Regenerative Endodontics: A Review of Current Status and a Call for Action

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Abstract

Millions of teeth are saved each year by root canal therapy. Although current treatment modalities offer high levels of success for many conditions, 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. Researchers are working toward this objective. 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. *(J Endod 2007;33:377–390)*

Key Words

Growth factors, pulp regeneration, scaffolds, stem cells, tissue engineering

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Each year approximately $400 billion is spent treating Americans suffering some type of tissue loss or end-stage organ failure. This includes 20,000 organ transplants, 500,000 joint replacements, and millions of dental and oral craniofacial procedures, ranging from tooth restorations to major reconstruction of facial soft and mineralized tissues (1). The regeneration or replacement of oral tissues affected by inherited disorders, trauma, and neoplastic or infectious diseases is expected to solve many dental problems. Within the next 25 years, unparalleled advances in dentistry and endodontics are set to take place, with the availability of artificial teeth, bone, organs, and oral tissues (2, 3); as well as the ability to stimulate endodontic regeneration (4), replace diseased tissues (5) produce vaccinations against viruses (6), and genetically alter disease pathogens to help eradicate caries and periodontitis (7). Patient demand for tissue engineering therapy is staggering both in scope and cost. The endodontic specialty may be able to adopt many of these new scientific advances emerging from regenerative medicine, thereby developing regenerative endodontic procedures and improving patient care.

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. Regenerative dental procedures have a long history, originating around 1952, when Dr. B. W. Hermann reported on the application of $Ca(OH)_2$ in a case report of vital pulp amputation (8). Subsequent regenerative dental procedures include the development of guided tissue or bone regeneration (GTR, GBR) procedures and distraction osteogenesis (9); the application of platelet rich plasma (PRP) for bone augmentation (10), Emdogain for periodontal tissue regeneration (11), and recombinant human bone morphogenic protein (rhBMP) for bone augmentation (12); and preclinical trials on the use of fibroblast growth factor 2 (FGF2) for periodontal tissue regeneration (13). Despite these applications and the considerable evolution of certain medical procedures of tissue regeneration, particularly bone marrow transplants, 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. The importance of the endodontic aspect of tissue engineering has been highlighted by the National Institute for Dental and Craniofacial Research (http://www.nidr.nih.gov/spectrum/NIDCR4/4menu.htm).

An Overview of Regenerative Medicine

Regenerative medicine holds promise for the restoration of tissues and organs damaged by disease, trauma, cancer, or congenital deformity. Regenerative medicine can perhaps be best defined as the use of a combination of cells, engineering materials, and suitable biochemical factors to improve or replace biological functions in an effort to effect the advancement of medicine. The basis for regenerative medicine is the utilization of tissue engineering therapies. Probably the first definition of tissue engineering was by Langer and Vacanti (14) who stated it was "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." MacArthur and Oreffo (15) defined tissue engineering as "understanding the principles of tissue growth, and applying this to produce functional replacement tissue for clinical use." Our own description goes on to say that "tissue engineering is the employment of

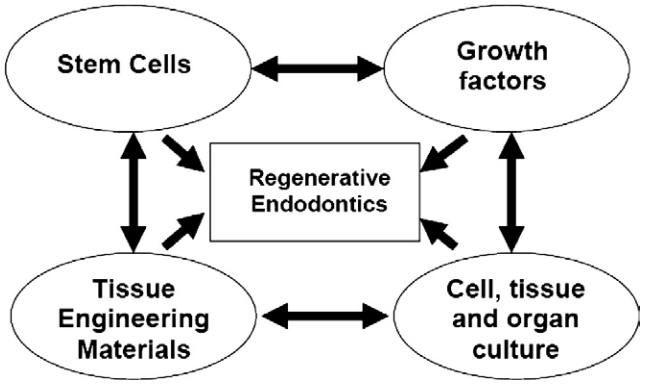


Figure 1. The major domains of research required to develop regenerative endodontic procedures.

biologic therapeutic strategies aimed at the replacement, repair, maintenance, and/or enhancement of tissue function." The changes to the definition of tissue engineering over the years are driven by scientific progress. Although there can be many differing definitions of regenerative medicine, in practice the term has come to represent applications that repair or replace structural and functional tissues, including bone, cartilage, and blood vessels, among organs and tissues (16).

The counterargument to the development of regenerative endodontic procedures is that the pulp in a fully developed tooth plays no major role in form, function, or esthetics, and thus its replacement by a filling material in conventional root canal therapy is the most practical treatment; respectfully, we disagree with this view. In terms of esthetics, there is a potential risk that endodontic filling materials and sealers may discolor the tooth crown (17, 18). In addition, an in vitro study of endodontically treated human teeth found the long-term intracanal placement of calcium hydroxide may reduce the fracture resistance of root dentin (19). A retrospective study of tooth survival times following root canal filling versus tooth restoration found that although root canal therapy prolonged tooth survival, the removal of pulp in a compromised tooth may still lead to tooth loss in comparison with teeth with normal tissues (20). On the other hand, although the replacement pulp has the potential to revitalize teeth, it may also become susceptible to further pulp disease and may require retreatment. The implantation of engineered tissues also requires enhanced microbiological control methods required for adequate tissue regeneration. Thus, additional research in regenerative therapies must be conducted to establish reliable and safe methods for all teeth requiring root canal treatment. In the future, the scope of regenerative endodontics may be increased to include the replacement of periapical tissues, periodontal ligaments, gingiva, and even whole teeth. This would give patients a clear alternative to the artificial tooth implants that are currently available (21). Thus, the potential for this area is indeed vast.

