**Title: Current overview on challenges in Regenerative Endodontics**

**Abstract:**

Regenerative endodontic procedures aim to offer high levels of success by replacing diseased or necrotic pulp tissues with healthy pulp tissue to revitalize teeth. Although current procedures successfully produce root development and in some cases histological evidence of pulp like connective tissues are also found to be filling the root canals but still fail to re-establish real healthy pulp tissue and give unpredictable results. There are several drawbacks that need to be addressed so that the application of regenerative dentistry in dental clinics can produce wonderful treatments to dramatically improve patients’ quality of life.

Keywords: Endodontics**,** Regeneration, Stem Cells**,** Tissue engineering

**Introduction:**

Historically, non-biological materials limited the ability of dentist to treat or replace diseased or lost tissues. However recent understanding about regeneration of dental tissues has the potential to revolutionize the complete dental health provision. Regenerative dental procedures that have been tried include guided tissue or bone regeneration(GTR,GBR) procedures and distraction osteogenesis[1], application of platelet rich plasma[2] and recombinant human bone morphogenic protein [3] for bone augmentation, use of Emdogain[4] and fibroblast growth factor 2 (FGF2)[5] for periodontal tissue regeneration.

Regenerative therapy promises numerous other clinical dental benefits,including biological strategies to repair teeth after carious damageand possibly even regrowing lost teeth.Wei F et al. successfully regenerated a functional bio-root structure for artificial crown restoration by using allogeneic dental stem cells and Vc-induced cell sheet.[6] Researchers at Toyko University reported successfully functioning tooth in a mouse achieved through the transplantation of bioengineered tooth germ into the alveolar bone.[7] Scientists led by Prof Cheng-Ming Chuong from the University of Southern California studied American alligator’s ability to renew teeth and found that stem cells in the mouth of alligators may regenerate teeth in humans.[8]

Regenerative dentistry is the future of dentistry and endodontists are leading the way in this new concept of dentistry. Regenerative Endodontics, a rising field, provides the hope of converting the non-vital tooth into vital once again. It focuses on substituting traumatized and pathological pulp with functional pulp tissue. 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.”

This review article discusses focuses on major priorities that ought to be dealt before translating laboratory results to thriving clinical applications heralding the dawn of regenerative endodontics.

**Method:**

A web-based research on MEDLINE (www.pubmed.gov) was done. To limit our research to relevant articles, the search was filtered using terms Review, published in the last 10 years & Dental journals. Keywords used for research were "regenerative endodontics" (found 36 articles), "dental stem cells"(found 111 articles), “growth factor regeneration” (found 184 articles), “scaffolds” (found 30 articles), and “challenges in regeneration” (found 24 articles). Relevant articles were chosen to get the desired knowledge update. This review article screened about 150 articles and then the relevant information was compiled.

**A look into History:**

The foundation of tooth regeneration was laid when stomatologist G. L. Feldman (1932) proposed that through biological-aseptic principle of tooth therapy, regeneration of pulp might be achieved and used dentine fillings for stimulating pulp regeneration [9]. In 1957 Gavrilov demonstrated regeneration of dentin and cementum of tooth root in dogs[9]. Regeneration of pulp that was key to regenerative endodontic procedures was conceptualized by ostby in 1961[10]. Subsequent researchers’ i.e Rule and winter (1966)[11],Nygaard-Ostby and Hjortdal (1971)[12] ,Ham et al. 1972 [13] further worked in this regard. In 2001Iwaya et al. described a procedure termed revascularization that resulted in thickening of the root canal walls and continued root development [14]. In 2004 Banchs and Trope proposed a clinical protocol for revascularization of infected immature teeth [15] . These two can be credited for sparking interest in regenerative endodontics.

**Present scenario of regenerative endodontics:**

Various regenerative approaches used in endodontics areRoot canal revascularization, Postnatal stem cell therapy, Scaffold implantation, Injectable scaffold delivery, Pulp implantation, 3-D cell printing and Gene therapy [16]. Out of all these only pulp revascularisation approach is presently a clinically feasible while rest other exist in research fields.

The 2011-2012 ADA Current Dental Terminology recognized pulp regeneration as an endodontic procedure and gave it code (D3354).

