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RESEARCH ARTICLE

REGENERATIVE ENDODONTICS

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Abstract

Regenerative endodontics has been defined as "biologically based procedures designed to replace damaged structures, including dentin and root structures, as well as cells of the pulp-dentin complex". Presently, two concepts exist in regenerative endodontics to treat non-vital infected teeth - one is the active pursuit of pulp-dentine regeneration to implant or regrow pulp (tissue engineering technology), and the other in which new living tissue is expected to form from the tissue present in the teeth itself, allowing continued root development(revascularisation). Regenerative endodontic procedures (REPs) have evolved in the past decade, being incorporated into endodontic practice and becoming a viable treatment alternative for immature teeth. The authors have summarized the status of regenerative endodontics on the basis of the available published studies and provide insight into the different levels of clinical outcomes expected from these procedures.

Introduction:-

Immature teeth with pulp necrosis and apical periodontitis are common dental diseases as a consequence of caries and trauma, and it's treatment has always been a challenge in endodontics, as due to open apex any infections because of trauma, anatomic anomalies or caries makes it difficult to manage. Though, traditional endodontic therapies for immature teeth, such as apexification procedures, promote resolution of the disease and prevent future infections. However, these procedures fail to promote continued root development, leaving teeth susceptible to fractures. To overcome these drawbacks, a biological approach in the form of Regenerative endodontics has been introduced, which is an exciting and developing field in the treatment of immature teeth with infected root canals that has been described as a "paradigm shift" in the management of these teeth and can result in continued root maturation and apical closure.[1]

Regenerative endodontics has been defined as "biologically based procedures designed to replace damaged structures, including dentin and root structures, as well as cells of the pulp-dentin complex". Nygaard-Östby pioneered the use of Regenerative endodontic therapies in necrotic teeth and investigated the potential for repair when bleeding was induced by over-instrumentation beyond the apex prior to partial root filling of the canal in the year 1961 but with limited success. Forty years later, in 2001 Iwaya et al. reported a case using a procedure termed

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'revascularization', on an infected necrotic immature premolar tooth that showed continued root maturation and thickening of root canal walls with mineralized tissue.[2]

Presently, two concepts exist in regenerative endodontics to treat non-vital infected teeth - one is the active pursuit of pulp-dentine regeneration to implant or regrow pulp (tissue engineering technology), and the other in which new living tissue is expected to form from the tissue present in the teeth itself, allowing continued root development (revascularization).[3]

The greatest benefit of these biological approaches for dental tissue restoration over many conventional dental materials is that the reparative matrices become an integral part of the tooth, overcoming any of the problems of retention of a restoration and possible marginal bacterial microleakage. This treatment approach strengthens the root walls of immature teeth.[3]

Future regenerative endodontics may involve the cleaning and shaping of root canals followed by the implantation of vital dental pulp tissue constructs created in laboratory. The success of regenerative endodontic therapy is dependent on the ability of researchers to create a technique that will allow clinicians to create a functional pulp tissue within cleaned and shaped root canal systems. The source of pulp tissue may be from root canal revascularization, stem-cell therapy and pulp implantation.[3] Thus, keeping the above mentioned in mind, it is our humble effort to explain and understand more about regenerative endodontics.

History

Treatment of necrotic immature teeth has always been considered a challenge in endodontics. Calcium hydroxide apexification and Mineral Trioxide Aggregate (MTA) apexification were classical treatments for necrotic immature permanent teeth but the first tend to fail for lack of compliance given the high number of sessions needed; the second has technical difficulties such as material manipulation and overfilling. With both techniques, the root development is interrupted leaving the tooth with a fragile root structure, a poor crown-to-root ratio, periodontal breakdown, and high risk of fracture, compromising long-term prognosis of the tooth. Therefore, a new scientific approach known as Regenerative Endodontics has been proposed that allows complete root development and had shown to induce root extension and radicular reinforcement.

Pioneering work supporting the concept of regenerating dental tissues was reported more than 50 years ago when **Dr.B.W. Hermann**[3] described the application of calcium hydroxide (Ca[OH]2) for vital pulp therapy and Regeneration of pulp that was key to regenerative endodontics procedures was conceptualized by **Professor Nygaard Østby** in 1961, in which he studied the role of blood clot in endodontic therapy by experimenting in dogs and human beings. It was observed that the blood clot in the root canal was organized probably by granulation tissue growing in from the periapical area and not from the blood cells originally contained in the clot. One setback of this experiment is that, the root canal walls showed signs of resorption during the organization of the clot.[5]

In 1966, **Rule** and **Winter** documented root development and apical barrier formation in cases of pulpal necrosis in children. Subsequently, in 1971 **Nygaard – Østby** and **Hjortdal** attempted revascularization of pulp space in a necrotic ,infected tooth with apical peridontitis. However, in teeth with necrotic pulp no repair tissue was formed in the apical canal space however, it was not successful due to limitations in technologies, material, and instruments available in those times. In 1996, **Hoshino et al.** reported that complete disinfection in the root canal space can be achieved using a combination of three antibiotics (ciprofloxacin, metronidazole, and minocycline).

In 2001, **Iwaya et al.** reported a procedure termed revascularization of an immature permanent tooth with apical periodontitis and sinus tract. Their treatment resulted in elimination of clinical symptom/sign and apical periodontitis as well as promoting thickening of the canal walls and apical closure of immature permanent tooth.

