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Potential Technologies for Regenerative Pediatric Endodontics-An Updated Review

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ABSTRACT

The regeneration endodontics procedures of immature permanent teeth are beneficial to reduce the risk of fracture and loss of teeth. Regenerative endodontic procedures include revascularization, partial pulpotomy, and apexogenesis. The appropriate dental care is needed to deliver regenerative endodontic procedures and to maintain or restore the vitality of teeth. Regeneration can be accomplished through the activity of the progenitor cells from the pulp, periodontium, vascular, and immune system. Most therapies use the host's own pulp or vascular cells for regeneration, but other types of dental stem cell therapies are under development. There are no standardized treatment protocols for endodontic regeneration. The purpose of this article is to review the recent literature and suggest guidelines for using regenerative endodontic procedures including several major areas of research that might have application in the development of regenerative endodontic techniques.

Keywords: endodontics, regenerative, vitality, teeth, immature.

INTRODUCTION

Regenerative endodontic procedures (REPs) can be defined as biologically based procedures designed to replace damaged structures including dentin and cells of the pulp-dentin complex[1]. 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. Several major areas of research that might have application in the development of regenerative endodontic techniques have been identified. These 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 cellprinting
(g) Gene delivery

The developmental approaches for regenerative endodontic techniques includes[2]:

Root Canal Revascularization via Blood Clotting
Several case reports have documented revascularization of necrotic root canal systems by disinfection followed by establishing bleeding into the canal system via over instrumentation. 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. This particular combination of antibiotics effectively disinfects root canal systems [3] and increases revascularization of avulsed and necrotic teeth, suggesting that this is a critical step in revascularization.

The selection of various irrigants and medicaments is worthy of additional research, because these materials may confer several important effects for regeneration in addition to their antimicrobial properties. For example, tetracycline enhances the growth of host cells on dentin, not by an antimicrobial action, but via exposure of embedded collagen fibres or growth factors. However, it is not yet know if minocycline shares this effect and whether these additional properties might contribute to successful revascularization.

Although these case reports are largely from teeth with incomplete apical closures, it has been noted that reimplantation of avulsed teeth with an apical opening of approximately 1.1 mm demonstrate a greater likelihood of revascularization [4]. 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 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.

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. However, several concerns need to be addressed in prospective research: Firstly, 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. Generally, tissue engineering does not rely on blood clot formation, because the concentration and composition of cells trapped in the fibrin clot is unpredictable. This is a critical limitation to a blood clot revascularization approach; because tissue engineering is founded on the delivery of effective concentrations and compositions of cells to restore function. It is very possible that variations in cell concentration and composition, particularly in older patients (where circulating stem cell concentrations may be lower) may lead to variations in treatment outcome. On the other hand, some aspects of this approach may be useful; plasma-derived fibrin clots are being used for development as scaffolds in several studies[5]. Second, enlargement of the apical foramen is necessary to promote vascularisation and to maintain initial cell viability via nutrient diffusion. Related to this point, 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 before angiogenesis. Interestingly, endothelial cells release soluble factors under hypoxic conditions that promote cell survival and angiogenesis, whereas other cell types demonstrate similar responses to low oxygen availability.

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[6]. 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) . Technical obstacles include the development of methods for harvesting and any necessary ex vivo methods required to purify and/or expand cell numbers sufficiently for regenerative endodontic applications.

One possible approach would be to use 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. It is important to note that no purified pulp stem cell lines are presently available, and that the mucosal tissues have not yet been evaluated for stem cell therapy. Although umbilical cord stem cell collection is advertised primarily to be used as part of a future medical therapy, these cells have yet to be used to engineer any tissue constructs for regenerative medical therapies.

There are several advantages to an approach using postnatal stem cells. First, autogenous stem cells are relatively easy to harvest and to deliver by syringe, and the cells have the potential to induce new pulp regeneration. Second, this approach is already used in regenerative medical applications, including bone marrow replacement, and a recent review has described several potential endodontic applications. However, there are several disadvantages to a delivery method of injecting cells. Firstly, the cells may have low survival rates. Second, the cells might migrate to different locations within the body, possibly leading to aberrant patterns of mineralization. A solution for this latter issue may be to apply the cells together with a fibrin clot or other scaffold material. This would help to position and maintain cell localization.

Pulp Implantation

The majority of in vitro cell cultures grow as a single monolayer attached to the base of culture flasks. However, some stem cells do not survive unless they are grown on top of a layer of feeder cells. In all of these cases, the stem cells are

grown in two dimensions. In theory, to take two-dimensional cell cultures and make them three-dimensional, the pulp cells can be grown on biodegradable membrane filters. Many filters will be required to be rolled together to form a three dimensional pulp tissue, which can be implanted into disinfected root canal systems.

