

REVIEW ARTICLE

Regenerative Endodontics: An Evidence Based Review

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Abstract

Endodontic treatment of a tooth in its present state offers a high degree of success for most cases, but it leaves the tooth in a physiologically “dead” state. Regenerative endodontics is an emerging field which is trying to regenerate natural tissues associated with the tooth generated by nature. This article reviews the present status of regenerative endodontics, why is it important to regenerate the pulp, the materials and methods used for the same and progress made so far, along with the various challenges being faced and the future lines of research based on the published literature.

Keywords: Regenerative, endodontics, teeth, pulp, stem cells, growth factors

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Introduction

Endodontics has come a long way since 1687 when Charles Allen authored the first book in the English language exclusively on the subject of dentistry describing the techniques of dental transplant^[1]. Since then there has been remarkable development in dentistry particularly in the field of Endodontics. Utilizing the reparative capacity of the dentinopulpal complex for the treatment of pulpal and periapical pathologies has become the “holy grail” of endodontics. This has been made possible because of rapid development in the field of regenerative medicine and tissue engineering. The ultimate aim of regenerative endodontics is to create practical, predictable and economical treatment protocol in which the damaged pulp tissue and the dentinoenamel complex can be restored using biomedically engineered or regenerated material to its natural undamaged state. Though it is still far away from this goal but there have been marked developments in this direction.

Why to regenerate the pulp?

The importance of a healthy vital pulp cannot be over stressed in an immature developing tooth,

where root completion to the full length and an increase in the root dentin thickness, makes the tooth more resistant to the functional forces. Trauma, which is common in the age from 7 to 15 years, can leave an immature tooth non vital with thin, fragile dentinal walls and a wide open and incompletely formed apex^[2]. Though traditionally, a multiple visit apexification technique has been use to create an apical barrier, but this technique leaves the dentinal walls weak and liable to fracture^[3]. Regeneration of dental pulp in this case will ensure the increase in both the length and the thickness of the dentinal walls thus providing an ideal treatment option. The importance of pulp vitality in mature tooth can be understood from the biological rational of endodontics which is to prevent or treat apical periodontitis. Apical periodontitis cannot exist if the pulp is healthy and vital. Therefore maintaining the vitality of the pulp prevents apical periodontitis, and the potential to regenerate an injured or necrotic pulp would be the best root filling possible^[4].

History

There have been multiple attempts at pulpal regeneration, of which, one of the first attempt at regeneration of the pulp tissue and the

importance of blood clot was reported in the literature by Nygaard-Ostby in 1961 further reestablished in a landmark article in 1971 [5]. The article described the procedure where in the pulp tissue was removed from vital teeth and bleeding was induced within the canal followed by partial root canal filling. They reported a connective tissue formation in the pulp space in the histological section [6]. In 1972, Ham et al demonstrated induced apical closure of immature pulpless teeth in monkeys [7]. In 2001, Iwaya *et al* demonstrated the use of antimicrobial agents (metronidazole and ciprofloxacin) without mechanical instrumentation for the continued development of the root in a 13 year old patient with an immature mandibular second molar with a sinus tract associated with it [8]. In 2004, Francisco Banchs and Martin Trope described a technique of revascularization in immature permanent mandibular second premolar with the use of triple antibiotic paste, intentional apical irritation and the use of good coronal seal [9].

Components of Regenerative Endodontic Therapy

The three components which play an important role in regenerative endodontics are:

1. Stem cells
2. Growth factors
3. Scaffolds

Stem cells

Stem cells are undifferentiated cells which have the capability to produce cells of the same type or more differentiated cells. Depending on the ability of the stem cells to produce the different types of cells they are classified into pluripotent or multipotent. Pluripotent stem cells are those which are capable of differentiating into specialized cells of any three germ layers. Truly pluripotent stem cells are found in the developing embryos. Stem cells, found in adults are restricted in their capacity to differentiate and are thus termed multipotent. The mesenchymal tissues (e.g. bone, dental pulp, periodontal ligament) appear to have an enriched population of adult stem cells [10, 11]. Most stem cells in the oral region are of mesenchymal origin. It is this multipotent capacity of the mesenchymal stem cells which

forms the basis of all regenerative endodontic procedures.