In 2004, **Banchs** and **Trope** proposed a clinical protocol for revascularization of infected immature teeth. They used the root canal disinfection (Hoshino et al. 1996), and induction of blood clot in the canal space (Nygaard -Ostby & Hjortdal 1971) and they added the antibiotic minocycline to that used by Iwaya et al. (2001) and since then, it has been known as triple antibiotic paste. Furthermore, mineral trioxide aggregate (MTA) was used as an intracanal barrier instead of glass ionomer cement. Their treatment also showed elimination of clinical symptom/sign and apical periodontitis in addition to promoting thickening of the canal walls and apical closure of immature permanent teeth with apical periodontitis. Therefore, regenerative endodontics was recommended consequently as a treatment

alternative when compared to traditional apexification for immature permanent teeth with necrotic pulp. These two scientists can be credited for sparking interest in regenerative endodontics.[5]

Definitions

Regenerative Endodontics:

The American Association of Endodontists' Glossary of Endodontic Terms (2012) defines regenerative endodontics as "biologically based procedures designed to physiologically replace damaged tooth structures, including dentin and root structures, as well as cells of the pulp-dentin complex."[1]

Tissue engineering:

It 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.'[3]

Revascularization:

It may be defined as the invagination of undifferentiated periodontal cells from the apical region in immature teeth.[6]

Revitalization:

An ingrowth of tissue that may not be the same as the original lost tissue.[7]

Regenerative Procedural Approach In Endodontics

The concept of the endodontic therapy has started with the idea of retrieving the vitality of dental pulp as a potential treatment option, as it focuses on substituting injured and diseased pulp with functional pulp tissue with the use of biologically based procedures which restores the normal function of pulp-dentin structure. Though the outcomes are difficult to predict, deposition of hard tissue and continued root development are expected to occur under ideal conditions after this treatment. The following are the various procedural approach of regenerative endodontics:[8]

1. Root canal revascularization via blood clotting
2. Pulp Regeneration
3. Scaffold Implantation
4. Injectable Scaffold Delivery
5. Gene therapy

Root Canal Revascularization Via Blood Clotting:

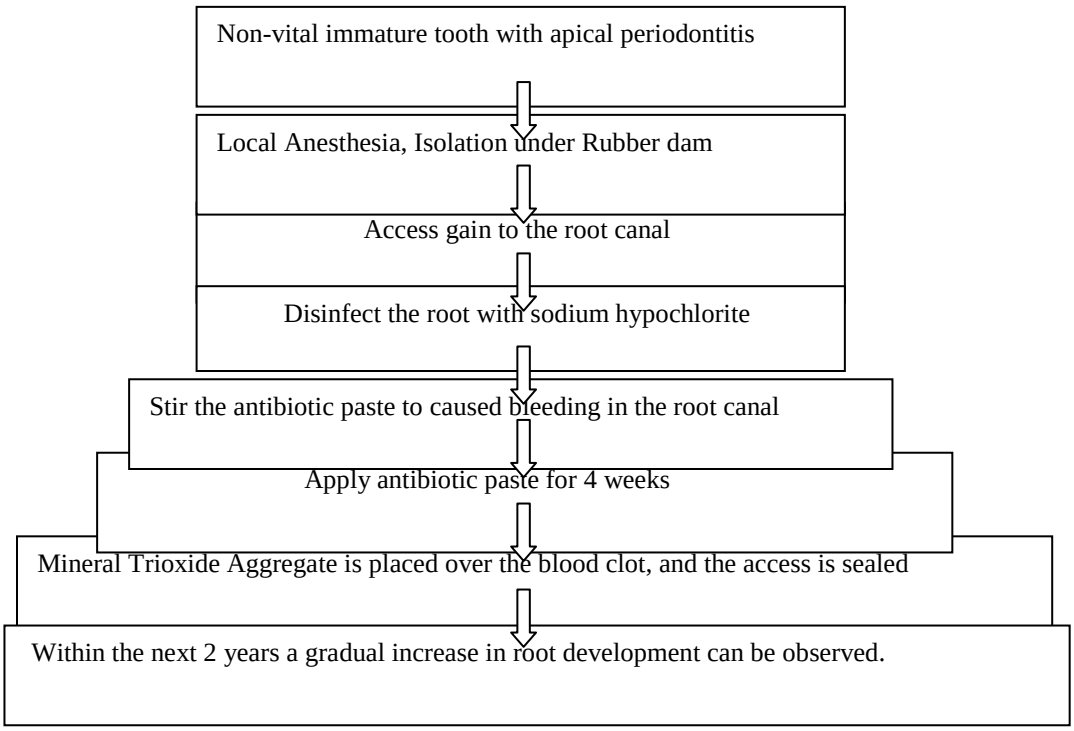
Revascularization is a surgical procedure for the provision of a new, additional, or augmented blood supply to a body part or organ. The term derives its name from the prefix 'Re' which in this case means restoration and vasculature, which refers to the circulatory structures of an organ.[9]Iwaya and associates were the first to coin the term "revascularization" in their endodontic treatment of an immature permanent tooth with apical periodontitis and a sinus tract.[8]The concept of revascularization was introduced by ostby in 1961 and in 1966, Rule and Winter documented root development and apical barrier formation in cases of pulpal necrosis on children. In 1971, Nygaard-Ostby and Hjortdal evaluated the role of the blood clotting in healing, in which their finding suggested that the blood clot seemed to be essential for the connective tissue healing at the apex of an immature tooth but the histologic analysis was unable to confirm the regeneration of the pulp-dentin complex. In 2001, Iwaya and collegues presented a 13-year old girl with a diagnosis of necrosis and chronic apical abscess on tooth 29 that showed the revascularization potential of the immature permanent tooth