ADA Codes for Pulpal Regeneration Procedures

1. First Phase of Treatment(D3351): consists of debridement and antibacterial medication
2. Interim Phase(D3352): consist of interim medication replacement
3. Final Phase(D3354): completion of regenerative treatment in an immature permanent tooth with a necrotic pulp. It does not include final restoration

**CHALLENGE REGENERATIVE ENDODONTICS IS FACING:**

Inspite of the impressive growth in regenerative endodontic field, numerous challenges remain unaddressed as discussed below:

1. **To obtain a sufficient number of autogenous cells for scaffold seeding.**

To date, five kinds of human dental stem cells are isolated and characterized. These are Dental pulp stem cells (DPSCs), Stem cells from exfoliated deciduous teeth (SHED), Stem cells from apical papilla (SCAP), Periodontal ligament stem cells (PDLSCs) and Tooth germ progenitor cell (TGPCs).[17]

Although human dental stem cells have promising regenerative therapeutic applications but from a practical prospect, retrieval of autologous dental stem cells is challenging and the prospect of obtaining a sub population of stem cells is even more difficult. Although stem cells are present in all teeth but only limited no. of teeth fulfill the criteria of eligibility for stem cell extraction. Deciduous incisors and canines with no pathology and at least one third of root left are candidates of SHED but most clinical cases possess more than one carious tooth and also if the teeth take longer time to exfoliate, it may result in more than required resorption of root that contains no pulp, and thus, no stem cells. The DPSCs in adult humans are limited to the availability of the third molars and are not replenished after extraction like the bone marrow.[18] The cells isolated from adult tissues are often difficult to expand in vitro and generally do not maintain their phenotype.[19]

To overcome these issues, other stem cell sources have to be explored. Recent reports describe the presence of mesenchymal stem/progenitor cells with regenerative capabilities in human inflamed pulps [20] and inflamed periapical tissue [21] present intriguing possibilities yet to be explored. RS et al. 8 investigated the possibility of using somatic mesenchymal stem cells (MSCs) from other sources using a biomimetic dental pulp extracellular matrix (ECM) incorporated scaffold and found that the dental pulp stem derived ECM scaffold stimulated odontogenic differentiation of PDLSCs and HMSCs without the need for exogenous addition of growth and differentiation factors. Epithelial rests of Malassez(ERMs) are also shown to be capable of undergoing epithelial–mesenchymal transition[22]

**Non-dental stem cells for dental application:** Researchers led by Dr. Duanqing Pei reported a possible method for growing teeth from stem cells obtained in urine[23]. In their study, pluripotent stem cells (iPSCs) derived from human urine were induced to generate tooth-like structures in a group of mice in the laboratory. Success rates up to 30% were reported. The generated teeth had physical properties similar to that of normal human teeth except hardness, which was about one-third the hardness of human teeth. The reported advantages to such an approach were being non-invasive technique, low cost, and use of somatic cells (instead of embryonic) that are flushed down the toilet daily. Also Urine-derived stem cells do not form tumors when transplanted in the body unlike other stem cells. And sourcing cells from the patient’s own body reduces the likelihood of rejection.

1. **Scaffolds:**

Scaffolds act as carriers for specific cell types and they guide and support tissue regeneration. Scaffolds that have been commonly used for regenerative procedures are Natural scaffolds such as collagen, chitosan, silk, and fibrin, Synthetic scaffolds such as polyglycolide[PGA], PLGA, and polyglycerol sebacate [PGS][24], blood clot (15) as well as platelet-rich plasma[25]. Recently the platelet rich fibrin (PRF) has found its applications in regenerative endododontics.[26] Many other types of scaffolds that include natural nanotolith/bacterial cellulose scaffolds[27], nanofibers with the microalga Spirulina as scaffolds[28], chemically modified bacterial cellulose nanocomposite[29], nanofiber scaffolds[30], peptide hydrogel nanofibers and various fibrin gels[31] have been investigated as potential scaffolds.

Various problems that must be addressed are:

1. Requirement of an appropriate vascularized scaffold to promote formation of large tissue constructs: The size of most tissue engineered constructs is small (1-2 mm) due to limited diffusion of nutrients and metabolites in non-vascularized scaffolds. As a consequence, studies using scaffold-based approaches often rely upon *in vivo* maturation of a small scaffold [32] followed by implantation into the jaw to develop a tooth-like structure. *In vitro* approaches overcome the problem of limited diffusion by relying upon perfusion or flow-based bioreactors that facilitate a deeper exchange of molecules within the scaffold[33,34].

Microscale technologies that support vascularization and enhance diffusion might help in development of large tissue constructs. Microfabrication has been used to fabricate tissue-engineered scaffolds with micro-engineered capillary beds[35]. Micro- and nanochannels provide passage for diffusion of oxygen and nutrients to support cells in tissue-engineered constructs. Photolithography is one technique in which vascular networks in scaffolds are created by selectively exposing a light-sensitive solution to light by means of a photomask. The exposed solution polymerizes whereas the unpolymerized masked solution gets washed away resulting in production of micro-channels[36].