The stem cells which have been isolated from the oral region are Stem Cells of The Apical Papilla (SCAP), Inflammatory Periapical Progenitor Cells (iPAPCs), Dental Follicle Stem Cells (DFSCs), Dental Pulp Stem Cells (DPSCs), Periodontal Ligament Stem Cells (PDLSCs), Bone Marrow Stem Cells (BMSCs), Tooth Germ Progenitor Cells (TGPCs), Salivary Gland Stem Cells (SGSCs), Stem Cells From Human Exfoliated Deciduous Teeth (SHED), Oral Epithelial Stem Cells (OESCs), Gingival-Derived Mesenchymal Stem Cells (GMSCs), and Periosteal-Derived Stem Cells (PSCs) [12, 13]. One more noteworthy property of these stem cells is their survival in chronically inflamed environment and in the presences of necrotic infected tissue and bacteria. These two properties of stem cells allows the disinfected canal to get repopulated with differentiated cells while allowing time for reorganization, angiogenesis and innervation to take place [14].

Growth factors

Growth factors are polypeptides which have the ability to bind to specific receptors on the target cells (in this case the stem cells and other pulpal cells) and modulate or facilitate certain activities like migration, proliferation, differentiation, and apoptosis [15, 16]. Unlike hormones which act systemically, growth factors have a local action on target cells. They play an important role in attraction and differentiation of pulpal and stem cells within the canal. The main events and the growth factors which cause them are as follows

1. **Repair and regeneration:** PDGF (Platelet Derived Growth Factor) [17-20], TGF (Transforming Growth Factor) [21,22], BMP (Bone morphogenic protein) [23], VEGF (Vascular endothelial growth factor) [24], FGF (Fibroblast Growth Factor) [22] and IGF [25, 26] (Insulin like growth factor)
2. **Angiogenesis:** FGF2 [27], PDGF [28,29], VEGF
3. **Neuronal growth:** NGF [30] (Neuronal growth factor)
4. **Differentiation:** TGF β , PDGF, FGF2, BMP 2, 4,7,11, IGF, NGF
5. **Proliferation:** FGF2, SDF-1, TGF β 1, VEGF, PDGF
6. **Chemotaxis:** SDF-1, TGF β 1, PDGF, FGF2

Scaffold

A scaffold denotes a temporary platform for repairing or erecting a building. In a similar manner, all multicellular living organisms have natural scaffolds that surround the cells and provide structural support for formation and maintenance of tissues and organs^[31]. Collagen, vitronectin, fibronectin, and laminin are the main extracellular matrix proteins (ECMP) which forms the natural scaffold. These ECMP have functions ranging from providing cell anchorage, sequestration of growth factors, and signal cells to migrate, differentiate, and proliferate through integrin receptor-mediated signaling pathways^[32]. ECMP have an important role in dental tissue regeneration. Collagen is an important structural component of all tissues and helps in the immobilization of growth factors^[33]. Vitronectin acts as structural framework^[34], while laminin promotes odontoblastic differentiation^[35]. Fibronectin promotes ameloblastic growth and differentiation^[34]. Scaffolds for tissue engineering have also been created to mimic the natural scaffold. These scaffolds have been created with a range of physical properties like porosity, weight, and pore size^[31].

The important requirements of a scaffold for use in tissue engineering are that it should be nontoxic, biocompatible, biodegradable, nonimmunogenic, low concentration and high rate of resorption and ease of introduction into the canal^[36]. They should also provide nutrition to the stem cells for their growth and survival^[37]. The scaffolds for tissue engineering have been created from synthetic materials like polymers or naturally occurring ECMP like collagen or calcium phosphate.

