://doi.org/10.1155/2015/972313
27. Zheng W, Wang S, Ma D, Tang L, Duan Y, Jin Y. Loss of proliferation and differentiation capacity of aged human periodontal ligament stem cells and rejuvenation by exposure to the young extrinsic environment. Tissue Engineering Part A. 2009;15(9):2363-71.
Available:https://doi.org/10.1089/ten.tea.2008.0562
28. Handa K, Saito M, Yamauchi M, Kiyono T, Sato S, Teranaka T, Narayanan AS. Cementum matrix formation in vivo by cultured dental follicle cells. Bone. 2002; 31(5):606-11.
Available:https://doi.org/10.1016/S8756-3282(02)00868-2
29. Morsczeck C, Götz W, Schierholz J, Zeilhofer F, Kühn U, Möhl C, Sippel C, Hoffmann KH. Isolation of precursor cells (PCs) from human dental follicle of wisdom teeth. Matrix Biology. 2005;24(2):155-65.
Available:https://doi.org/10.1016/j.matbio.2004.12.004
30. Morsczeck C, Völlner F, Saugspier M, Brandl C, Reichert TE, Driemel O, Schmalz G. Comparison of human dental follicle cells (DFCs) and stem cells from human exfoliated deciduous teeth (SHED) after neural differentiation *in vitro*. Clinical Oral Investigations. 2010;14(4):433-40.
Available:https://doi.org/10.1007/s00784-009-0310-4
31. Saito MT, Silvério KG, Casati MZ, Sallum EA, Nociti Jr FH. Tooth-derived stem cells: Update and perspectives. World Journal of Stem Cells. 2015;7(2):399.
DOI: 10.4252/wjsc.v7.i2.399
32. Maiorano E, Renne G, Tradati N, Viale G. Cytogical features of calcifying epithelial odontogenic tumor (Pindborg tumor) with abundant cementum-like material. Virchows Archiv. 2003;442(2):107-10.
Available:https://doi.org/10.1007/s00428-002-0722-x
33. Chalisserry EP, Nam SY, Park SH, Anil S. Therapeutic potential of dental stem cells.

Journal of tissue engineering. 2017;8:2041731417702531.
Available:https://doi.org/10.1177/2041731417702531

34. Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, Shi S. SHED: Stem cells from human exfoliated deciduous teeth. Proceedings of the National Academy of Sciences. 2003;100(10):5807-12.
Available:https://doi.org/10.1073/pnas.0937635100
35. Wang X, Sha XJ, Li GH, Yang FS, Ji K, Wen LY et al. Comparative characterization of stem cells from human exfoliated deciduous teeth and dental pulp stem cells. Archives of oral biology. 2012; 57(9):1231-40.
Available:https://doi.org/10.1016/j.archoralbio.2012.02.014
36. Wang J, Wang X, Sun Z, Wang X, Yang H, Shi S et al. Stem cells from human-exfoliated deciduous teeth can differentiate into dopaminergic neuron-like cells. Stem Cells and Development. 2010;19(9):1375-83.
Available:https://doi.org/10.1089/scd.2009.0258
37. Arora V, Arora P, Munshi AK. Banking stem cells from human exfoliated deciduous teeth (SHED): Saving for the future. Journal of Clinical Pediatric Dentistry. 2009;33(4):289-94.
Available:https://doi.org/10.17796/jcpd.33.4.y887672r0j703654
38. Saber SE. Tissue engineering in endodontics. Journal of Oral Science. 2009;51(4):495-507.
Available:https://doi.org/10.2334/josnusd.51. 495
39. Nakashima M. Bone morphogenetic proteins in dentin regeneration for potential use in endodontic therapy. Cytokine & Growth Factor Reviews. 2005;16(3):369-76.
Available:https://doi.org/10.1016/j.cytogfr.2005.02.011
40. Gathani KM, Raghavendra SS. Scaffolds in regenerative endodontics: A review. Dental Research Journal. 2016;13(5): 379.
41. Huang GT, Sonoyama W, Chen J, Park SH. In vitro characterization of human dental pulp cells: various isolation methods and culturing environments. Cell and Tissue Research. 2006;324(2):225.
Available:https://doi.org/10.1007/s00441-005-0117-9
42. Sharma S, Mittal N. A comparative evaluation of natural and artificial scaffolds in regenerative endodontics: A clinical study. Saudi Endodontic Journal. 2016; 6(1):9.
DOI: 10.4103/1658-5984. 171995
43. Nör JE. Buonocore memorial lecture: Tooth regeneration in operative dentistry. Operative dentistry. 2006;31(6):633-42.
Available:https://doi.org/10.2341/06-000
44. Malhotra N, Mala K. Regenerative endodontics as a tissue engineering approach: Past, current and future. Australian Endodontic Journal. 2012;38: 137-48.
Available:https://doi.org/10.1111/j.1747-4477.2012.00355.x
45. American Association of Endodontists. AAE clinical considerations for a regenerative procedure.
[Accessed on 20/4/2020]
Available:https://f3f142zs0k2w1kg84k5p9i1o-wpengine.netdnassl.com/specialty/wpcontent/uploads/sites/2/2018/06/ConsiderationsForRegEndo_AsOfApril2018.pdf
46. Kim SG, Malek M, Sigurdsson A, Lin LM, Kahler B. Regenerative endodontics: A comprehensive review. International Endodontic Journal. 2018;51(12):1367-88.
Available:https://doi.org/10.1111/iej.12954
47. Liu ZN, Jiang T, Wang YX. Platelet-riched plasma promotes potential mineralizing capacity of human dental pulp cells in vivo. Beijing da xue xue bao. Yi xue ban= Journal of Peking University. Health Sciences. 2011;43(2):276-9.
48. Jadhav GR, Shah N, Logani A. Platelet-rich plasma supplemented revascularization of an immature tooth associated with a periapical lesion in a 40-year-old man. Case Reports in Dentistry. 2014; 2014.
Available:https://doi.org/10.1155/2014/479584
49. He L, Kim SG, Gong Q, Zhong J, Wang S, Zhou X, Ye L, Ling J, Mao JJ. Regenerative endodontics for adult patients. Journal of Endodontics. 2017; 43(9):S57-64.
Available:https://doi.org/10.1016/j.joen.2017.06.012
50. Paryani K, Kim SG. Regenerative endodontic treatment of permanent teeth after completion of root development: A report of 2 cases. Journal of Endodontics. 2013;39(7):929-34.