Indications

1. Infected non-vital immature teeth
2. Teeth with necrotic pulp and immature apex
3. Pulp space not needed for post/core, final restoration
4. Patient compliance
5. No allergy to the medicaments to be used.[7]

Contraindication

1. In case post and core are the final restorative treatment plan
2. Deciduous (baby teeth) as it risks retaining teeth, which could impair the eruption pattern of adult teeth.
3. Patients who are allergic to medicaments and antibiotics to complete the procedure.[4]



Advantages:

1. It strengthens the root walls of immature teeth.
2. Continued root development (root lengthening) and strengthening due to reinforcement of lateral dentinal walls with deposition of new dentin/hard tissue.
3. Obturation of the canal is not required unlike in calcium hydroxide-induced apexification.
4. After control of infection, the procedure can be completed in a single visit.
5. Cost effective.
6. The regeneration of tissue in root canal systems avoids the possibility of immune rejection and pathogen transmission from replacing the pulp with a tissue engineered construct by a patient's own blood cells. [10]

Disadvantage

1. Discoloration due to minocycline if used in triple antibiotic paste
2. Prolonged treatment period and more appointments (compared with one-visit MTA apical barrier technique).[11]

Pulp Regeneration

Tissue regeneration in dental pulp entails resolution of chronic inflammation and restoration of damaged dento-alveolar tissues, including the organized pulp-dentin complex. The underlying rationale for endodontic regeneration is reinstitution of normal physiologic function in an otherwise necrotic pulp, including the protective mechanisms—for example, innate pulp immunity, pulp repair through tertiary dentin mineralization, and sensation of occlusal pressure and pain. Therefore, restoration of these pulpal functions will enhance long-term survival of teeth and help patients to retain their natural dentition. Hence, it is vital to find out a novel regenerative approach that will restore not only pulp vitality but organized pulp-dentin structure with the full spectrum of normal physiologic functions. In general, tissue engineering encompasses 3 requirements— namely, scaffold, growth differentiation signals, and stem cells (Alsberg et al. 2001)[12]

Definitions

1. Pulp regeneration means transplanting exogenous stem cells into the root canal system of the host to allow regeneration to take place. (Huang et al. 2013)[13]
2. Stem cells also known as "progenitor or precursor" cells are defined as clonogenic cells capable of both self-renewal and multi-lineage differentiation and are responsible for normal tissue renewal, healing and regeneration after injuries (van der Kooy and Weiss, 2000).[14]

Requisite preconditions for pulp regeneration

Two preconditions are requisite to achieve pulp regeneration:[15]

(i) Efficient root canal disinfection and (Figure 6)

(ii) Proper size of the apical foramen.

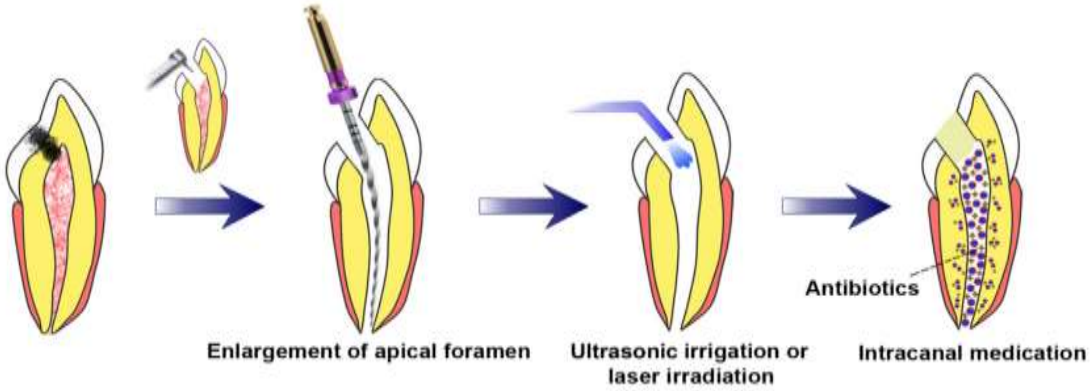


Figure 6:- Requisite preconditions for pulp regeneration.

