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Regenerative Endodontics

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Abstract
Regenerative endodontics is the creation and delivery of tissues to replace diseased, missing, and traumatized pulp. This review provides an overview of regenerative endodontics and its goals, and describes possible techniques that will allow regenerative endodontics to become a reality. These potential approaches include root-canal revascularization, postnatal (adult) stem cell therapy, pulp implant, scaffold implant, three-dimensional cell printing, injectable scaffolds, and gene therapy. These regenerative endodontic techniques will possibly involve some combination of disinfection or debridement of infected root canal systems with apical enlargement to permit revascularization and use of adult stem cells, scaffolds, and growth factors. Although the challenges of introducing endodontic tissue engineering therapies are substantial, the potential benefits to patients and the profession are equally ground breaking. Patient demand is staggering both in scope and cost, because tissue engineering therapy offers the possibility of restoring natural function instead of surgical placement of an artificial prosthesis. By providing an overview of the methodological issues required to develop potential regenerative endodontic therapies, we hope to present a call for action to develop these therapies for clinical use.

Key Words *Growth factors; pulp regeneration; scaffolds; stem cells; tissue engineering*

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The treatments and outcomes of regenerative endodontics are different from traditional endodontic therapy; therefore, it has attracted enormous interest and attention in the field of endodontics in recent years.

Procedures that involve the usage of materials that encourage healing and repair of the pulp dentin complex after restoring the infectious or diseased tooth tissue are known as regenerative procedures. According to the concepts of regenerative therapy in dentistry, this procedure can turn a dead tooth into a vital one.Regenerative endodontics has introduced numerous procedures such as pulp implantation, revascularization and postnatal stem cell therapy. Revascularization has been successfully implemented clinically nowadays, thus providing dentists with outrageous results. Due to the increased rate of root canal treatment failure and posttreatment complications, regenerative endodontics plays an essential role in overcoming these complications. The goal has to prevent aggressive invasive instrumentation and radiographic exposure. This is performed by the reimposition of B $ T lymphocytes that aid in defense against the pathogens leading to pulp damage. The canal is completely sealed, and the vitality of the tooth is sustained, leading to the prevention of tooth fracture and periapical reinfection.

Healing is known to be a demand in surgical procedures, and it is achieved by a series of events: cellular organization, chemical signals, and extracellular matrix for tissue repair. Platelet-rich fibrin used either alone or along with bone graft promotes bone growth and vascularization. This matrix promotes migration, cell attachment, and proliferation of osteoblast that leads to bone formation. Cytokines released by PRF play a significant role in blood vessel formation and immune system stimulation to fight foreign pathogens. Studies claim that PRF that is prepared using low centrifugal forces leads to the effective concentration of leukocytes and growth factors related to those prepared at high centrifugal forces. A study has proved the enhanced results of PRF consumption along with iliac crest bone graft in patients with cleft alveolar ridge defects; relatively, in cases where iliac graft alone was used, results were unsatisfactory.

Similarly, an orthodontic surgical case was pursued using PRF, cancellous bone allograft, bovine bone matrix, and metronidazole, leading to complete healing with no complication.Results obtained from studies have shown that when PRF is combined with the biomaterials, the respective substitute's revitalization power increases and is more suitable and acceptable for the defected tissue space. PRF develops the cell-to-cell interaction; thus, proper incorporation of biomaterial is attained. The current review aims at the uses of PRF in regenerative endodontics and dentistry and its applications with future application and limitations.

Tissue engineering is the field of functional restoration of tissue structure and physiology of impaired or damaged tissues because of cancer, diseases and trauma. Revascularization refers to the re-establishment of vascularity in the pulp space post-injury to the original vascularity of the pulp of a traumatized immature tooth. Revitalization, on the other hand, describes non-specific vital tissues rather than just blood vessels. For immature teeth with non-vital pulp, such revascularization/ revitalization treatment induces apexogenesis, which thus results in tissue regeneration. The strength of the root and long-term retention of the tooth is increased by restoring root development and reinforcing dentinal walls. Such treatment modality has proven to be an efficient alternative to conventional apexification procedures.

The tooth is a complex organ that is formed by highly organized mineralized tissues encasing the dental pulp. Different mineralized tissues have different regenerative capabilities. Ameloblasts are derived from ectoderm and produce enamel after being stimulated by the odontoblasts. These cells do not have regenerative capacity and undergo apoptosis after the formation of enamel matrix. Odontoblasts and cementoblasts are derived from ectomesenchyme and lead to the formation of dentin and cementum respectively. These cells have limited regenerative capacity. In mild injuries, progenitor cells derived from pulp produce tertiary dentin at the pulp-dentin interface. Tertiary dentin helps in maintaining the pulp vitality by separating the damaged tooth structure from pulp. Similar to dentin, cellular cementum is also laid down throughout life at the root apex to compensate for passive eruption of the tooth.

