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Regenerative endodontics: rising ray of dentistry- A review

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ABSTRACT

When pulp tissue becomes necrotic in immature teeth, the prognosis of the teeth is compromised. Disinfection of the root presents several challenges including difficulties in cleaning and shaping of large canals with open apices, obturation of canals with open apices, and potential root fractures caused by thin and/or weakened root walls. The regeneration of immature permanent teeth following trauma could be beneficial to reduce the risk of fracture and loss of millions of teeth each year. The purpose of this article is to review these biological procedures and the hurdles that must be overcome to develop regenerative endodontic procedures.

Keywords: Stem cells, Morphogenes, Scaffold, Revasculrization, Various methods of regeneration technique.

INTRODUCTION

The goal of modern restorative dentistry is to functionally and cosmetically restore the tooth structure. Till recently, a variety of synthetic materials were developed to restore the damaged tooth structure. Although these materials have proved to be effective, they do not exhibit the same mechanical and physical properties as naturally formed dentine and enamel [1].

The success of regenerative endodontic procedures (REPs) is undoubtedly related to the attainment of specific biological purposes [2]. Healing of apical periodontitis, thickening and/or lengthening of root walls, and, finally, regaining a positive response to pulp sensibility tests are strongly correlated with a successful outcome of regenerative endodontic therapy [3].

The objectives of regenerative endodontic procedures are to regenerate pulp-like tissue, ideally, the pulp-dentin complex; regenerate damaged coronal dentin, such as following a carious exposure, and regenerate resorbed root, cervical or apical dentin [4].

Regenerative Endodontic procedures can be defined as biologically based procedures designed to create and deliver tissues to replace diseased, missing and traumatized pulp-dentin complex. The

science of regenerative endodontics has a long history dating back to 1952 when Dr. BW Hermann reported on the application of calcium hydroxide in a case report of vital pulp amputation [5]. Tissue engineering can be defined as 'an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function [6].

Objectives of regenerative endodontics

The pulp-dentin complex regenerates the damaged coronal dentin, resorbed root, cervical or apical dentin [4]. Regenerative procedures are to be done with the use of tissue engineering materials, stem cells and suitable biochemical factors that will enhance or replace biological functions. Largely the objective of tissue engineering is the functional restoration of tissue structures. Clinical applications depends on the use of a potential material which would be anti-inflammatory, antibacterial and can simultaneously enhance the proliferation and induce the differentiation of the present dental Pulp Stem Cells (DPSC) into odontoblast-like cells leading to dentin formation. [7] Formation of a reparative dentin layer would provide an optimal barrier to avoid any bacteria infiltration to the pulp tissue, which is not provided by any artificial restorative materials. So application of a scaffold on an open pulp enabling odontoblast-like cells to grow into the scaffold and to convert it into dentin would be an ideal goal. [8] The ultimate objective of regenerative endodontics would be creation of a replacement pulpal tissue [9]

Components

The three key components for tissue engineering are: [10]

- Stem cells – to respond to growth factors.
- Scaffold of extracellular matrix (ECM).
- Growth factors (signals for morphogenesis)

Stem cells

General Characteristics

Stem cells are undifferentiated embryonic or adult cells that continuously divide. A fundamental property of stem cells is self-renewal or the ability to go through numerous cycles of cell division while maintaining the undifferentiated state. In addition, stem cells produce intermediate cell types (called progenitor or precursor cells) that have the capacity to differentiate into different cell types and generate complex tissues and organs. [11] Differentiation occurs when a stem cell acquires the features of a specialized cell (e.g. odontoblast).

Stem cells can be either embryonic or adult (postnatal). Thomson and colleagues [12] first reported human embryonic stem cell lines in 1998. Embryonic stem cells are isolated from the blastocyst during embryonic development and give rise to the 3 primary germ layers: ectoderm, endoderm, and mesoderm. These cells are totipotent or pluripotent with an unlimited capacity to differentiate and can develop into each of the more than 200 cell types of the adult body.