Essential features of the regenerated pulp tissue

The regenerated tissues should be connective tissues that:

1. Produce new dentin with a controlled rate similar to the normal pulp,
2. Exhibit similar cell density and architecture to the natural pulp,
3. Vascularized, and
4. Are innervated (Fawzy El-Sayed et al., 2015).[15]

Dental stem cells

Stem cells also known as "progenitor or precursor" cells are defined as clonogenic cells capable of both self-renewal and multi-lineage differentiation. They are divided in two groups: 1) the embryonic stem cells and 2) the adult stem cells. The latter are located in human tissues such as bone marrow, skin, adipose tissue and dental pulp. Dental stem cells are of mesenchymal in origin and are non hematopoietic, multipotent cells that can proliferate and differentiate into a range of cell types comprising various tissues.[14]

Various mesenchymal stem cell populations exist in the tooth. According to their position in the tooth they can be grouped as (Figure 7):

1. Dental pulp stem cells (DPSCs.)
2. Stem cells from human exfoliated deciduous teeth (SHED.)
3. Stem cells from apical papilla (SCAP)
4. Periodontal ligament stem cells (PDLSCs)

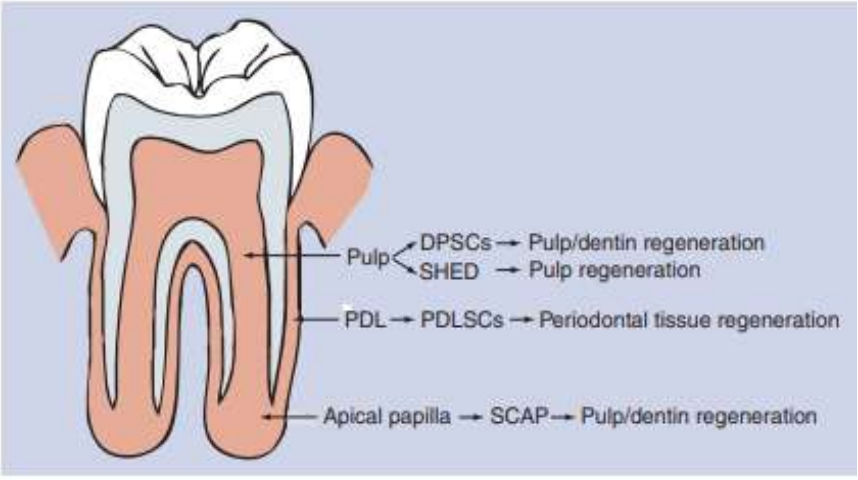


Figure 7:- Schematic image of Source of dental stem cells.

Dental pulp stem cells

They are mesenchymal type of stem cells present inside dental pulp which have osteogenic and chondrogenic potential in vitro and can differentiate into dentin, in vivo and also differentiate into dentin-pulp-like complex.

Dental pulp stem cells are putative candidate for dental tissue engineering due to:

1. Easy surgical access to the collection site and very low morbidity after extraction of the dental pulp.
2. Ability to generate much more typical dentin tissues within a short period than nondental stem cells.
3. Can be safely cryopreserved and recombined with many scaffolds.
4. Possess immuno-privilege and anti-inflammatory abilities favorable for the allotransplantation experiments.[14]

Identification of dental pulp stem cells

Four commonly used stem cell identification techniques are:[14]

1. Fluorescent antibody cell sorting: Stem cells can be identified and isolated from mixed cell populations by staining the cells with specific antibody markers and using a flow cytometer.
2. Immunomagnetic bead selection.
3. Immunohistochemical staining.
4. Physiological and histological criteria, including phenotype, proliferation, chemotaxis, mineralizing activity and differentiation.

Stem cells from human exfoliated deciduous teeth (SHED)

Stem cells from human exfoliated deciduous teeth (SHED) are mesenchymal cells, originating from the neural crest, which reside within exfoliated deciduous tooth pulp tissue. These cells have a high growth potential and can differentiate into osteoblasts, adipocytes, and neuron cells. They are isolated based on their high proliferation capacity and their mesenchymal surface markers.[16]

Dr. Songtao Shi discovered Stem cells from human exfoliated deciduous teeth in 2003. Miura et al. confirmed that Stem cells from human exfoliated deciduous teeth were able to differentiate into a variety of cell types to a greater extent than DPSCs, including osteoblast-like, odontoblast-like cells, adipocytes, and neural cells. Abbas et al. investigated the possible neural crest origin of SHED. The main task of these cells seems to be the formation of mineralized tissue, which can be used to enhance orofacial bone regeneration.[17]

General characteristics of Stem cells from human exfoliated deciduous teeth includes:

1. The ability to differentiate into odontoblasts in vivo
2. Postnatal stem cells with the capabilities of extensive proliferation and multipotential differentiation
3. The ability to develop into other cell lineages such as neural cells and adipocytes, to induce bone formation during the eruption of permanent teeth
4. It is an ideal resource for repairing damaged tooth structures, inducing bone regeneration, and possibly treating neural tissue injury or degenerative diseases.

Methods of Harvesting, Isolation and Culture

The surgical procedure of deciduous teeth extraction aged 7–9 years (shedding period), is done under standard conditions and local anesthesia after treatment with a disinfection solution (Figure 9). Posteriorly, the surgeon uses an excavator to release the dental pulp from the pulp chamber, after wide access opening [Suchánek et al., 2010].[17]