In human dentistry, regenerative endodontics become a part of therapeutic endodontics and is considered to be an alternative to apexification.

Presently two concepts are seen in regenerative endodontics to treat non-vital teeth- one is the active persuit of pulp-dentin regeneration to implant or regrow pulp (Tissue engineering technique) and the other in which new living tissue is expected to form from tissue present in the teeth itself, allowing continued root development (Revascularization).The major attempt in regenerative endodontics seems to employ various types of stem cells positioned on a scaffold within the diseased root canal system of the tooth along with the addition of growth factors, externally or from dentin and/ or remaining dental pulp, aids in formation of a dental pulp like tissue.

Potential technologies for Regenerative Endodontic therapy include root canal revascularization, postnatal stem cell therapy, pulp implant, scaffold implant, threedimensional cell printing, injectable scaffold and gene therapy. A growing body of evidence is demonstrating the possibility for regeneration of tissues within the pulp space and continued root development in teeth with necrotic pulps and open apices.

GOALS

1. The primary goal is to eliminate the clinical symptoms/signs and resolution of apical periodontitis.
2. Secondary goals (which are desirable but not essential) include increased root wall thickness and/ or increased root length i.e. continued root maturation.
3. A tertiary goal (which if achieved indicates a high level of success) is a positive response to vitality testing.
4.Pinnacle of regenerative endodontic treatment goals:- Histologic confirmation for structural and functional restoration.

Therefore it can be stated that the primary goal of regenerative endodontic procedure and traditional non-surgical RCT is similar, the difference is that the disinfected root canal space in regenerative endodontic procedure is filled with hosts own vital tissue and in RCT it is filled with biocompatible foreign materials.

ELEMENTS OF REGENERATIVE ENDODONTICS

Stem cells

Stem cells are defined as cells possessing the capacity of self-renewal and differentiation. This means that cells are able to give rise to daughter cells with one being identical to the mother (self- renewal) and the other one being differentiated into a more specialized cell.

Stem cells have the ability to continuously divide to either replicate themselves (self-replication), or produce specialized cells that can differentiate into various other types of cells or tissues (multi lineage differentiation). Different types of stem cells are Embryonic stem cells, Blastocyst embryonic stem cells, Fetal stem cells, Umbilical cord stem cells, Adult stem cells, Dental pulp stem cells, Stem cells from human exfoliated deciduous teeth, Stem cells from apical papilla& Periodontal ligament stem cells.

Growth Factors:

To restore the vitality and functions of pulp dentin complex that has been lost to trauma or infections, another alternative approach is present, apart from the regular practice of delivering dental or nondental stem/progenitor cells. This alternative approach for pulp-dentin regeneration relies on growth factor delivery. The growth factors and cytokines may act as signalling molecules that modulate cell behaviour by mediating intracellular communication. Growth factors are polypeptides or proteins that bind to specific receptors on the surface of target cells. They can initiate a cascade of intracellular signalling, an act in either an autocrine or paracrine manner. Morphogens regulates the rate of tissue proliferation, cell differentiation into another cell type and matrix production. Various growth factors are Platelet derived growth factor, Transforming growth factor β, Vascular endothelial growth factor, Fibroblast growth factor, Insulin like growth factor and bone morphogenic protein.

Scaffold biomaterial

A scaffold surrounds cells and provides structural support for the formation and maintenance of tissues and organs. The scaffold is mainly composed of extracellular matrix proteins (ECMPs). The key ECMPs are collagen, vitronectin, and laminin, which provides cell with anchorage, sequestration of growth factors, and signal cells to migrate, differentiate and proliferate through integrin receptor- mediated signalling pathways. ECMPs have important role in dental regeneration. COLLAGEN is the predominant structural factors to regulate cell proliferation and differentiation. LAMININ promotes odontoblast differentiation, and a recent study by howard and colleagues claim it to be an important factor in dental pulp stem cell migration. FIBRONECTIN is known to increase ameloblast growth and differentiation, VITRONECTIN provides a structural framework.

Natural pulp wound healing versus tissue engineering approaches

The scope of regenerative endodontics ranges from procedures aimed at promoting natural wound healing events in the pulp to application of tissue engineering principles to treatment protocols. At this stage, there is insufficient evidence that either is superior, but advances in pulp biology will likely lead to clinical introduction of tissue engineering techniques, either for the pulp or the whole tooth in the future. There is already proofof-principle at the laboratory level that such techniques are feasible and such studies offer exciting future possibilities for novel treatment modalities in endodontics. Currently, there are attempts to apply tissue engineering principles within regenerative endodontics. In tissue engineering, cells (often stem or progenitor cells) are combined with scaffolds and appropriate signalling molecules to construct tissues resembling their physiological counterparts. Although application of cells for regenerative endodontic procedures has been restricted to experimental studies, there have been attempts to introduce scaffolds through blood clot formation – termed 'revascularisation' procedures. This emulates the role of the blood clot as a scaffold for tissue regeneration in natural soft-tissue wound healing. While encouraging results have been observed with this approach, there is insufficient evidence to determine whether it is better than other regenerative endodontic approaches. This perhaps highlights the challenges of haemostasis during endodontic procedures and the problems of controlling tissue events in a carefully defined manner within revascularisation procedures.