Adult stem cells exist throughout the body in different tissues, including bone marrow, brain, blood vessels, liver, skin, retina, pancreas, peripheral blood, muscle, adipose tissue, and dental tissues. They are localized to specific niches where the regulation of stem cell proliferation, survival, migration, fate, and aging occur [11, 13]. Whether cells undergo either prolonged self-renewal or differentiation depends on intrinsic signals modulated by extrinsic factors in the stem cell niche. [14]

An adult stem cell can divide and create another cell like itself, and also a cell more differentiated than itself, but the capacity for differentiation into other cell types is limited. This capability is described as being multipotent and is a distinguishing feature of adult stem cells compared with the pluripotency of embryonic stem cells [15, 16]

We prefer the term embryonic, rather than fetal, because the majority of these cells are embryonic. We also prefer the term postnatal, rather than adult, because these same cells are present in babies, infants, and children. The reason why it is important to distinguish between embryonic and postnatal stem cells is because these cells have a different potential for developing into various specialized cells (i.e. plasticity).[4] Although early research suggested that adult stem cells were limited in the types of tissues they produced, it is increasingly apparent that adult stem cells have greater plasticity than previously thought and can generate a tissue different to the site from which they were originally isolated [17,18] An example with potential clinical applications is the ability of dental pulp cells to generate heart tissue in rats. [19]

Fundamental properties of stem cells [20]

Undifferentiated cells: Have not developed into a specialized cell type
Long-term self-renewal: The ability to go through numerous cycles of cell division while maintaining the undifferentiated state
Production of progenitor cells: Capacity to differentiate into specialized cell types (e.g., odontoblast, osteoblast, adipocyte, fibroblast)

Stem cell potency [20]

Embryonic stem cells: from inner cell mass of 3-to5 day embryo (blastocyst)	Totipotent	Can give rise to all the cell types of the body, Including those cells making the extra embryonic tissues (e.g., placenta), Unlimited capacity to divide.
Embryonic stem cells Induced pluripotent stem cells	Pluripotent	Can form derivatives of all the embryonic germ layers (ectoderm, mesoderm, and endoderm) from a single cell
Adult stem cells (postnatal)	Multipotent	Can give rise to more than one cell type of the body
Induced pluripotent stem cells	Pluripotent	Pluripotent Derived from somatic cells

Stem cells are often categorized by their source [10]

- Autologous stem cells – are obtained from the same individual to whom they will be implanted.
- Allogenic stem cells – originate from a donor of the same species.
- Xenogenic cells – are those isolated from individuals of another species.

Various sources for postnatal dental stem cells have been successfully studied

- Permanent teeth – Dental pulp stem cells (DPSC): derived from third molar. [2]
- Deciduous teeth – Stem cells from human-exfoliated deciduous teeth (SHED): stem cells are present within the pulp tissue of deciduous teeth. [21]
- Periodontal ligament - Periodontal ligament stem cells (PDLSC). [22]
- Stem Cells from apical papilla (SCAP). [23]
- Stem cells from supernumerary tooth – Mesiodens. [24]
- Stem cells from teeth extracted for orthodontic purposes. [25]
- Dental follicle progenitor cells. [26]
- Stem cells from human natal dental pulp-(hNDP). [27]

DPSCs

DPSCs were first isolated from human permanent third molars in 2000. [28] The cells were characterized as clonogenic and highly proliferative. Colony formation frequency was high and produced densely calcified, albeit sporadic, nodules. [28] Dentin and pulp like tissues were generated following the transplantation of DPSCs in hydroxyapatite/ tricalcium phosphate (HA/TCP) scaffolds into immune deficient mice. [28,29]

DPSCs have also been shown to express the bacterial recognition toll-like receptors, TLR4 and TLR2, and vascular endothelial growth factor in response to lipopolysaccharide, a product of gram-negative bacteria. [30,31,32] When compared with normal pulps, DPSCs in inflamed pulp tissues have reduced dentinogenesis activity, [33] and an in vitro investigation has shown reduced dentinogenic potential of DPSCs exposed to a high bacterial load that can be recovered after the inhibition of the bacterial recognition toll-like receptor TLR2.[31] Taken together, these studies support the existence of interactions between DSCs and immune cells in pulps affected by dental caries, [34] a better

understanding of which has significant implications for the future management of teeth affected by dental caries.