Available:https://doi.org/10.1016/j.joen.2013.04.029

51. Saoud TM, Sigurdsson A, Rosenberg PA, Lin LM, Ricucci D. Treatment of a large cystlike inflammatory periapical lesion associated with mature necrotic teeth using regenerative endodontic therapy. Journal of Endodontics. 2014;40(12):2081-6.
Available:https://doi.org/10.1016/j.joen.2014.07.027
52. Fang Y, Wang X, Zhu J, Su C, Yang Y, Meng L. Influence of apical diameter on the outcome of regenerative endodontic treatment in teeth with pulp necrosis: A review. Journal of Endodontics. 2018; 44(3):414-31.
53. Huang GT. A paradigm shift in endodontic management of immature teeth: conservation of stem cells for regeneration. J Dent. 2008;36:379–386.
Available:https://doi.org/10.1016/j.jdent.2008.03.002
54. Diogenes A, Ruparel NB, Shiloah Y, Hargreaves KM. Regenerative endodontics: A way forward. The Journal of the American Dental Association. 2016; 147(5):372-80.
Available:https://doi.org/10.1016/j.adaj.2016.01.009
55. Alobaid AS, Cortes LM, Lo J, Nguyen TT, Albert J, Abu-Melha AS, Lin LM, Gibbs JL. Radiographic and clinical outcomes of the treatment of immature permanent teeth by revascularization or apexification: A pilot retrospective cohort study. Journal of Endodontics. 2014;40(8):1063-70.
Available:https://doi.org/10.1016/j.joen.2014.02.016
56. Reynolds K, Johnson JD, Cohenca N. Pulp revascularization of necrotic bilateral bicuspids using a modified novel technique to eliminate potential coronal discolouration: A case report. Int Endod J. 2009;42(1):84-92.
Available:https://doi.org/10.1111/j.1365-2591.2008.01467.x
57. Kim JH, Kim Y, Shin SJ, Park JW, Jung IY. Tooth discoloration of immature permanent incisor associated with triple antibiotic therapy: A case report. J Endod. 2010; 36(6):1086-1091.
Available:https://doi.org/10.1016/j.joen.2010.03.031
58. Petrino JA, Boda KK, Shambarger S, Bowles WR, McClanahan SB. Challenges in regenerative endodontics: A case series. J Endod. 2010;36(3):536-541.
Available:https://doi.org/10.1016/j.joen.2009.10.006
59. Krastl G, Allgayer N, Lenherr P, Filippi A, Taneja P, Weiger R. Tooth discoloration induced by endodontic materials: A literature review. Dental Traumatology. 2013;29(1):2-7.
Available:https://doi.org/10.1111/j.1600-9657.2012.01141.x
60. Bose R, Nummikoski P, Hargreaves K. A retrospective evaluation of radiographic outcomes in immature teeth with necrotic root canal systems treated with regenerative endodontic procedures. J Endod. 2009;35:1343–1349.
Available:https://doi.org/10.1016/j.joen.2009. 06.021
61. Nagy MM, Tawfik HE, Hashem AA, Abu-Seida AM. Regenerative potential of immature permanent teeth with necrotic pulps after different regenerative protocols. Journal of Endodontics. 2014;40(2):192-8.
Available:https://doi.org/10.1016/j.joen.2013.10.027
62. Weisleder R, Yamauchi S, Caplan DJ, Trope M, Teixeira FB. The validity of pulp testing: A clinical study. JADA 2009; 140(8):1013-1017.
Available:https://doi.org/10.14219/jada.archive.2009.0312
63. Torabinejad M, Turman M. Revitalization of tooth with necrotic pulp and open apex by using Platelet rich plasma: A case report. J Endod. 2011;37(2):265–268.
[PubMed: 21238815]
Available:https://doi.org/10.1016/j.joen.2010.11.004
64. Torabinejad M, Corr R, Buhrley M, Wright K, Shabahang S. An animal model to study regenerative endodontics. Journal of endodontics. 2011;37(2):197-202.
Available:https://doi.org/10.1016/j.joen.2010.10.011
65. Thibodeau B, Teixeira F, Yamauchi M, Caplan DJ, Trope M. Pulp revascularization of immature dog teeth with apical periodontitis. Journal of endodontics. 2007; 33(6):680-9.
Available:https://doi.org/10.1016/j.joen.2007.03.001
66. Zhu X, Zhang C, Huang GT, Cheung GS, Dissanayaka WL, Zhu W. Transplantation of dental pulp stem cells and platelet-rich plasma for pulp regeneration. Journal of Endodontics. 2012;38(12):1604-9.