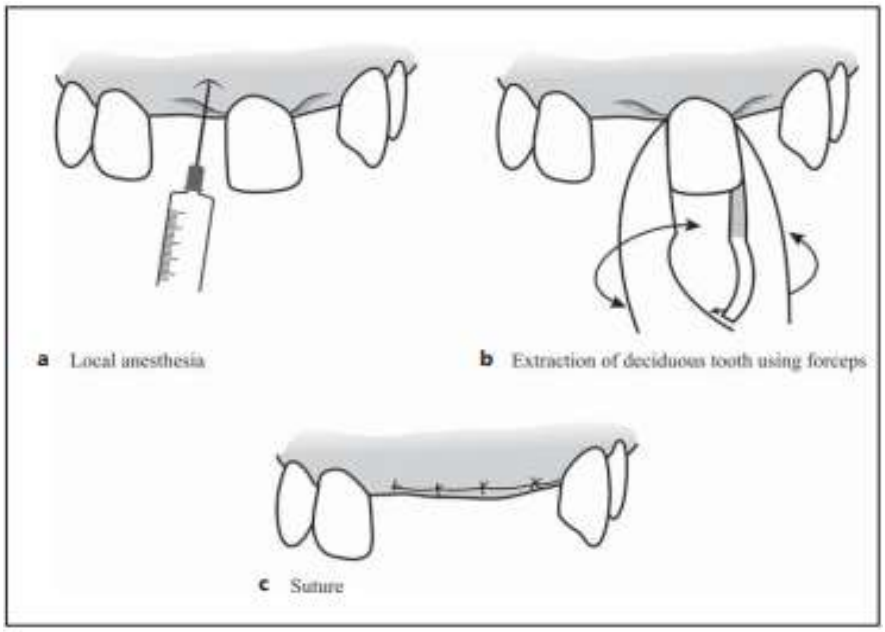


Figure 9:- Surgical procedure of deciduous teeth extraction.

For SHED isolation, the dental pulp is then enzymatically treated with collagenase type I (3 mg/ml) and dispase (4 mg/ml) for 1 h at 37°C to completely digest the pulp tissue. Following centrifugation, the supernatant is aspirated and the single-cell suspensions are seeded in a Petri dish and cultivated in basal culture medium(Figure 10).

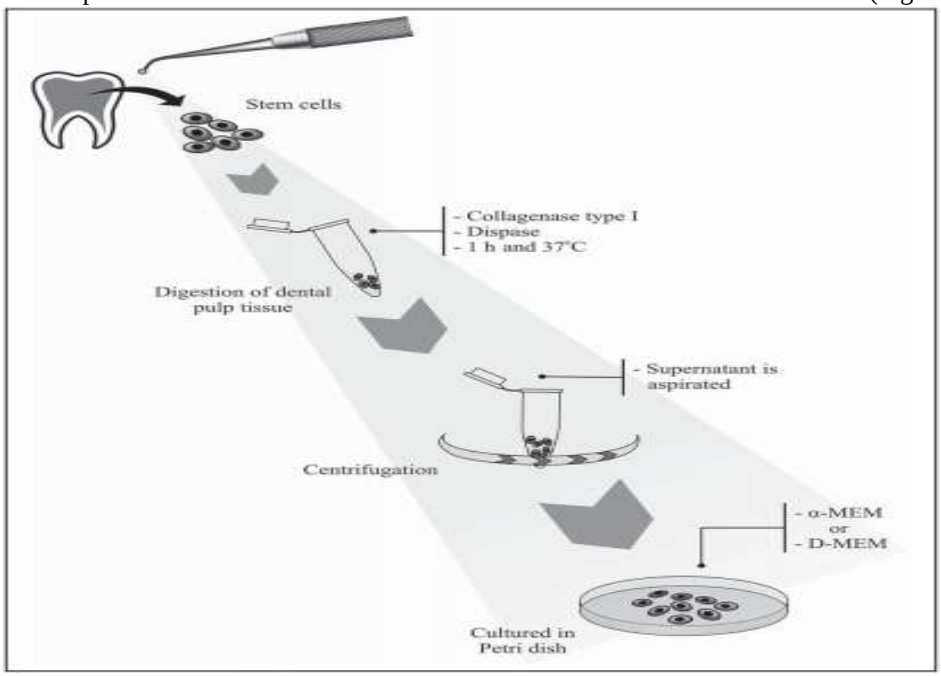


Figure 10:- Method of harvest, isolation and culture of SHED.
D-MEM = Dulbecco's modified Eagle's medium; α-MEM = α-minimum essential medium.

Stem cells from apical papilla (SCAP)

Mesenchymal Stem Cells residing in the apical papilla of permanent teeth with immature roots are known as Stem cells from apical papilla. They are biological cells that can self-renew and can diferentiate into multiple cell lineages and are residing in the apical papilla of immature permanent teeth represent a novel population of dental Mesenchymal Stem Cells that possesses the properties of high proliferative potential, the self-renewal ability, and immunogenicity.

It was discovered by Sonoyama et al. in 2006 from the dental papilla of wisdom teeth or incisors of 4 month old mini-pigs. The dental papilla is an embryonic-like tissue that becomes the dental pulp during maturation and formation of the crown. Therefore, SCAPs can only be isolated at a certain stage of tooth development.

Isolation of SCAPs

Currently, there are two common approaches to isolate and culture SCAPs.[81]

1. The first method is enzyme digestion. The apical papilla tissue is separated from the tip of the root, minced into pieces, and then digested in a solution of collagenase type I and dispase with gentle agitation. Afer digestion, tissue clumps are collected and passed through a cell strainer to obtain single cell suspension of SCAPs, which is then seeded in culture dishes.
2. Another method is explant culture, in which the apical papilla tissue is cut into samples about 1 mm^3 in size and then plated on culture dishes

Therapeutic Potential of SCAPs

SCAPs have the ability to diferentiate into various cell types and possess low immunogenicity, which could contribute to the regeneration and repair of tissues. Hence they can be considered as an attractive alternative cell source for stem cell-based therapy.[18]

Potential of SCAPs for Pulp-Dentin Regeneration

SCAPs are characterized by a high proliferation rate and odontogenic diferentiation potential, which makes them suitable for stem cell-based regeneration and producing dentin-pulp complex.

