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Regenerative Endodontics In The Light Of Recent Research

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Abstract
Current treatment modalities offer high levels of success for many conditions, however, an ideal form of therapy might consist of regenerative approaches in which diseased or necrotic pulp tissues are removed and replaced with healthy pulp tissue to revitalize teeth. Regenerative endodontics is the creation and delivery of tissues to replace diseased, missing and traumatized pulp. This review describes possible techniques that will allow regenerative endodontics to become a reality. These include root-canal revascularization, postnatal (adult) stem cell therapy, pulp implant, scaffold implant, three-dimensional cell printing, injectable scaffolds and gene therapy.

Key Words
pulp regeneration, tissue engineering, stem cells, growth factors

Introduction

The regeneration or replacement of oral tissues affected by inherited disorders, trauma, and neoplastic or infectious diseases is expected to solve many dental problems. Unparalleled advances in dentistry and endodontics are set to take place, with the availability as well as the ability to stimulate endodontic regeneration.[1],[2] Regenerative endodontic procedures can be defined as biologically based procedures designed to replace damaged structures, including dentin and root structures, as well as cells of the pulp-dentin complex. Dr. B. W. Hermann reported the application of calcium hydroxide in a case report of vital pulp amputation. Subsequent regenerative dental procedures include the development of guided tissue or bone regeneration (GTR, GBR) procedures and distraction osteogenesis;[3] the application of platelet rich plasma (PRP) for bone augmentation,[4] Emdogain for periodontal tissue regeneration,[5] and recombinant human bone morphogenic protein (rhBMP) for bone augmentation;[6] and preclinical trials on the use of fibroblast growth factor 2 (FGF2) for periodontal tissue regeneration.[7] However, there has not been significant translation of any of these therapies into clinical endodontic practice. 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.

Potential Technologies for Regenerative Endodontics

Major areas of research that might have application in the development of regenerative endodontic techniques. Are (a) root canal revascularization via blood clotting, (b) postnatal stem cell therapy, (c) pulp implantation, (d) scaffold implantation, (e) injectable scaffold delivery, (f) three-dimensional cell printing, and (g) gene delivery.

Root Canal Revascularization

Several case reports have documented revascularization of necrotic root canal systems by disinfection followed by establishing bleeding into the canal system via overinstrumentation.[8] An important aspect of these cases is the use of intracanal irrigants (NaOCl and chlorhexdine) with placement of antibiotics (e.g. a mixture of ciprofloxacin, metronidazole, and minocycline paste) for several weeks. It disinfects and increases revascularization of avulsed and necrotic teeth.[9] It has been noted that reimplantation of avulsed teeth with an apical opening of approximately 1.1 mm demonstrate a greater likelihood of revascularization.[10] This finding suggests that revascularization of necrotic pulps with fully formed (closed) apices might require instrumentation of the tooth apex to approximately 1 to 2mm in apical diameter to allow systemic bleeding into root canal systems. The development of regenerative endodontic procedures may require reexamination of many of the closely held precepts of traditional endodontic procedures. The revascularization method assumes that the root canal space has been disinfected and that the formation of a blood clot yields a matrix (e.g., fibrin) that traps cells capable of initiating new tissue formation. It is not clear that the regenerated tissue's phenotype resembles dental pulp; however, case reports published to date do demonstrate continued root formation and the restoration of a positive response to thermal pulp testing. Another important point is that younger adult patients generally have a greater capacity for healing.[11]

There are several advantages to a revascularization approach. First, this approach is technically simple and can be completed using currently available instruments and medicaments without

expensive biotechnology. Second, the regeneration of tissue in root canal systems by a patient's own blood cells avoids the possibility of immune rejection and pathogen transmission from replacing the pulp with a tissue engineered construct. The case reports of a blood clot having the capacity to regenerate pulp tissue are exciting, but caution is required, because the source of the regenerated tissue has not been identified. Animal studies and more clinical studies are required to investigate the potential of this technique before it can be recommended for general use in patients. However, plasma-derived fibrin clots are being used for development as scaffolds in several studies. Enlargement of the apical foramen is also necessary to promote vascularizaton and to maintain initial cell viability via nutrient diffusion. Cells must have an available supply of oxygen; therefore, it is likely that cells in the coronal portion of the root canal system either would not survive or would survive under hypoxic conditions.