Available:https://doi.org/10.1016/j.joen.2012.09.001

67. Torabinejad M, Faras H, Corr R, Wright KR, Shabahang S. Histologic examinations of teeth treated with 2 scaffolds: A pilot animal investigation. Journal of Endodontics. 2014;40(4):515-20. Available:https://doi.org/10.1016/j.joen.2012.03.006
68. Yoo YJ, Lee W, Cho YA, Park JC, Shon WJ, Baek SH. Effect of conditioned medium from preameloblasts on regenerative cellular differentiation of the immature teeth with necrotic pulp and apical periodontitis. Journal of endodontics. 2014;40(9):1355-61. Available:https://doi.org/10.1016/j.joen.2014.02.009
69. Nosrat A, Kolahdouzan A, Hosseini F, Mehrizi EA, Verma P, Torabinejad M. Histologic outcomes of uninfected human immature teeth treated with regenerative endodontics: 2 case reports. Journal of Endodontics. 2015;41(10):1725-9. Available:https://doi.org/10.1016/j.joen.2015.05.004
70. Nosrat A, Kolahdouzan A, Khatibi AH, Verma P, Jamshidi D, Nevins AJ, Torabinejad M. Clinical, radiographic and histologic outcome of regenerative endodontic treatment in human teeth using a novel collagen-hydroxyapatite scaffold. Journal of Endodontics. 2019;45(2):136-43. Available:https://doi.org/10.1016/j.joen.2018.10.012
71. Gupta P, Gada S, Shetty H. Regenerative endodontics: An evidence based review. J Cont Med A Dent. 2015;3(1):12-9. Available:http://dx.doi.org/10.18049/jcmad/312
72. Witherspoon DE, Small JC, Regan JD, Nunn M. Retrospective analysis of open apex teeth obturated with mineral trioxide aggregate. J Endod. 2008;34:11711176. Available:https://doi.org/10.1016/j.joen.2008.07.005
73. Andreasen JO, Farik B, Munksgaard EC. Long-term calcium hydroxide as a root canal dressing may increase risk of root fracture. Dent Traumatol. 2002;18:134-137. Available:https://doi.org/10.1034/j.1600-9657.2002.00097.x
74. Athirasala A, Tahayeri A, Thrivikraman G, França CM, Monteiro N, Tran V, Ferracane J, Bertassoni LE. A dentin-derived hydrogel bioink for 3D bioprinting of cell laden scaffolds for regenerative dentistry. Biofabrication. 2018;10(2):024101. Available:https://doi.org/10.1088/1758-5090/aa9b4e
75. Kim HJ, Kim UJ, Leisk GG, Bayan C, Georgakoudi I, Kaplan DL. Bone regeneration on macroporous aqueous-derived silk 3-D scaffolds. Macromolecular Bioscience. 2007;7(5):643-55. Available:https://doi.org/10.1002/mabi.200700030
76. HS Shilpashree, Shriprasad Sarapur. Gene therapy in dentistry: A review. New York State Dental Journal. 2013;79(5):60.
77. Lovelace TW, Henry MA, Hargreaves KM, Diogenes A. Evaluation of the delivery of mesenchymal stem cells into the root canal space of necrotic immature teeth after clinical regenerative endodontic procedure. Journal of Endodontics. 2011;37(2):133-8.