After transplantation of SCAPs combined with hydroxyapatite/tricalcium phosphate (HA/TCP) scafolds into immunocompromised mice, a layer of dentin tissue is generated on the surface of the HA/TCP. After transplantation of SCAPs combined with hydroxyapatite/tricalcium phosphate (HA/TCP) scafolds into immunocompromised mice, a layer of dentin tissue is generated on the surface of the HA/TCP. When SCAPs are seeded onto synthetic scafolds consisting of polyD, L-lactide/glycolide, inserted into tooth fragments and transplanted into immunocompromised mice, a continuous layer of dentin-like tissue is deposited on the dentin surface and vascularized pulp-like tissue is formed in the root canal.[18]

Scaffold Implantation

Scaffolds are three-dimensional structures that provide an initial framework for cells, and can be used to deliver morphogenic molecules and, it also provides an environment that allows both cell migration and proliferation, and may be fabricated in pre-determined shapes and composition.

Ideally, a scaffold should accurately reproduce the features of the native Extra Cellular Matrix at the nanoscale to regulate cellular responses and encourage and regulate specific events at the cellular and tissue level. Furthermore, it has been well established that the synthesis of scaffolds should involve the use of biocompatible and biodegradable material(s) to avoid immunologically mediated reactions.[19]

They are available in the form of:

1. Natural polymer: It provides better biocompatibility in general.
2. Synthetic polymer: It allowed improved control of physicochemical properties, such as degradation rate, microstructure, and mechanical strength.

Scaffold for Pulp Tissue Regeneration

Scaffold for revascularization of immature permanent teeth

Root canal revascularization procedure aims to restore the blood supply of necrotic pulp tissues of permanent immature teeth. The revascularization technique depends on the induction of bleeding through the open apical foramen through the chemically cleaned canal. The canal dentin and the blood clot provide scaffolds in the root canal revascularization. More recently, platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) are suggested as further possible scaffolds.[20]

Blood clot:

The utilization of a blood clot to regenerate dental pulp tissues was first practiced by Ostby and resulted in a growth of granulation tissues, fibrous tissues or cementum-like tissues into the root canals. In 1974, Myers and Fountain

succeeded to generate 0.1-1.0 mm of soft connective tissues into the root canal using blood clots. Later on, successful clinical landmark cases of pulp tissue revascularization were reported.[20]

Since it is believed that tissues are not able to grow into empty spaces with the absence of suitable scaffolds, it can be suggested that blood clots yield good scaffolds to fill intracanal spaces and aid the growth of new tissues. The blood clot consists of fibrin matrix that traps cells necessary for tissue regeneration. It also provides a suitable pathway for cells from the periapical area including macrophages and fibroblasts to migrate into the root canal and enhance the new tissue growth. The rich content of growth factors allows the blood clot to play an important role in cell differentiation and thus, promotion of tissue regeneration. The growth factors include:

1. Platelet-derived growth factor (PDGF)
2. Vascular endothelial growth factor (VEGF), and
3. Platelet-derived epithelial growth factor, known also as vascular permeability factor.[20]

Disadvantage:

1. Failure to initiate bleeding or inadequate bleeding in the root canal.
2. Apical bleeding is the injury of the periapical tissues during the push and pull motion of the file.
3. The composition of a clot is variable.
4. The concentrations of cells trapped in a clot might differ leading to unpredictable outcomes.

Advantage:

1. Cost effective
2. Less time consuming

Platelet Rich Plasma

The Platelet Rich Plasma was introduced to dentistry world in 1997 by Whitman, Since then it was widely used to promote wound healing after oral maxillofacial, implant, and endodontic surgery, and currently it is referred as a first-generation platelet concentrate. It is prepared from autologous plasma with concentrated platelets. It has been nominated as a scaffold for pulp tissue regeneration because it is rich with important growth factors. These factors have the ability to enhance wound healing and stimulate matrix remodeling and angiogenesis. In addition, they have a role in controlling local inflammatory response and promoting cell proliferation and differentiation during osteogenesis. They also have the ability to attract stem cells from surrounding periapical tissues and when it is combined with dental pulp cells and also increased vital tissue regeneration.[20]

Some of the growth factors and cytokines found in platelets are:[20]

1. Transforming growth factor-β (TGF-β) 1 and 2
2. Platelet Derived Growth Factor
3. Vascular Endothelial Growth Factor
4. epidermal growth factor
5. fibroblast growth factor
6. Insulin-like growth factor-2 (IGF-2), IGF-1
7. Keratinocyte growth factor
8. Interleukin-8, and
9. Connective tissue growth factor.