Postnatal Stem Cell Therapy

The simplest method to administer cells of appropriate regenerative potential is to inject postnatal stem cells into disinfected root canal systems after the apex is opened. Postnatal stem cells can be derived from multiple tissues, including skin, buccal mucosa, fat, and bone.[12] A major research obstacle is identification of a postnatal stem cell source capable of differentiating into the diverse cell population found in adult pulp (e.g., fibroblasts, endothelial cells, odontoblasts). Dental pulp stem cells derived from autologous (patient's own) cells that have been taken from a buccal mucosal biopsy, or umbilical cord stem cells that have been cryogenically stored after birth; an allogenic purified pulp stem cell line that is disease and pathogen-free; or xenogneic (animal) pulp stem cells that have been grown in the laboratory.

There are several advantages to an approach using postnatal stem cells.(i) autogenous stem cells are relatively easy to harvest and to deliver by syringe, and the cells have the potential to induce new pulp regeneration. (ii) this approach is already used in regenerative medical applications, including bone marrow replacement, and a recent review has described several potential endodontic applications. Disadvantages (i) the cells may have low survival rates. (ii) the cells might migrate to different locations within the body, possibly leading to aberrant patterns of mineralization.

In general, scaffolds, cells, and bioactive signaling molecules are needed to induce stem cell differentiation into a dental tissue type. Therefore, the probability of producing new functioning pulp tissue by injecting only stem cells into the pulp chamber, without a scaffold or signaling molecules, may be very low. Instead, pulp regeneration must consider all three elements (cells, growth factors, and scaffold) to maximize potential for success.

Pulp Implantation

The majority of in vitro cell cultures grow as a single monolayer 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. 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.[13] 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 nanofibers or on sheets of extracellular matrix proteins such as collagen I or fibronectin.[14] So far, growing dental pulp cells on collagens I and III has not proved to be successful. The advantage of having the cells aggregated together is that it localizes the postnatal stem cells in the root canal system. The disadvantage of this technique is that implantation of sheets of cells may be technically difficult.

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[15]. A scaffold should contain growth factors to aid stem cell proliferation and differentiation, leading to improved and faster tissue development. The scaffold may also contain nutrients promoting cell survival and growth, and possibly antibiotics to prevent any bacterial in-growth in the canal systems. In pulp-exposed teeth, dentin chips have been found to stimulate reparative dentin bridge formation.[16] Dentin chips may provide a matrix for pulp stem cell attachment[17] and also be a reservoir of growth factors.[18] 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. Most of the scaffold materials used in tissue engineering have had a long history of use in medicine as bioresorbable sutures and as meshes used in wound dressings. The types of scaffold materials available are natural or synthetic, biodegradable or permanent. The synthetic materials include polylactic acid (PLA), polyglycolic acid (PGA), and polycaprolactone (PCL), which are all common polyester materials that degrade within the human body.[19] These scaffolds have all been successfully used for tissue engineering applications because they are degradable fibrous structures with the capability to support the growth of various different stem cell types.

Injectable Scaffold Delivery

Tissue engineered pulp tissue is to be administered in a soft three-dimensional scaffold matrix, such as a polymer hydrogel. Hydrogels are injectable scaffolds that can be delivered by syringe.[20] Hydrogels have the potential to be noninvasive and easy to deliver into root canal systems. The hydrogel may promote pulp regeneration by providing a substrate for cell proliferation and differentiation into an organized tissue

structure. Hydrogels at are at an early stage of research, and this type of delivery system, although promising, has yet to be proven to be functional in vivo.

Three-Dimensional Cell Printing

The final approach for creating replacement pulp tissue may be to create it using a three-dimensional cell printing technique.[21] In theory, an ink-jet-like device is used to dispense layers of cells suspended in a hydrogel to recreate the structure of the tooth pulp tissue. The three-dimensional cell printing technique can be used to precisely position cells, 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. Theoretically, the disadvantage of using the three-dimensional cell printing technique is that careful orientation of the pulp tissue construct according to its apical and coronal asymmetry would be required during placement into cleaned and shaped root canal systems.