Root canal revascularisation

Revascularization is the procedure to re-establish the vitality in a non-vital tooth to allow repair and regeneration of tissues.Several case reports have documented revascularization of necrotic root canal systems by disinfection followed by establishing bleeding into the canal system via over instrumentation. In literature, revascularization for necrotic, infected, immature permanent teeth was disinfected with a topical antibiotic paste, a blood clot scaffold from the periapical tissues was induced. It was revealed that the treatment approach offered great potential to avoid the need for traditional apexification with calcium hydroxide or the need to achieve an artificial apical barrier with mineral trioxide aggregate.An important aspect of these cases is the use of intracanal irrigants (NaOCI and chlorhexidine) with placement of antibiotics (e.g. a mixture of ciprofloxacin, metronidazole,and minocycline paste) for several weeks. This particularcombination of antibiotics effectively disinfects root canal systems and increases revascularization of necrotic teeth, suggesting that this is a critical step in revascularization. However, owing to the potential of minocycline to stainthe triple antibiotic paste resulting in a antibiotic paste, or use of Arestin substitution of minocycline with amoxicillin.

Protocol for preparation of the triple antibiotic paste

Antibiotic (3mix)

Make sure to not cross-contaminate and remove sugar coating from tablets with surgical blade and crush individually in separate mortars. Then open capsules, crush individually in separate mortars. Grind each antibiotic to a fine powder and combine equal amounts of antibiotics (1:1:1) on mixing pad.

Carrier (MP)
Equal amounts of macrogol ointment and propylene glycol (1:1) have to take using clean spatula and mix together on pad. Result should be opaque.
- Separate out small portions of 3mix and incorporate into MP using the following
- 1:5 (MP 3Mix) – creamy consistency
- 1:7 (standard mix) – smears easily but does not crumble
- If result is flaky or crumbly, then too much 3mix has been incorporated

Storage
Antibiotics mustbe kept separately in moisture tight porcelain containers. Macrogol ointment and propylene glycol must be stored separately. Discard if mixture is transparent (evidence of moisture contamination).
Novel approaches towards apical regeneration:
a) Treatment with calcium enriched mixture (CEM)
b) Using soft tissue diode laser.
-Diode laser are soft tissue lasers with wavelength of 810980nm.
-Laser act by ablation of damaged pulp tissue in the immediate vicinity of the beam, disinfection of the remnant tissue by bacterial cell lysis and biostimulation of surrounding tissue,which promotes healing.
Challenges in regenerative procedures
- **Biological challenges:**Isolation of stem cell, Characterization of stem cell, Control of stem cell differentiation into specialized tissue, Interaction with microenvironment, Immune rejection, Long term sequelae and neoplastic potential.
- **Technical challenges:** Culture conditions and mediums, Maintaining the cultures free of pathogens and infection, Timing of processing, Biocompatible scaffolds and delivery system, Neo vascularization and nerve regeneration.
- **Clinical challenges:**Cost effective and efficiency, Immune reaction, Genomic stability, Risk of tumourigenesis, Long term follow-up.
- **Ethical challenges:**To use an embryo as a source of body cells is of very different notion both scientifically and ethically. It treats the embryo purely functionally as a―ball of cells as are source and no longer as a whole. The notion that it is okay to destroy embryos because they were created for research is wrong.

FUTURE SCOPE

The scope for regenerative endodontics includes research on the ability of stem cells to trigger regeneration hard tissues of the tooth, vitalization of a nonvital pulp, replacement of periapical tissues and periodontal ligaments. The ability to generate biological tooth substitutes from autologous human tissues would be a valuable clinical tool. The triad for dentine regeneration is responding cells, inductive morphogenic signals like BMPs, and an extracellular matrix scaffold. The human post-natal stem cells from accessible resources, like the ones derived from exfoliated primary teeth, can constitute in a potential clinical application, providing cells for stem cell therapies including cell transplantation and tissue engineering.

Tertiary dentinogenesis is a form of regeneration that is naturally engineered by the body. There are two types of tertiary dentinogenesis. Reactionary dentinogenesis represents the focal regulation of a group of primary odontoblasts surviving injury to the tooth, while reparative dentinogenesis represents the response of tertiary dentin secretion by a new generation of odontoblast like cells after death of the primary odontoblast cells. Reparative dentinogensis involving progenitor cell recruitment and differentiation prior to matrix secretion at the site of injury. Dentin extracellular matrix does contain bioactive molecules potentially available for release during pulp healing and repair. Carious demineralization of the dentin causes the release bioactive molecules, which in turn signal the cascade of dentinogenic events.