Steps to prepare PRP:

1. Blood is extracted, collected with anticoagulant and immediately centrifuged for a variable time which is completed within an hour.
2. By first centrifugation process, blood is separated into three layers, the bottom is red blood cells, the second is a buffy coat layer and the supernatant layer which is acellular plasma known as platelet-poor plasma.
3. The aim of the preparation is to collect the buffy coat layer and discard the other layers.[20]

Advantage

1. Unlike blood clot procedure, anesthesia is not necessary for Platelet Rich Plasma application since periapical bleeding is not indicated.
2. Platelet Rich Plasma has an additional value in patients where bleeding into root canal cannot be established. [84]

Disadvantage

1. Blood extraction from young patients is at times difficult
2. Additional specialised equipment is needed which is the main disadvantages of Platelet Rich Plasma procedure.[20]

Platelet Rich Fibrin

Platelet Rich Fibrin was first developed in France by Choukroun et. al.for specific use in oral and maxillofacial surgery. This technique requires neither anticoagulant nor bovine thrombin (nor any other gelling agent) and it is also known as the second-generation platelet concentrate. It is prepared by the centrifugation of venous blood for once without the need for an anticoagulant material. This technology requires a PC-02 table centrifuge and a collection kit from Process (Nice, France).[21]

Steps to prepare PRF:

1. A blood sample is taken without anticoagulant in 10-mL tubes as (Figure 22).
2. They are immediately centrifuged at 3000 rpm (approximately 400g) for 10 minutes.
3. Fibrinogen is initially concentrated in the high part of the tube, before the circulating thrombin transforms it into fibrin.
4. A fibrin clot is then obtained in the middle of the tube, just between the red corpuscles at the bottom and acellular plasma at the top.[21]

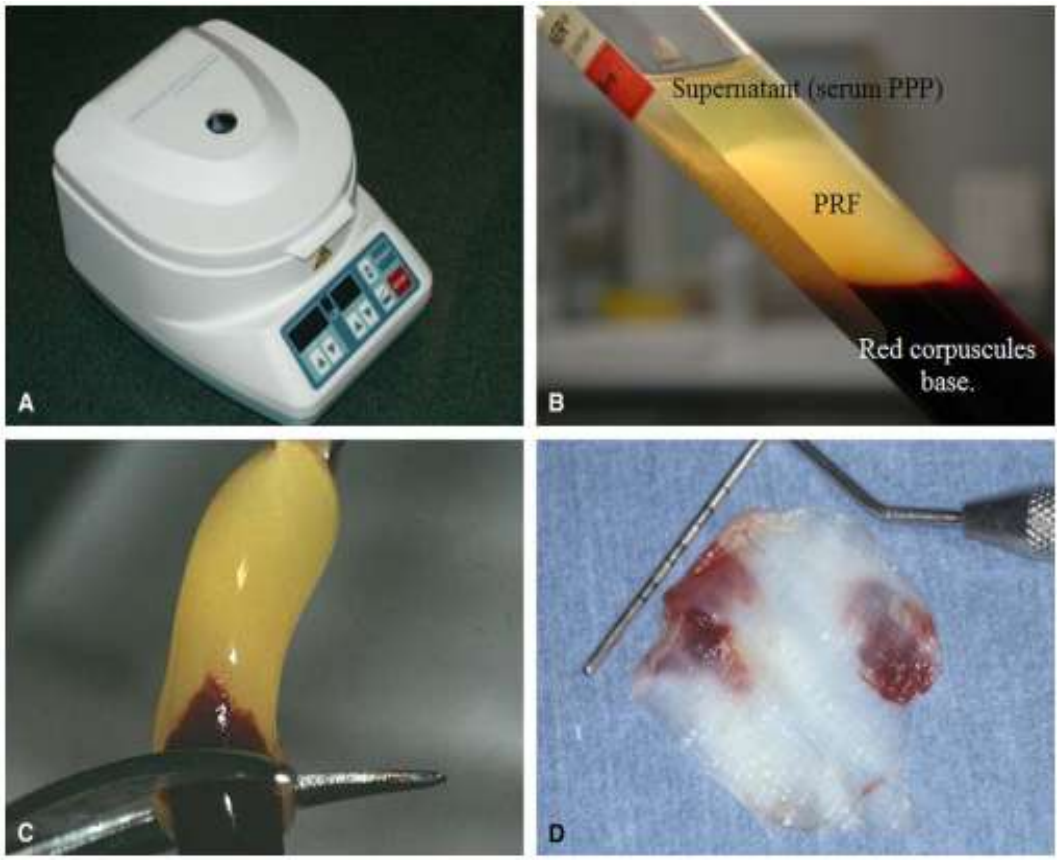


Figure 22:- Blood processing with a PC-O2 centrifuge for PRF (A; Process, Nice, France) (B). After collection of the PRF itself (C), resistant autologous fibrin membranes are easily obtained by driving out the serum from the clot (D).