Gene Therapy

New techniques involving viral or nonviral vectors can deliver genes for growth factors, morphogens, transcription factors, and extracellular matrix molecules into target cell populations, such as the salivary gland. Viral vectors are modified to avoid the possibility of causing disease. Several viruses have been genetically modified to deliver genes, including retroviruses, adenovirus, adeno associated virus, herpes simplex virus, and lentivirus.[22] Nonviral gene delivery systems include plasmids, peptides, gene guns, DNA-ligand complexes, electroporation, sonoporation, and cationic liposomes.[23] The choice of gene delivery system depends on the accessibility and physiological characteristics of the target cell population. A recent review has discussed the use of gene delivery in regenerative endodontics. One use of gene delivery in endodontics would be to deliver mineralizing genes into pulp tissue to promote tissue mineralization. there remains much to be accomplished to use gene therapy as part of endodontic treatment. Moreover, potentially serious health hazards exist with the use of gene therapy; these arise from the use of the vector (gene transfer) system. Researchers must learn how to accurately control gene therapy and make it very cell specific to develop a gene therapy that is safe to be used clinically. Because of the apparent high risk of health hazards, the development of a gene therapy to accomplish endodontic treatment seems very unlikely in the near future. Gene therapy is a relatively new field, and evidence is lacking to demonstrate that this therapy has the potential to rescue necrotic pulp.

Methods to Disinfect and Shape Root Canal System

The simplest approach to pulp tissue regeneration would be to regrow pulp over remaining pulp tissue. However, attempts to regenerate pulp tissue under conditions of inflammation or partial necrosis have proved unsuccessful.[24] In the presence of infection, the pulp stem cells that survive appear to be incapable of mineralization and deposition of a tertiary dentin bridge. Therefore, the majority of the available evidence suggests that necrotic and infected tooth pulp does not heal. Therefore, in the foreseeable future, it will be necessary to disinfect the root canal systems and remove infected hard and soft tissues before using regenerative endodontic treatments. The literature contains no or few reports of pulp stem cell attachment and adherence to root canal dentin. To successfully attach and adhere to root canal dentin, the stem cells must be supported within a polymer or hydrogel scaffold. For regenerative endodontics to be successful, the disinfection of necrotic root canal systems must be accomplished in a fashion that does not impede the healing and integration of tissue engineered pulp with the root canal walls. Moreover, the inclusion of a small local amount of antibiotics may need to be considered in developing these biodegradable scaffolds. Thorough disinfection removes microorganisms, permits better adaptation of filling materials, and enhances the action of the intracanal medicaments. The choice of an irrigant is of great importance, because the irrigant acts as a lubricant during instrumentation, flushes debris and microorganisms out of the canal, and reacts with pulp, necrotic tissues, and microorganisms and their subproducts. Sodium hypochlorite has been extensively used for several decades for this purpose.[25] Its excellent properties of tissue dissolution and antimicrobial activity make it the irrigant of choice for the treatment of teeth with pulp necrosis.

Removal of the Smear Layer

The presence of a smear layer on root canal walls may inhibit the adherence of implanted pulp stem cells, potentially causing the regenerative endodontic treatment to fail. Improved methods to remove the smear layer from the root canal walls appear to be necessary to help promote the success of regenerative endodontics. Its removal provides better sealing of the endodontic filling material to dentin, and avoids the leakage of microorganisms into oral tissues.

Engineering a Functional Pulp Tissue

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 that appears capable of forming hard tissue under certain conditions; stem cell therapy, involving the delivery of autologous or allogenic stem cells into root canals; or pulp implantation, involving the surgical implantation of synthetic pulp tissue grown in the laboratory.

Delivery of Regenerative Endodontic Procedures

Ideally, the delivery of regenerative endodontic procedures must be more clinically effective than current treatments. The method of delivery must also be efficient, cost-effective, and free of health hazards or side-effects to patients. A promising cellular source for regenerative endodontic procedures is autogenous stem cells from oral mucosa. The oral mucosa cells are readily accessible as a source of oral cells, which avoids the problem of patients being required to store umbilical cord blood or third molars immediately after extraction. It also avoids the need for bone biopsies. The oral mucosa cells may be maintained using in vitro cell culture with antibiotics to remove infection.[26] The cells may then be seeded in the apical 1 to 3 mm of a tissue engineering scaffold with the remaining coronal 15 mm containing an acellular scaffold that supports cell growth and vascularization.

By seeding cells only in the apical region, there is reduced demand for large numbers of cells derived from the host. Instead, most of the cellular proliferation would occur naturally in the patient. This would reduce the need to grow large quantities of cells in the laboratory.

Conclusions

The clinical success rates of endodontic treatments can exceed 90%.[27], [28], [29]. However, many teeth are not given the opportunity to be saved by endodontic treatment and instead are extracted, with subsequent placement of an artificial prosthesis, such as an implant. Regenerative endodontic methods have the potential for regenerating both pulp and dentin tissues and therefore may offer an alternative method to save teeth that may have compromised structural integrity.

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