Shed

SHED cells are highly proliferative stem cells isolated from exfoliated deciduous teeth capable of differentiating into a variety of cell types, including osteoblasts, neural cells, adipocytes, and odontoblasts, and inducing dentin and bone formation.[21] Like DPSCs, SHED cells can generate dentin-pulp like tissues with distinct odontoblast like cells lining the mineralized dentin-matrix generated in HA/TCP scaffolds implanted in immune deficient mice.[29] However, SHED cells have a higher proliferation rate than DPSCs and BMMSCs, suggesting that they represent a more immature population of multipotent stem cells. [21, 35, 36]

SHED cells have shown different gene expression profiles from DPSCs and BMMSCs; genes related to cell proliferation and extracellular matrix formation, such as transforming growth factor (TGF)-b, fibroblast growth factor (FGF)2, TGF-b2, collagen (Col) I, and Col III, are more highly expressed in SHED cells compared with DPSCs. [36]

SCAP Cells

SCAP cells are found in the apical papilla located at the apices of developing teeth at the junction of the apical papilla and dental pulp.[23,37,38] The apical papilla is essential for root development.

SCAP cells were first isolated in human root apical papilla collected from extracted human third molars.[37] The cells are clonogenic and can undergo odontoblastic/ osteogenic, adipogenic, or neurogenic differentiation. Compared with DPSCs, SCAP cells show higher proliferation rates and greater expression of CD24, which is lost as SCAP cells differentiate and increase alkaline phosphate expression. [37, 38, 39]

SCAP cells seeded onto synthetic scaffolds consisting of poly-D, L-lactide/glycolide inserted into tooth fragments, and transplanted into immunodeficient mice, induced a pulp like tissue with well-established vascularity, and a continuous layer of dentin like tissue was deposited onto the canal dentinal wall. [40]

PDLSCs

McCulloch [41] reported the presence of progenitor/stem cells in the periodontal ligament of mice in 1985. Subsequently, the isolation and identification of multipotent MSCs in human periodontal ligaments were first reported in 2004. [42]

PDLSCs have the capability to differentiate into cementoblast like cells, adipocytes, and fibroblasts that secrete collagen type I. [43] As with BMMSCs, PDLSCs can undergo osteogenic, adipogenic, and chondrogenic differentiation. [44]

PDLSCs have also been shown to differentiate into neuronal precursors. [45] A recent retrospective pilot study showed evidence of the therapeutic potential of autologous periodontal ligament progenitor cells obtained from third molar teeth implanted on bone grafting material into intrabony defects in 2 patients. [46] After 32 to 70 months, a marked improvement was found in all sites. The progenitor cells behaved like PDLSCs, although they did not express the same markers. [46]

DFPCs

The dental follicle forms at the cap stage by ectomesenchymal progenitor cells. It is a loose vascular connective tissue that contains the developing tooth germ, and progenitors for periodontal ligament cells, cementoblasts, and osteoblasts. [47]

DFPCs were first isolated from the dental follicle of human third molars

Induced Pluripotent Stem cell and Dental Pulp Pluripotent like Stem Cells

In breakthrough studies in 2006 and 2007, investigators described methods to reprogram somatic cells from mice, [48] and subsequently humans, [49, 50] by the insertion of 4 genes (OCT3/4, SOX2, KLF4, and MYC) that reprogrammed the somatic cells and returned them to an embryo like state. The resultant induced pluripotent stem (iPS) cells have embryonic stem cell characteristics: they are capable of generating cells from each of the 3 embryonic germ layers and can propagate in culture indefinitely.