The advantages of this delivery system are that the cells are relatively easy to grow on filters in the laboratory. The growth of cells on filters has been accomplished for several decades, as this is how the cytotoxicity of many test materials is evaluated. Moreover, aggregated sheets of cells are more stable than dissociated cells administered by injection into empty root canal systems. The potential problems associated with the implantation of sheets of cultured pulp tissue is that specialized procedures may be required to ensure that the cells properly adhere to root canal walls. Sheets of cells lack vascularity, so only the apical portion of the canal systems would receive these cellular constructs, with coronal canal systems filled with scaffolds capable of supporting cellular proliferation[7]. 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. So far, growing dental pulp cells on collagens I and III has not proved to be successful[8], but other matrices, including vitronectin and laminin, require investigation. 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. The sheets are very thin and fragile, so research is needed to develop reliable implantation techniques. The sheets of cells also lack vascularity, so they would be implanted into the apical portion of the root canal system with a requirement for coronal delivery of a scaffold capable of supporting cellular proliferation. Cells located more than 200 um from the maximum oxygen diffusion distance from a capillary blood supply are at risk of anoxia and necrosis. The development of this endodontic tissue engineering therapy appears to present low health hazards to patients, although concerns over immune responses and the possible failure to form functioning pulp tissue must be addressed through careful in vivo research and controlled clinical trials.

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[9]. A scaffold should contain growth factors to aid stem cell proliferation and differentiation, leading to improved and faster tissue development. Growth factors were described in the previous section. The scaffold may also contain nutrients promoting cell survival and growth, and possibly antibiotics to prevent any bacterial in-growth in the canal systems. The engineering of nanoscaffolds may be useful in the delivery of pharmaceutical drugs to specific tissues. In addition, the scaffold may exert essential mechanical and biological functions needed by replacement tissue.

In pulp-exposed teeth, dentin chips have been found to stimulate reparative dentin bridge formation. Dentin chips may provide a matrix for pulp stem cell attachment and also be a reservoir of growth factors. The natural reparative activity of pulp stem cells in response to dentin chips provides some support for the use of scaffolds to regenerate the pulp-dentin complex.

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

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 and 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 . 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.

The principal drawbacks are related to the difficulties of obtaining high porosity and regular pore size. This has led researchers to concentrate efforts to engineer scaffolds at the nanostructural level to modify cellular interactions with the scaffold. Scaffolds may also be constructed from natural materials; in particular, different derivatives of the extracellular matrix have been studied to evaluate their ability to support cell growth. Several proteic materials, such as

collagen or fibrin, and polysaccharidic materials, like chitosan or glycosaminoglycans (GAGs), have not been well studied. However, early results are promising in terms of supporting cell survival and function, although some immune reactions to these types of materials may threaten their future use as part of regenerative medicine.

Injectable Scaffold Delivery

Rigid tissue engineered scaffold structures provide excellent support for cells used in bone and other body areas where the engineered tissue is required to provide physical support. However, in root canal systems a tissue engineered pulp is not required to provide structural support of the tooth. This will allow tissue engineered pulp tissue 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[10]. Hydrogels have the potential to be noninvasive and easy to deliver into root canal systems. In theory, the hydrogel may promote pulp regeneration by providing a substrate for cell proliferation and differentiation into an organized tissue structure.

Past problems with hydrogels included limited control over tissue formation and development, but advances in formulation have dramatically improved their ability to support cell survival. Despite these advances, 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. To make hydrogels more practical, research is focusing on making them photopolymerizable to form rigid structures once they are implanted into the tissue site.

Three-Dimensional Cell Printing

The final approach for creating replacement pulp tissue may be to create it using a three-dimensional cell printing technique[11]. 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. However, early research has yet to show that three-dimensional cell printing can create functional tissue in vivo.

Gene Therapy

The year 2003 marked a major milestone in the realm of genetics and molecular biology. That year marked the 50th anniversary of the discovery of the double-helical structure of DNA by *Watson and Crick*. On April 14, 2003, 20 sequencing centres in five different countries declared the human genome project complete. This milestone will make possible new medical treatments involving gene therapy[12].

All human cells contain a 1-m strand of DNA containing 3 billion base pairs, with the sole exception of non-nucleated cells, such as red blood cells. The DNA contains genetic sequences (genes) that control cell activity and function; one of the most well known genes is p53 . New techniques involving viral or non-viral 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, but still retain the capacity for infection. Several viruses have been genetically modified to deliver genes, including retroviruses, adenovirus, adenoassociated virus, herpes simplex virus, and lentivirus. Non-viral gene delivery systems include plasmids, peptides, gene guns, DNA-ligand complexes, electroporation, sonoporation, and cationic liposomes . 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. However, a literature search indicates there has been little or no research in this field, except for the work of Rutherford [13]. He transfected ferret pulps with cDNA-transfected mouse BMP-7 that failed to produce a reparative response, suggesting that further research is needed to optimize the potential of pulp gene therapy. Our own unpublished observations of inserting mineralizing genes by electroporation into cultures of pulp stem cells have yet to prove successful, suggesting 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, rather than the genes expressed.

The FDA did approve research into gene therapy involving terminally ill humans, but approval was withdrawn in 2003 after a 9-year-old boy receiving gene therapy was found to have developed tumors in different parts of his body[14]. 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.

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