78. Nakashima M, Iohara K, Zheng L. Gene therapy for dentin regeneration with bone morphogenetic proteins. Current gene therapy. 2006;6(5):551-60. Available:https://doi.org/10.2174/156652306778520665
79. Rutherford RB. BMP-7 gene transfer to inflamed ferret dental pulps. European Journal of Oral Sciences. 2001;109(6):422-4. Available:https://doi.org/10.1034/j.1600-0722.2001.00150.x
80. Bansal R, Bansal R. Regenerative endodontics: A state of the art. Indian Journal of Dental Research. 2011;22(1):122. Available:http://www.ijdr.in/text.asp?2011/22/1/122/79977
81. Rutherford RB. BMP-7 gene transfer to inflamed ferret dental pulps. European Journal of Oral Sciences. 2001;109(6):422-4. Available:https://doi.org/10.1034/j.1600-0722.2001.00150.x
82. Duailibi SE, Duailibi MT, Vacanti JP, Yelick PC. Prospects for tooth regeneration. Periodontology 2000. 2006;41(1):177-87. Available:https://doi.org/10.1111/j.1600-0757.2006.00165.x
83. Ikeda E, Morita R, Nakao K, Ishida K, Nakamura T, Takano-Yamamoto T, Ogawa M, Mizuno M, Kasugai S, Tsuji T. Fully functional bioengineered tooth replacement as an organ replacement

therapy. Proceedings of the National Academy of Sciences. 2009;106(32): 13475-80.
Available:https://doi.org/10.1073/pnas.0902944106

84. Oshima M, Mizuno M, Imamura A, Ogawa M, Yasukawa M, Yamazaki H, Morita R, Ikeda E, Nakao K, Takano-Yamamoto T, Kasugai S. Functional tooth regeneration using a bioengineered tooth unit as a mature organ replacement regenerative therapy. PloS One. 2011;6(7).
Available:https://doi.org/10.1007/s10266-014-0168-z
85. Mao JJ, Kim SG, Zhou J, et al. Regenerative endodontics: Barriers and strategies for clinical translation. Dental Clinics. 2012;56:639-649.
Available:https://doi.org/10.1016/j.cden.2012.05. 005
86. Huang GT, Al-Habib M, Gauthier P. Challenges of stem cell-based pulp and dentin regeneration: A clinical perspective. Endodontic Topics. 2013;28(1):51-60.
Available:https://doi.org/10.1111/etp.12035
87. Lenherr P, Allgayer N, Weiger R, et al. Tooth discoloration induced by endodontic materials: A laboratory study. Int Endod J. 2012;45:942–9
Available:https://doi.org/10.1111/j.1365-2591.2012.02053.x
88. Shimizu E, Jong G, Partridge N, Rosenberg PA, Lin LM. Histologic observation of a human immature permanent tooth with irreversible pulpitis after revascularization/regeneration procedure. J Endod. 2012;38(9):1293-1297.
DOI: 10.1016/j.joen.2012.06.017
89. Tong HJ, Rajan S, Bhujel N, Kang J, Duggal M, Nazzal H. Regenerative endodontic therapy in the management of nonvital immature permanent teeth: A systematic review-outcome evaluation and meta-analysis. J Endod. 2017;43(9):1453-1464.
DOI: 10.1016/j.joen.2017.04.018

© 2020 Mallishery and Shah; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/57036