Scaffold

These are three dimensional structures that provide physico-mechanical and biological

environment for cell growth and differentiation. Natural and Synthetic scaffolds can be used that have biocompatibility, non-toxicity and proper physical and mechanical strength. [51] Natural and synthetic scaffolds can be used. Natural polymers such as collagen and glycosaminoglycans are used and synthetic polymers like polylactic acid (PLA), poly glycolic acid (PGA), and their co polymers polylactic-co-glycolic acid (PLGA). Synthetic hydrogels include poly ethylene glycol

(PEG) based polymers, and those modified with cell surface adhesion peptides such as arginine glycine and aspartic acid that can improve cell adhesion and matrix synthesis with three dimensional network. [52]

Morphogenes

Stem cells require external stimuli to undergo differentiation. Morphogenes or the signaling molecules are protein in nature that binds to the specific cell membrane receptor and induce a cascade of processes that result in generation of new tissue. [52, 53] Morphogenes regulates the rate of tissue proliferation, cell differentiation into another cell type and matrix production. Growth factors play an important role in regenerative endodontics. Dentin contains many proteins capable of stimulating tissue responses. Demineralization of dentinal tissue can lead to release of growth factors following the application of cavity etching agents, restorative materials and even caries. List of growth factors are elaborated in table. [54]

Table- List of growth factors [55]

Abbreviation	Factor	Usefulness
BMP	Bone morphogenic protein	Differentiation of osteoblasts and odontoblasts, mineralization of bone and dentin
CSF	Colony stimulating factor	Increases stem cell number
EGF	Epidermal growth factor	Proliferation of mesenchymal, glial and epithelial cells
FGF	Fibroblast growth factor	Increases stem cell Number
IGF	Insulin like growth factor	Increases stem cell Number
IL	Interleukins	Promotes inflammatory cell activity
PDGF	Platelet derived growth factor	Proliferation of connective tissues, glial and smooth muscle cells
TGFa	Transforming growth factor Alpha	Epithelial and tissue structure development
TGFß	Transformation growth factor beta	Present in dentin matrix and promotes mineralization of pulp tissues
NGF	Nerve growth factor	Promotes neuron outgrowth and neural cell survivor

Regenerative Endodontics

The ultimate aim of tissue engineering in endodontics is regeneration of pulp. Various techniques adapted for regenerative endodontics adapted are

1. Root canal revascularization
2. Post natal stem cell therapy
3. Pulp implantation
4. Scaffold implantation
5. Injectable scaffolds delivery
6. Three dimensional cell printing
7. Gene therapy

Case reports in the early 2000s [56, 57] there have been several case studies showing the successful regeneration of tissue in the necrotic canal space of permanent teeth with immature apices.

These case studies have several commonalities including the following: [58-79]

1. Younger patients (6–18 years)
2. Permanent teeth with immature apices
3. Minimal to no canal instrumentation
4. Placement of an intracanal medicament
5. Placement of a bacteria-tight seal at the completion of the treatment

They varied in the following ways

1. Type and concentration of irrigants (1.25–5.25% NaOCl with or without use of Peridex or 3% hydrogen peroxide).
2. The type and concentration of the intracanal medicament (e.g., TAP vs double antibiotic paste vs calcium hydroxide paste)
3. Number of appointments and the length of time between appointments (none to 3 months)
4. Creation of a blood clot versus the use of another scaffold type (e.g., PRP)
5. Type of pulp space barrier
6. Final restoration

Root canal revascularization

Necrosis of immature tooth with open apex would render the tooth weak and prone to fracture. Ideal treatment would be to induce the root completion followed by restoration. Revascularization method assumes that once root canal space had been disinfected and the formation of blood dot is initiated by over instrumentation results in fibrin matrix formation that traps cells that are capable of initiating new tissue formation. [54]

In 2001, Iwaya et al described a procedure, which they termed revascularization that was undertaken on a necrotic immature mandibular second premolar with a chronic apical abscess. [56]

There have been several terms used to describe the introduction of new living tissue into the canal space. These include regeneration, revascularization, and revitalization. There has been debate as to which of these 3 terms (i.e.revascularization, revitalization, or regeneration) is most appropriate to describe the outcome of procedures used to regenerate pulp tissue. [80-82] The term revascularization describes the re-establishment of the vascular supply to existing pulp in immature permanent teeth. [83] Revitalization describes the ingrowth of tissue that may not resemble the original lost tissue. [84]