The oldest scaffold used in the field of regenerative endodontics is the blood clot itself. The limitation of using blood clot as scaffold is that it does not provide predictably in cell concentration and composition which goes against the basic principle of tissue engineering. It been observed that erythrocytes in blood clot undergo degradation and adversely affect its properties^[38]. One of the most popular natural scaffold is Platelet rich plasma (PRP) which was introduced in the oral surgical practice by Whitmann and colleagues in 1997^[39]. A concentrated form of platelets, termed as platelet rich plasma, is a volume of autologous plasma

derived from the patient's blood, withdrawn from venous puncture, centrifuged twice to separate red blood cells from plasma, that has platelet concentrate above baseline This separated plasma has a gel like consistency and can easily be introduced into the pulp space. Other natural scaffolds used are bone siloprotein, alginate hydrogel while the synthetic scaffolds used are polylactic acid (PLA), polyglycolic acid (PGA), and polycaprolactone (PCL).

Established Methods of Regenerative Endodontic Procedure

I. For immature permanent non vital tooth with open apex

1. The regenerative endodontic protocol using blood clot

The regenerative endodontic protocol was first given by Martin Trope in 2007^[4] and has been incorporated as a standardized treatment protocol by the American association of Endodontics in 2014, based on the current research^[40].

Case selection

Regeneration in endodontics works best in teeth with necrotic pulp with wide open apex in young patients with or without a periapical infection. The restorative prognosis of the tooth should be assessed before the regenerative procedure is attempted as the pulp space will not be available for any restorations, like post and core. The medical history of the patient should also be reviewed thoroughly for any allergies to antibiotics and for systemic diseases which may adversely affect the prognosis of the procedure. Though the prognosis of regenerative endodontic procedures have not been well defined on the basis of etiology, but intrusive luxation injuries seem to have a poorer prognosis than other etiologies. This is because of the fact that the apical papilla, which is a rich source of stem cells, gets badly injured in intrusive luxation^[41].

Access cavity preparation and disinfection

First visit

A conventional access cavity is prepared for the tooth in question and copious irrigation is done. Little or no instrumentation is done as the walls of the canals are weak. The main objective of irrigation is to disinfect the root canal and

provide an environment which is conducive for stem cells to proliferate and differentiate in. A well-structured analysis of studies done on regenerative endodontics suggests that 80% of reported cases used sodium hypochlorite in a concentration of 1 to 6%^[42]. As is well known that the disinfection potential of sodium hypochlorite is concentration dependent and higher concentration translates into greater degree of disinfection but it has been found that higher concentration of sodium hypochlorite leads to decreased survival of stem cells due to denaturation of growth factor^[12]. Therefore it is recommended that a minimal concentration of sodium hypochlorite (about 1-2%) be used for irrigation and the needle be kept 1 mm short of the working length or a negative pressure irrigation system be used to prevent the extrusion of sodium hypochlorite in the periapical space and adversely affect the stem cells of the apical papilla. It has also been observed that use of 17% EDTA as the last irrigant has shown to increase the survival and differentiation potential of stem cells^[43]. This effect of irrigants on the survival potential of stem cells can be explained by their interactions with the dentin. While 6% sodium hypochlorite denatures the growth factors (e.g. TGF- β) embedded in the dentin^[44], EDTA stabilizes them and increases their bioavailability^[45, 46]. It is therefore recommended to use a low concentration of sodium hypochlorite along with a final rinse of 17% EDTA. Chlorhexidine in the concentration of 2% has also been used as an irrigant but has been reported to reduce the proliferation potential of the stem cells of the apical papilla^[47].

After thorough irrigation the canals are dried with paper points and a disinfecting agent is introduced into the canal space. The most commonly used medicament for disinfection of the canal space is Triple Antibiotic Paste (TAP)^[42]. Calcium hydroxide has also been used. Both have their merits and demerits. Triple antibiotic paste which consists of ciprofloxacin, metronidazole, and minocycline in equal proportion was first proposed for use by Hoshino *et al*^[48, 49] is the most popular intracanal medicament for regenerative endodontic procedures. Though double antibiotic pastes (ciprofloxacin, metronidazole) modified triple antibiotic paste (ciprofloxacin,