Application of a scaffold on an open pulp enabling odontoblast-like cells to grow into the scaffold and to convert it into dentin.Thus, a deep carious lesion is turned into a rather small dentin wound that could easily be covered by a common restorative material acting as a substitute for enamel.

The future advancement of pulp regeneration will continuously focus on three essential components: dental stem cells, scaffolds and growth factors. Several major challenges need to be addressed:

(1) Microbial control,Since the microorganism and biofilm are the fundamental etiologic factors of endodontic diseases, similar to conventional root canal treatment, microbial control is a key step for the success of pulp regeneration. (2) Spatial and temporal control of the release of growth factors from the scaffold remains as a major challenge when we design a new scaffold for tissue engineering. (3) It still remains a challenge to regenerate a real and functional pulp- dentin complex. A functional pulp- dentin complex would ideally contain an inside soft core highly vascularized with sufficient nerve supply and a peripheral layer of odontoblasts, located against the existing dentin wall, synthesizing matrix to produce new dentin.Regenerative endodontic

methods offer an alternative method to save teeth that may have compromised structural integrity.

The basis for cell proliferation most likely lies in the response of cells to a group of molecules known as polypeptide mitogens (or polypeptide growth factors) in conjunction with extracellular matrix molecules. Studies have indicated that primary dentin is far more effective than reparative dentin (tertiary dentin) in protecting the pulp from bacterial threats and so regeneration of primary like dentin should be the ultimate goal for regenerative measures.[107] This may be possible only by tissue-engineering methods by the formulation of biologically active matrices and molecules which can induce the differentiation. The goal of vital pulp therapy aims to maintain pulp vitality and function. Ideally under clinical conditions, complete healing of the exposed pulp with formation of dentin (complete dentin regeneration) cannot normally occur, due to total destruction of primary odontoblasts. Incomplete dentin regeneration often takes place at the exposure site when a new generation of odontoblast-like cells differentiates and reparative dentin is formed at the pulp- capping material interface.So research is focused on the prospect of inducing more extensive mineralized area that can fill the crown and root pulp partially or totally. Calcium hydroxide and MTA are time tested bioactive material which induces the formation of reparative dentinal bridge. New molecules such as bone morphogenic proteins (BMPs) or transforming growth factors – beta (TGF-β) have been implicated to cause dentin repair. Gene therapy is another tool being employed in dentistry to regenerate lost tissue structures. It is a science of manipulation of the developmental processes that direct organ/tissue formation in the embryo, a source of cells with multipotential that can be easily cultured, and an ability of an organ rudiment to form the complete organ in the adult environment. In endodontic‘s stem cell therapy would consist of the transfer of materials that contain pulp stem cells grown in laboratory to generate new tooth tissues.; PGA and PLGA scaffolds supported the growth of mature tooth structures.Such approaches provide potential for restoration of the structural integrity of the dental tissues where the new tissues become an integral part of the tooth, thus minimizing some of the problems of restoration failure with traditional dental materials through interface failure and bacterial microleakage.

In the future, the challenge of generating tissues that mimic the original pulp and dentin-like structure might be more effectively addressed by using tissue engineering approaches under more controlled clinical conditions. Such approaches might rely more on therapies that utilize autologous stem cells combined with customized scaffolds and delivery of appropriate growth factors at the right time and in the right sequence. Further translational research is needed to learn about these processes and, importantly, ensure that new protocols are clinically practical.

The future for regenerative and tissue-engineering applications to dentistry is of enormous prospective, capable of bringing quantum advances in treatment for our patients. Till date the design of suitable growth factor delivery system meeting all requirements and mimicking a natural biological environment still remains as one of the most important subjects in tissue engineering.

Conclusion:

Regenerative endodontics offers a number of exciting opportunities for preservation of pulp vitality following episodes of trauma and disease and the many biological advances have helped to underpin the development of this approach. From a semantics viewpoint, regenerative endodontics as it is currently performed represents more of a reparative than regenerative therapeutic strategy. Treatment outcomes from techniques, such as revascularisation procedures, generally give rise to a vital biological tissue, albeit not necessarily representative of the physiological structure of pulp. Despite these current limitations, such treatment strategies still offer significant clinical benefits, especially for immature teeth. True pulp regeneration will emerge as a viable clinical treatment strategy from the many recent advances being reported at the experimental level. Such approaches will likely target recruitment of specific stem/progenitor cell populations and exploit endogenous signalling molecules sequestered in dentine to regenerate dentine-pulp tissue with physiological characteristics.

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