Endodontic regeneration is the replacement of ‘‘damaged structures, including dentin and root structures, as well as cells of the pulp-dentin complex’’.4 Subsequent Banchs and Trope [85] described a revascularization procedure for the treatment of a necrotic immature mandibular second premolar with an open apex and a large apical lesion. They stated that many thought that regeneration of pulp tissue in a necrotic infected tooth with apical periodontitis was impossible. Nonetheless, because it had been radio graphically proven that regeneration was possible in a re-implanted tooth, the same could be accomplished in an infected tooth if a favorable environment was established.

Jeeruphan et al [79] evaluated radiographic and clinical outcomes of 61 immature teeth treated with Ca (OH) 2 apexification (n = 22), MTA apexification (n = 19), or revascularization procedure (n = 20) and found that the percentage changes in root width and length were significantly greater in the revascularization group (28.2% and 14.9%) when compared with the MTA apexification group (0.0% and 6.1%) and Ca (OH)2 apexification group (1.5% and 0.4%). Moreover, the survival rate of teeth in the revascularization group (100%) and MTA apexification group (95.5%) was greater than the survival rate observed in the Ca(OH)2 group (77.2%). They concluded that revascularization protocols offered a favorable outcome for resolving the infection and promoting root development in the management of infected immature permanent teeth.

Guidelines for revascularization that have been recommended for the treatment of infected immature permanent teeth, with or without apical pathosis[87]

Appointment 1

Immature permanent teeth with necrotic pulp, with or without apical pathosis, and an incomplete developed root with an apical opening that measures 1 mm or larger are considered suitable candidates for treatment, providing the crown, when damaged, is restorable. [87] An informed consent must be signed by the patients’ parents/guardians, who must be informed that this is a relatively new procedure with no standardized guidelines.

After obtaining consent, the tooth should be anesthetized, a rubber dam placed, the tooth and

working field disinfected, and straight line access made to allow the necrotic tissue in the pulp chamber to be removed after initial irrigation of the root canal treatment. Mechanical cleaning is contraindicated because it may weaken the thin dentinal root walls [88], as well as remove vital tissue remnants that might be present in the apical part of the canal [89]. A K-file, or alternatively a gutta-percha cone, should be introduced into the canal to establish a working length [67, 73]. Removal of necrotic tissue from the root canal is accomplished by gently irrigating the root canal with a minimum of 20 mL 2.5% NaOCl dispensed through a syringe and a 20-gauge needle. [57,58,62,66,85,89,90,91] Although higher concentrations are potentially toxic to periapical tissue, Trevino et al [92] found that the survival rate of human stem cells of the apical papilla (SCAP) exposed to 6% NaOCl, followed by 17% EDTA and then 6% NaOCl again, was 74%. Concentrations of NaOCl ranging from 1.25%–6% have also been used and have reportedly yielded favorable results. [62, 66, 90, 91]

Root Canal Medication

After the root canal has been irrigated, it should be carefully dried with large, sterile paper points. The root canal can then be medicated with 1 of 2 dressings.

Antibiotic Combination: An intracanal antibiotic dressing can be placed into the root canal to a depth 2 mm short of the root apex and to allow room for reestablishment of a new vasculature and formation of new hard tissue on the root canal walls.[93] Hoshino et al [94] introduced a triple antibiotic combination of ciprofloxacin, metronidazole, and minocycline that they claimed was sufficiently potent to eradicate bacteria from the dentin of the infected root and promote healing of the apical tissues. The medicament is made by mixing equal doses of the 3 antibiotics with sterile saline to a paste-like consistency. [66, 95] Reynolds et al [66] used a mixture of 250 mg each of ciprofloxacin, metronidazole, and minocycline with sterile water and confirmed by x-ray.