Advantage

1. Ideal biomaterial for pulp-dentin complex regeneration
2. Prevents the early encroachment of undesired cells, therefore, perform as a viable barrier between desired and undesired cells

3. Healing and inter positional biomaterial .
4. Accelerates wound closure and mucosal healing due to fibrin bandage and growth factor release.[21]

Disadvantage
1. The final amount available is low because it is autologous blood.
2. PRF protocol success depends directly on the handling, mainly, related to blood collection time and its transference for the centrifuge.[21]

Injectable Scaffold Delivery
A scaffold is an artificial extracellular matrix (ECM) and serves as a template for cell growth and tissue regeneration, which ideally should be biocompatible and biodegradable, possess proper mechanical and physical properties, and mimic the in vivo microenvironment (niche) to facilitate cell adhesion, proliferation, differentiation, and neo tissue formation. Based on when a scaffold is shaped, it can be considered a pre-formed or an injectable scaffold, wherein a pre-formed scaffold has a definite shape prior to its application while an injectable scaffold forms the shape in situ.[22]

Advantages of injectable scaffold
1. It is performed in a minimally invasive manner
2. It decreases the risk of infection while improving comfort.
3. It can easily fill any irregularly-shaped defects.
4. It overcomes the difficulties of cell seeding and adhesion, and the delivery of bioactive molecules, as these factors can be simply mixed with the material solution before being injected in situ.

Pre-requisites of injectable scaffolds
Following are the pre-requisites of injectable scaffolds:[23]
1. Injectability
2. Cytotoxicity
3. Host response
4. Mechanical properties
5. Degradation

Types of injectable scaffolds
There are two types of injectable scaffolds:
Hydrogels:
Hydrogels are three-dimensional hydrophilic polymeric networks that are most widely explored injectable scaffolds and can be classified based on the method of crosslinking into physically and chemically cross-linked hydrogels.

Microsphere:
Microspheres are also another type of injectable scaffold with inherently small size and large specific surface area, which can also act as injectable cell carriers for tissue engineering. It allows faster nutrient and oxygen diffusion comparing to bulk hydrogels., and additionally, it also provide biomimetic three-dimensional (3D) extra-cellular microenvironment to generate microtissue stimulating positive cell-cell interactions and extracellular matrix (ECM) formation. The narrow, irregular anatomical structures of root canals and the poor blood supply gives more importance to the injectable microspheres as cell delivery vehicles in endodontic regeneration.[23]

Gene therapy
Gene therapy offers a novel approach toward the healing and regeneration of dental pulp tissue. Genes are located in the form of a genetic sequence in the DNA of nucleated cells that control cell activity and function. Vectors are used for delivery of required sequences to target tissues. These sequences may belong to morphogens, Extra Cellular Matrix components, or may be transcription factors. Although viral vectors are a highly efficient mode of gene transfer compared with nonviral vectors like plasmids, peptides, electroporation, sonoporation, and so forth, they pose a risk of infection.[24]

Rutherford (2001) encoded the sequence of Bone Morphogenic Protein 7 gene into a recombinant adenovirus and was able to induce reparative dentinogenesis in vitro but failed to do so in vivo.[25]

Nakashima et al. (2005) published a report wherein a three-dimensional pellet culture was electrotransfected with growth/differentiation factor 11(Gdf11). After 10 days, markers of odontoblast differentiation had a higher expression in transduced pellets than control. Based on this finding an in vivo investigation was done on a canine. The transplantation of transfected cells onto amputated pulp successfully induced reparative dentin formation.[26]

In a study by Yang et al.(2004) chitosan/collagen scaffolds were loaded with a plasmid encoding gene for Bone Morphogenic Protein 7. Dental Pulp Stem Cells were seeded into these scaffolds and were evaluated in vitro and in vivo. The cells were successfully transfected and secretion of Bone Morphogenic Protein 7 was observed until day 24. They also displayed better odontoblast differentiation and proliferation properties than non-transduced cells. In vivo, transfected cells lasted up to 4 weeks and showed upregulated expression of Dentin Sialophosphoprotein (DSPP).

The number of studies involving gene therapy as a means of endodontic tissue regeneration is limited. In case of necrotic pulps, gene therapy will have to be used in combination with stem cell therapy for treatment.

Conclusion:-

The field of regenerative endodontics is rapidly advancing and this progress is based on the principles of tissue engineering—namely, the spatial delivery of appropriate cells, scaffolds, and growth factors. The translational nature of regenerative endodontic research is allowing for changes to take place in the clinical practice in a relatively short time. This cross-talk between basic and clinical sciences is largely fueled by the realization that all three components of the tissue engineering triad are already present in revascularization procedures: stem cells, scaffold (blood clot), and growth factors (from dentin and blood). Preclinical studies that evaluated the effect of irrigants and medicaments on the survival of stem cells, release of growth factors from dentin, and odontoblastic differentiation are shaping the future generations of regenerative procedures. Further, the incorporation of other scaffolds such as PRP, PRF, and gelatin sponges has been used in the clinical practice with encouraging results.

Lately, there has been a high emphasis for pulp-dentin regeneration procedure using a cell-based approach, but the progress has been hampered primarily due to safety and regulatory issues regarding pulpal Mesenchymal Stem Cells production and transplantation in patients. However, several notable preclinical studies illustrate the efficacy of the cell transplantation approach for pulp-dentin regeneration, such as, stem cells from human exfoliated deciduous teeth–seeded scaffolds had the potential to form dental pulp–like tissue in vitro. There is also an in vivo evidence reported in a murine model, which showed pulp-dentin regeneration in human root fragments only after MSC transplantation.

Therefore, Dental practitioners and researchers are urged to focus their research and clinical observation practices on the exploration of the biological basis of this novel approach in order to determine a standardized therapeutic protocol leading to a more predictable treatment outcome. Hence, the need for further clinical studies in the field remains imperative.

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