1. Distribution of cells in scaffold:

The association of Bio-electrospraying (BES) with scaffold production techniques can produce biomaterials with cells homogeneously distributed in the entire structure.[37]

Three-dimensional cell printing technique[38] can be used to precisely position cells, and create tissue constructs that mimic the natural tooth pulp tissue structure.

Scaffold based approaches have the potential for rapid formation of a functional tooth of the correct shape and in the desired location but it has to overcome challenges associated with attachment to the jaw, infection, repetitive movement, and ability to withstand load during maturation.[39] Scaffold‐free stem‐cell sheet‐derived pellet (CSDPs) have greater odontogenic potential but require precise control over tooth shape and orientation. Sijia Na et al.proposed that SCAP‐CSDPs with a mount of endogenous Extracellular matrix can be used in the fabrication of bioengineered dental roots.[40]

1. **Growth factors:**

Growth factors act as signals to induce cellular proliferation and/or differentiation. Examples of key growth factors in regenerative dentistry include bone morphogenetic protein, transforming growth factor–beta, fibroblastic growth factor, Platelet-derived growth factor (PDGF), and Insulin-like growth factor (IGF).16 Growth factors found dentin[41] are also being investigated for their potential applications.

The major drawback in growth factors is that a different set of growth factors is required to induce stem cells from different sources to achieve specific differentiation. Along with this safety, quantity and time of delivery of the growth factors pose a significant challenge. This problem can be overcome by use of the biomimetic ECM embedded scaffold that can be produced in large quantities and are patient specific without complications of immune response and do not require any exogenous growth factor delivery[42].

Another drawback is application of higher loading levels of growth factors to compensate their physiologic solubility[43] can result in unwanted side-effects and limited spatial control. Microencapsulation[44] or binding of these factors to the scaffold[45] can relieve these problems. Also microparticles containing growth factors can be used control the activity of cells.[46]

1. **Advances in disinfection techniques:**

Disinfection of the root canal spaces of immature teeth is quite challenging, and more effective antimicrobial regimens are required to create a conducive environment. Although TAP is established antibiotic paste [47,48,49] but it has its own drawbacks. TAP is radiolucent[50], the vehicle of TAP (propylene glycol) may be difficult to remove from the dentin surface, an additional appointment is required to remove TAP and again opening the tooth to remove TAP introduces risk of recontamination. To overcome these problems better resorbable single or multiple antibiotics, compatible vehicles for delivery and radio-opaque material is necessary for achieving efficient and easy disinfection that could easily be monitored. Antibiotic containing scaffolds can answer such problems.

An electrospun nanofibrous polymeric scaffold with antibiotic incorporated into it, can serve in vitro drug delivery device, for canal disinfection. Its use can improve drug delivery due to its high surface area of the fibers arranged in an interconnecting structure that allows controlled drug release[51] and improve drug adaptation to the canal wall in the regeneration procedure. As the scaffold degrades over time,[52] it does not required to be removed, thus reduces appointments and subsequent risk of bacterial contamination Also, the drug release can be manipulated i.e. made rapid, intermediate or delayed depending on the polymer used.[53] The effectiveness of an electrospun scaffold as a biologically safe antimicrobial drug delivery system for regenerative endodontics is reported in the literature.[54] Synthetic electrospun polymeric nanofibers are under investigation as drug delivery modes.

**Can intracanal antibiotics be substituted for achieving disinfection??**

The sole purpose behind intracanal antibiotic medicament is to eliminate microbes. If this motive is achieved by some other means then antibiotics can be avoided. The EndoVac apical negative-pressure system of irrigation can answer. EndoVac delivers irrigating agents safely to the full extent of the root-canal terminus, thereby removing 100% of organic tissue and 100% of the microbial contaminants.[55] Also it is the only method capable of 100% cleaning the isthmus area.[56] Thus creating optimum conditions for regenerative endodontic procedures without the use of antibiotics. Studies have also shown that apical negative pressure with sodium hypochlorite irrigation resulted in similar bacterial reductions as with use of apical positive pressure irrigation and a triple antibiotic in immature teeth[57] and equivalent mineralized tissue formation and the repair process resulted.[58] Additionally using negative apical pressure and sodium hypochlorite also avoids the risk of drug resistance, tooth discoloration[58], and allergic reactions.