Temporary Restoration

Preventing coronal leakage of bacteria into the cleaned and medicated root canal is a primary prerequisite for successful revascularization. It is for this reason that a double coronal restoration is recommended. [86]

Appointment #2

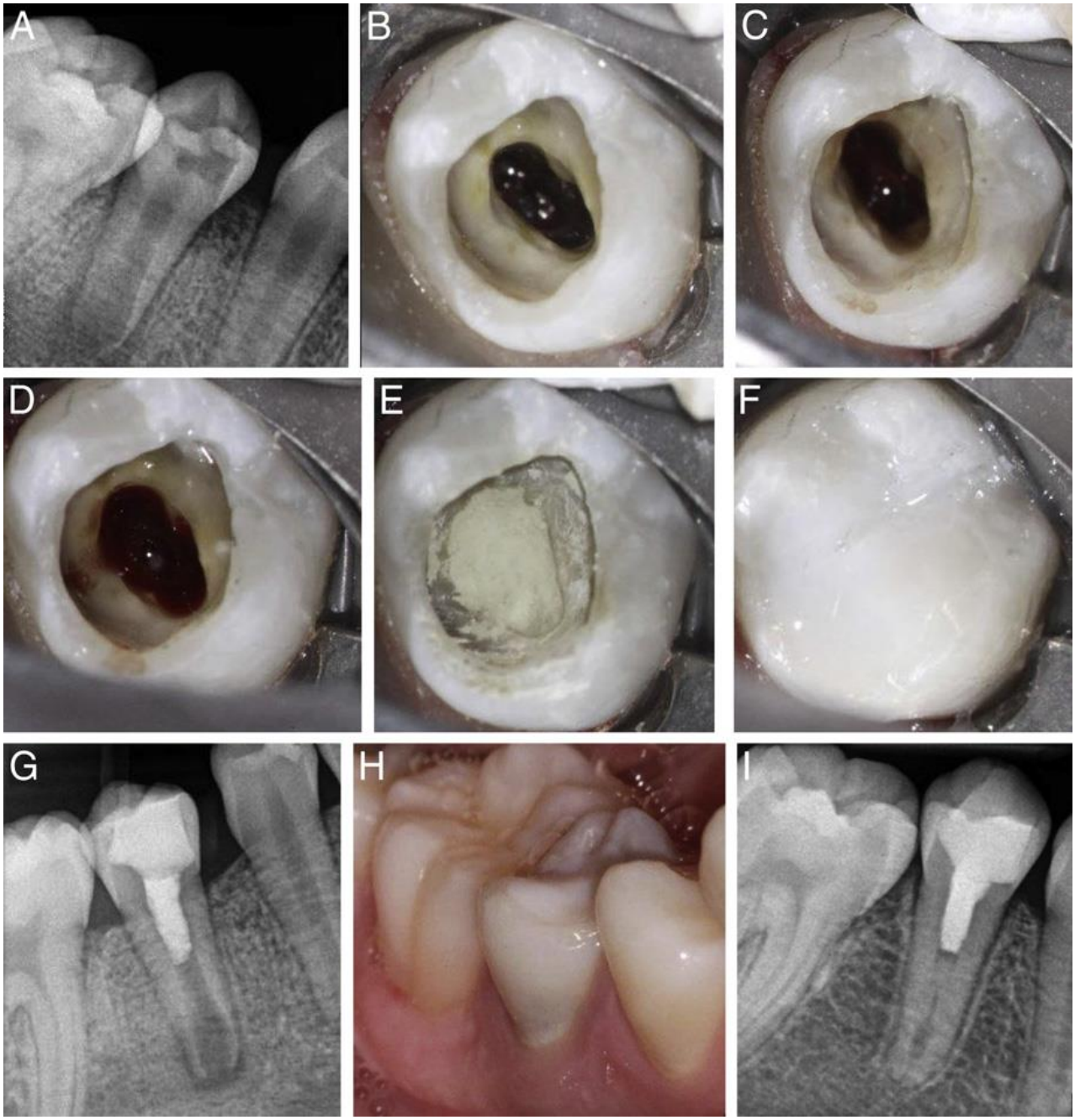
Before proceeding with the next phase of treatment, it is important to ensure that all clinical signs and symptoms have abated. If clinical signs or symptoms persist, the procedures performed in the first appointment should be repeated. If they continue to persist over several appointments, an apexification procedure should be considered. [96] When proceeding with the second appointment, the tooth should be anesthetized before the rubber dam is placed. An anesthetic without vasoconstrictor should be chosen to prevent constriction of the blood vessels in the apical region or a limited flow of blood when bleeding is mechanically induced. [97]