metronidazole and cefaclor) and a combination of amoxicillin with clavulanic acid have also been used. The main disadvantage of using TAP is the discoloration of the crown which has been proposed to be prevented by application of bonding agent in the access cavity. It has also been reported that the use higher concentration of the antibiotics (typically 1gm/ml which makes a creamy consistency of the paste) have an adverse effect on the survival and proliferation capability of the stem cells of the apical papilla. Interestingly the concentration of antibiotics suggested by Hoshino *et al* was 100 μ g/ml of each antibiotic was found to be non-toxic in the same study. It was further noted that calcium hydroxide was found to be nontoxic at all concentration and it even stimulated the growth of stem cells at certain concentrations^[50]. Thus it can be conclude that the concentration of the antibiotics in the triple antibiotic paste should be governed not by the physical consistency of the paste but by the optimal concentration necessary for disinfection of the canal space. After the intracanal medicament dressing has been given the access cavity is temporarily restored with IRMTM or CavitTM or Glass Ionomer cement and the patient is recalled after 2 to 4 weeks.

Second visit

On the second appointment, the patient is evaluated for symptoms like pain, swelling and sensitivity to percussion and palpation or any other sign of persistent infection. If the patient is asymptomatic the intracanal disinfective agent is washed off from the canal with copious saline irrigation and the patient is administered local anesthesia. The American association of endodontics recommends that a final irrigation with 17% EDTA be done^[40]. The canal is dried with paper points and bleeding is induced into the canals, upto the canal orifice, by deliberate over instrumentation, 2mm beyond the apex. Pressure is applied at the orifice level with wet cotton pellet till clot formation takes place. A resorbable matrix such as CollaPlugTM, CollacoteTM, CollaTapeTM or other material over the blood clot which acts as a matrix for the MTA. The orifice is sealed with a 3-4 mm layer of MTA and is restored with a dual cure glass ionomer cement or composite. As MTA has also been associated with discoloration, resin modified glass ionomer cement can be used

instead in esthetic zones and layered over by composite resin. The patient is recalled at 6, 12 and 24 months interval and a clinical and radiographic evaluation is done to check for absence of any periapical radiolucency, increase in root width and root thickness. A positive pulp vitality test is also a measure of success of the regenerative procedure though not a very reliable one. Advantages of revascularization by blood clot is that it can be executed without the need of specialized equipment and is less technique sensitive as compared to other revascularization methods. Also the use of autologous blood removes most of the immunological, ethical and legal problem.

2. Regenerative endodontic procedures with the use of Platelet Rich Plasma (PRP)

There have been numerous case reports on the use of PRP for regenerative endodontic procedures [51-56]. PRP basically acts as a scaffold and the basic premise for its use is that plasma which is segregated from whole blood is rich in platelets and growth factors and its insertion into the root canal provides for a more predictable quality and concentration of fibrin clot and associated growth factors. Second generation of platelet concentrates is termed PRF (Plasma Rich Fibrin) and unlike PRP does not require the use of anticoagulant and calcium chloride, but has to be used immediately. There is much less literature available on PRF [57, 58]. PRP is produced by venipuncture from the patient and transferring it into a test tube coated with anticoagulant and putting it through a soft spin (low RPM, about 1000) which produces three layers, the topmost layer is platelet poor plasma (PPP), the middle layer is platelet rich plasma (PRP) also termed as the "Buffy coat" and the bottom layer is red blood cells.

The PPP and PRP are segregated into another test tube and are subjected to a hard spin (higher RPM about 2500- 3500). This again gives the same three layers but it's much easier to segregate PRP at this stage. It is taken on a petri dish where it is mixed with 1ml of 10 % calcium chloride or thrombin or collagen which are used to activate the platelets and neutralize the acidity of the anticoagulant [59]. The resultant PRP is introduced into the canal with cotton pliers or soaked on a collagen sponge and carried to the apical portion with a plugger. The access cavity is sealed afterwards and the patient is recalled

every three months for radiographic and clinical examination. The most obvious advantage of using PRP is the fact that the concentration of platelets in PRP is about 1 million per microliter which is much more than normal blood clot [60]. It also has a much higher concentration of growth factors than normal blood clot [61]. This technique like the blood clot technique also doesn't have any immunological, pathological ethical and legal issues involved as the patient's own blood is used.