1. **DISCOLORATION**:

TAP is associated with severe discoloration [59,60] due to the presence of minocycline in it[61]  that binds with the calcium of dentin forming insoluble complexes. To avoid staining while using TAP, the pulp chamber should be sealed with dentine bonding agent and ensure that TAP remains below the CEJ. The clinician should remove residual paste from the pulp chamber and wipe clean it with cotton pellets soaked in absolute alcohol.[62]

Modified TAP in which minocycline is substituted with non-discoloring medicaments like clarithromycin[63] or fosfomycin[63] or cefuroxime[64] or Arestin[65] or cefaclor[66] have shown to be effective in eliminating endodontic pathogens and were able to avoid the permanent staining effect of the crown. Calcium hydroxide can also be used alternatively or EndoVac apical negative-pressure irrigating system system along with sodium hypochlorite irrigation can be used to avoid antibiotics completely as described in the disinfection section.

In addition, presence of gray MTA and white MTA might be another source for discoloration [67] which can be prevented by using alternative tooth-colored bioactive materials like CEM cement over the blood clot.[68]

1. **UNPREDICTABLE OUTCOME:**

Guidelines given by ADA for Follow-up Evaluation of pulp regeneration procedures include clinically asymptomatic and functional tooth. Radiographic evaluation at 6-12 months should show resolution of periapical radiolucency. Increased dentinal wall thickness might also be seen. At 12-24 months radiograph should show increased dentinal wall thickness along with increased root length

Based on these guidelines many success stories have been reported in literature. [69,70,71] Recently, Torabinejad and Faras[72]  presented clinical, radiographic, and histologic findings showing ‘‘pulp-like vital connective tissue from a tooth after regenerative endodontic treatment done using platelet-rich plasma [PRP] as a scaffold. Similar histological report was presented by Shimizu et al from a tooth extracted after the completion of regenerative endodontic treatment in which more than one half of the canal was found filled with pulp like loose connective tissue.[73] Positive response to cold and/or electric pulp tests occurs in some cases.[74] These findings indicate the success of regenerative endodontic procedures.

In contrast to this, literature also some case reports in which despite following proper protocol pup regeneration and root develoment failed. Lenzi and Trope[75] found empty root canal space after treatment of an immature maxillary central incisor with a necrotic pulp. Nosrat et al[67] showed the absence of vital tissue inside the root canal space of treated immature maxillary incisors with necrotic pulps after 6 years. Ali Nosrat et al. 2013[76] presented a case where root maturation occurred in a maxillary central incisor, even though a regenerative endodontic procedure resulted in an empty root canal space. Even after using tissue engineering strategies, cementum-like hard tissue was deposited on root canal walls, and bony islands were found throughout the root canals.[77] Formation of a hard-tissue barrier inside the canal between the coronal MTA plug and the root apex[78]  is another reported unfavorable outcome.

 These findings might not be considered as “clinical failures” but show that the outcome of the current protocol for pulp regeneration might be unpredictable.[79]

**FUTURE PROSPECTIVE:**

Regenerative endodontic strategies are continuously being updated and improved to benefit dentistry in every possible way. American Association of Endodontists Foundation has recently awarded a grant of $1.7 million[80] to evaluate the effectiveness of two regenerative approaches (REGENDO and REVASC ), compared with the conventional mineral trioxide aggregate (MTA) apexification. The trial will be carried out in collaboration with Loma Linda University, University of Texas Health Science Center at San Antonio and the University Of Maryland School Of Dentistry and is estimated to complete in December 2019. Iohara K et al[81] aims to use pulp stem cells with granulocyte-colony stimulating factor (G-CSF) for pulp/dentin regeneration to fully restore the tooth instead of filling, capping or extracting it. Misako Nakashima, (Japan) said that a clinical trial of pulp regeneration has already been initiated with the permission of the Japanese Ministry of Health, Labor and Welfare. Recently, PRF box has been announced[82] to produce homogenously thickened hydrated exudate rich in platelets, vitronectin, leukocytes, and fibronectin expressed from the fibrin clots that have improved the issues regarding the handling of the PRF clot. It is likely that the next advance in regenerative dentistry is the availability of regenerative dental kits, which will enable the dentists the ability to deliver regenerative therapies locally as part of routine dental practice.

**Conclusion:**

Regenerative endodontic strategies have tremendous potential to be an effective, safe and biological mode to save teeth which have compromised structural integrity provided the above discussed problems are dealt with. Considerable research and development efforts are required to advance the regenerative therapeutics to next level. With new discoveries, innovative ideas and high quality research, in the future, the scope of regenerative endodontics might increase to include the replacement of periapical tissues, gingiva, and even whole teeth.

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