After careful removal of the temporary restoration the medicament should be removed by gently irrigating root canal by using a minimum of 20 mL 2.5% NaOCl. The irrigation should be repeated until no medicament is evident in the canal. The use of EDTA as a final rinse was promoted by Yamauchi et al, [98] who concluded after their animal study that EDTA had no negative effect and helped in the formation of a calcified tissue that led to strengthening of the root walls. The suggested protocol begins with the introduction of a sterile #20 K-file into the apical tissues 2 mm past the apical foramen to initiate bleeding into the root canal. [57, 62,66,75,85] bleeding should be controlled so that it does not extend beyond a point approximately 3 mm apical to the CEJ. This is done by applying intracanal pressure with a sterile saline soaked cotton pellet until a clot is formed.

Estimated mean time for the establishment of a stable blood clot is 15 minutes. [57,62,66,85] The clot can be carefully touched with the reverse end of a large sterile paper point to confirm its stability. Once stability is confirmed, the clot should be carefully covered with MTA cement that is back-filled to the level of the CEJ. After its initial set, a wet cotton pellet should be placed over the MTA and the access opening sealed with a temporary restoration.

The third appointment is principally scheduled to remove the cotton pellet, confirm the set of the MTA, and place a permanent restoration into the access opening. Different clinicians have advised

different follow-up periods in their case reports, with some lasting as long as 5 years post treatment. In the majority of the cases, improvement or resolution of the apical lesion can be expected in approximately 6 months and root elongation and apical closure, with thickening of the root canal walls, within 12–24 months postoperatively. [57,58,62,66,89,96] Most clinicians suggest that during the first year, 3-month recalls should be scheduled, followed by 6-month recalls unless clinical symptoms develop.



Images from regenerative endodontic procedures (REPs). (A) Periapical radiograph of mandibular right second premolar before the initiation of treatment showing occlusal caries and an immature apex. (B) Clinical photograph from the second appointment showing a bluish color of TAP in the canal immediately after the removal of temporary, (C) vital tissue in the apical third of the canal evident after irrigation to remove TAP, (D) the formation of blood clot, (E) the placement of

white MTA, and (F) composite restoration. (G) A periapical radiograph made immediately after the placement of final restoration showing MTA Covering pulp space and composite restoration. (H) A photograph at the 14-month follow-up showing grayish discoloration in the cervical third of the crown. (I) a periapical radiograph made at the 14-month follow-up showing increased root wall thickness.

POST NATAL STEM CELL THEARPY

It involves injection of post natal stem cells into root canal. The best approach would be to use cells from autologous (patient's own) cells that has been taken from buccal mucosa or umbilical cord .[54] Advantages includes relative ease of harvesting and delivering with a syringe and the cells have potential to induce new pulp regeneration. Disadvantages of this technique includes low survival rates of cells and secondly the cells might migrate to different areas of body possibly leading to aberrant pattern of mineralization. [55]

Pulp Implantation

The majority of in vitro cell cultures grow as a single monolayer attached to the base of culture flasks. However, some stem cells do not survive unless they are grown on top of a layer of feeder cells. [99] In all of these cases, the stem cells are grown in two dimensions. In theory, to take two-dimensional cell cultures and make them three-dimensional, the pulp cells can be grown on biodegradable membrane filters. Many filters will be required to be rolled together to form a three dimensional pulp tissue, which can be implanted into disinfected root canal systems. The advantages of this delivery system are that the cells are relatively easy to grow on filters in the laboratory.