II. Regenerative endodontic procedures in adults

There are numerous reports which state that the failure of root canal treatment is the result of improper cleaning and shaping and not improper obturation [62]. Conventional root canal therapy leads to the loss of reparative capacity, increased loss of tooth structure, brittleness [63] and the loss of a protective mechanism against noxious stimuli. A search for better methods to treat pulp diseases is being researched. 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. In a series of cases reported by Shah *et al.* [64] in which the patient had the initial diagnosis of acute or chronic periapical abscess with or without radiographic signs. A conventional access cavity was prepared and disinfection by the use of triple antibiotic paste was done after which "apical clearing" (increase of the size of apical third by 3 to 4 size larger than the master apical file) was done. Apical foramen was enlarged to 25 to 30 number file and bleeding was induced into the canal and calcium sulphate based cement was placed in the access cavity followed by a coronal restoration. Recall was done every 6 months. All the cases in the study showed good healing and were clinically and radiographically asymptomatic. The size of the apical foramen has been much stressed upon when attempting regeneration in permanent tooth with closed apex. In a study by Andreasen *et al.* [65] it was concluded that regeneration is unpredictable if the size of the apical foramen is less than 1mm. This result has been contradicted in a recent study in which in growth of new tissue upto two third of the pulp chamber were observed at 90 days of implantation with an apical diameter of 0.32mm [66]. Further research is needed in this

area to determine if the size of the apical foramen actually influences the outcome of regenerative endodontic procedures in teeth with closed apex. Regeneration in mature permanent tooth requires more research as it has a potential to replace conventional root canal therapy in the future provided a predictable, feasible and economical protocol can be developed for the same.

Future line of development for endodontic regeneration:

Post natal stem cell implantation

It has been proposed to use post natal stem cells from the patient themselves which have been derived from various sources of stem cells like skin fat or bone and injecting them into the cleaned and disinfected root canal. This approach will not generate any immunological reaction. Though it has been difficult to develop techniques to isolate, purify and multiply stem cells to a sufficient number which can be used for implantation. It has also been noted that these cells may have a low survival rate and they may migrate to different locations into the body if they are used without a scaffold^[67].

Pulp implantation

In this technique, stem cells, grown in layers in the laboratory over feeder cell layers which provide them nutrition, can be rolled to make them into a three dimensional structure and implanted in the pulp space. The disadvantage of this technique is that the sheets of stem cells over a layer of feeder cell layers are extremely fragile and can break while being transported into the canal. In a recent study, scaffold free dental pulp stem cells were prevascularized by human umbilical vein endothelial cells and were implanted into the canal space of human tooth slices following which they were further implanted subcutaneously into immune deficient rats. A histological examination and immunohistochemical staining four weeks later revealed the presence of well-organized human pulp tissue^[68].

Scaffold implantation

In this technique, the stem cells are seeded onto a porous scaffold^[69] which can facilitate their proliferation and differentiation. These scaffolds support the stem cells and also can be

laced with growth factors. Most of the scaffolds are highly porous and are made of polymers.

3 D Cell Printing

Theoretically a three dimensional printer can be used to recreate the precise shape and size of the pulp tissue in a particular tooth. The biggest advantage of this is the precise placement of cells which can mimic the pulp tissue^[70].

Gene therapy

Specific mineralizing genes can be transferred into the root canal with the help of a carrier (vector)^[71]. Except for the work of Rutherford^[72], very little research has been done on this technique of regeneration.

Injectable scaffolds

These are very similar to the scaffolds described earlier in their functions, except for the fact that they are made of a material which can be injected directly into the pulp space. Polymer hydrogels have been used as injectable scaffold^[73]. This technique is still in its early stage of development.

Conclusion

Regenerative endodontic procedures have proven to be an invaluable tool for teeth with an open apex and necrotic pulp in young patient. These procedures have proven to produce a favorable biological outcome as compared to conventional apexification techniques. Research needs to be done in many areas so as to make the regenerative procedures more predictable. The procedures themselves can be expected to undergo major changes in the near future. The time is not far when the dentists, in general, and endodontist, in particular may have to unlearn what has been taught in the dental schools and undergo a paradigm shift in thinking, learning and treating a patient who presents with pain of pulpal and/or periapical region.

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