Moreover, aggregated sheets of cells are more stable than dissociated cells administered by injection into empty root canal systems. The potential problems associated with the implantation of sheets of cultured pulp tissue is that specialized procedures may be required to ensure that the cells properly adhere to root canal walls. Sheets of cells lack vascularity, so only the apical portion of the canal systems would receive these cellular constructs, with coronal canal systems filled with scaffolds capable of supporting cellular proliferation. [100] Because the filters are very thin layers of cells, they are extremely fragile, and this could make them difficult to place in root canal systems without breakage.

In pulp implantation, replacement pulp tissue is transplanted into cleaned and shaped root canal systems. The source of pulp tissue may be a purified pulp stem cell line that is disease or pathogen-free, or is created from cells taken from a biopsy, that has been grown in the laboratory. The cultured pulp tissue is grown in sheets in vitro on biodegradable polymer nano fibers or on sheets of extracellular matrix proteins such as collagen I or fibronectin . [101,102]

Scaffold Implantation

To create a more practical endodontic tissue engineering therapy, pulp stem cells must be organized into a three-dimensional structure that can support cell organization and vascularization. This can be accomplished using a porous polymer scaffold seeded with pulp stem cells. [103] A scaffold should contain growth factors to aid stem cell proliferation and differentiation, leading to improved and faster tissue development. [104] The scaffold may also contain nutrients promoting cell survival and growth, [105] and possibly antibiotics to prevent any bacterial in-growth in the canal systems. Dentin chips may provide a matrix for pulp stem cell attachment [106] and also be a reservoir of growth factors. [107] The natural reparative activity of pulp stem cells in response to dentin chips provides some support for the use of scaffolds to regenerate the pulp dentin complex.

To achieve the goal of pulp tissue reconstruction, scaffolds must meet some specific requirements. Biodegradability is essential, since scaffolds need to be absorbed by the surrounding tissues without the necessity of surgical removal. [108] A high porosity and an adequate pore size are necessary to facilitate cell seeding and diffusion throughout the whole structure of both cells and nutrients. The rate at which degradation occurs has to coincide as much as possible with the rate of tissue formation; this means that while cells are fabricating their own natural matrix structure around themselves, the scaffold is able to provide structural integrity within the body, and it will eventually break down, leaving the newly formed tissue that will take over the mechanical load.

Injectable scaffold delivery

Root canal have varied three dimensional anatomy and a rigid scaffold may not occupy all the canal space. So cells are injected with liquid scaffold which enables them to reach all the area of canal anatomy. An example of injectable scaffold is hydrogel. Hydrogels have the potential to be noninvasive and easy to deliver into root canal systems. In theory, the hydrogel may promote pulp regeneration by providing a substrate for cell proliferation and differentiation into an organized tissue structure. [110]

Three dimensional cell printing

The three-dimensional cell printing technique can be used to precisely position cells, [111] and this method has the potential to create tissue constructs that mimic the natural tooth pulp tissue structure. The ideal positioning of cells in a tissue engineering construct would include placing odontoblastoid cells around the periphery to maintain and repair dentin, with fibroblasts in the pulp core supporting a network of vascular and nerve cells.

Gene Therapy

Gene therapy includes modification or alteration of gene to regulate the cellular processes and responses and to utilize it for therapeutic benefit. Vector is used for this alteration which can be viral or non-viral. Viral vectors are modified to avoid the possibility of causing disease, but still retain the capacity for infection. Several viruses have been genetically modified to deliver genes, including retroviruses, adenovirus, adeno associated virus, herpes simplex virus, and lentivirus. [112,113] Non-viral gene delivery systems include plasmids, peptides, gene guns, DNA-ligand complexes, electroporation, sonoporation, and cationic liposomes. [114,115] The choice of gene delivery system depends on the accessibility and physiological characteristics of the target cell population.

SUMMARY

Although many of the above mentioned pulpal regenerative procedures are in initial stages of research and some are only hypothetical. Pulpal revascularization procedures have shown promising results clinically. A lot of research needs to be done for incorporation of growth factors in capping agents, and introduction of newer materials which would exploit endogenous growth factors.

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