The principles of regenerative medicine can be applied to endodontic tissue engineering. Regenerative endodontics comprises research in adult stem cells, growth factors, organ-tissue culture, and tissue engineering materials (Fig. 1). Often these disciplines are combined, rather than used individually to create regenerative therapies. Many aspects of regenerative endodontics are thought to be recent inventions; however, the long history of research in these fields may be surprising. A brief overview of the potential types of regenerative endodontic therapies is provided in this review.

Adult Stem Cells

Regenerative medicine solves medical problems by using living cells as engineering materials. Potential applications include artificial skin comprised of living fibroblasts (22), cartilage repaired with living chondrocytes (23), or other types of cells used in other ways. The most valuable cells for regenerative medicine are stem cells, with a translational emphasis on the use of postnatal or adult stem cells. Stem cells hold great promise in regenerative medicine, but there are still many unanswered questions that will have to be addressed before these cells can be routinely used in patients, especially in regard to the safety of the procedure. The potential for pulp-tissue regeneration from implanted stem cells has yet to be tested in animals and clinical trials. Extensive clinical trials to evaluate efficacy and safety lie ahead before it is likely the Food and Drug Administration (FDA) will approve regenerative endodontic procedures using stem cells.

All tissues originate from stem cells (24). A stem cell is commonly defined as a cell that has the ability to continuously divide and produce progeny cells that differentiate (develop) into various other types of cells or tissues (25). Stem cells are commonly defined as either embryonic/fetal or adult/postnatal (26). We prefer the term *embryonic*, rather than *fetal*, because the majority of these cells are embryonic. We also prefer the term *postnatal*, rather than *adult*, because these same cells are present in babies, infants, and children. The reason why it is important to distinguish between embryonic and postnatal stem cells is because these cells have a different potential for developing into various specialized cells (i.e. plasticity). Researchers have traditionally found the plasticity of embryonic stem cells to be much greater than that of postnatal stem cells, but recent studies indicate that postnatal stem cells are more plastic than first imagined (27). The plasticity of the stem cell defines its ability to produce cells of different tissues (28). Stem cells are also commonly subdivided into totipotent, pluripotent, and multipotent categories according to their plasticity, as shown in Table 1.

The greater plasticity of the embryonic stem cells makes these cells more valuable among researchers for developing new therapies (29). However, the sourcing of embryonic stem cells is controversial and is surrounded by ethical and legal issues, which reduces the attractiveness of these cells for developing new therapies. This explains why many researchers are now focusing attention on developing stem cell therapies using postnatal stem cells donated by the patients themselves or their close relatives. The application of postnatal stem cell therapy was launched in 1968, when the first allogenic bone marrow transplant was successfully used in the treatment of severe combined immunodeficiency (30). Since the 1970s, bone marrow transplants have been used to successfully treat leukemia, lymphoma, various anemias, and genetic disorders (31). Postnatal stem cells have been sourced from umbilical cord blood, umbilical cord, bone marrow, peripheral blood, body fat, and almost all body tissues (32), including the pulp tissue of teeth (33).

One of the first stem cell researchers was Dr. John Enders, who received the 1954 Nobel Prize in medicine for growing polio virus in

TABLE 1. Types of stem cells

Stem cell type	Cell Plasticity	Source of stem cell
Totipotent	Each cell can develop into a new individual	Cells from early (1–3 days) embryos
Pluripotent	Cells can form any (over 200) cell types	Some cells of blastocyst (5–14 days)
Multipotent	Cells differentiated, but can form a number of other tissues	Fetal tissue, cord blood, and postnatal stem cells including dental pulp stem cells

human embryonic kidney cells (34). In 1998, Dr. James Thomson, isolated cells from the inner cell mass of the early embryo and developed the first human embryonic stem cell lines (35). In 1998, Dr. John Gearhart derived human embryonic germ cells from cells in fetal gonadal tissue (primordial germ cells) (36). Pluripotent stem cell lines were developed from donated embryonic cells. In 2001, the president of the United States, George W. Bush, restricted federal funding to preexisting embryonic cell lines. Of these 78 preexisting embryonic cells lines, 7 were duplicates, 31 were not available, 16 died after thawing, and 2 were withdrawn or are still in development, and the remaining 22 available cell lines did not prove to be very useful to many scientists (37). This restricted most U.S.-based researchers from working on embryonic stem cells. The legal limitations and the great ethical debate related to the use of embryonic stem cells must be resolved before the great potential of donated embryonic stem cells can be used to regenerate diseased, damaged, and missing tissues as part of future medical treatments. Accordingly, there is increased interest in autogenous postnatal stem cells as an alternative source for clinical applications, because these cells are readily available and have no immunogenicity issues, even though they may have reduced plasticity.

Stem cells are often categorized by their source: The most practical clinical application of a stem cell therapy would be to use a patient's own donor cells. Autologous stem cells are obtained from the same individual to whom they will be implanted. Bone marrow harvesting of a patient's own stem cells and their reimplantation back to the same patient represents one clinical application of autogenous postnatal stem cells. Stem cells could be taken from the bone marrow (38), peripheral blood (39), fat removed by liposuction (40), the periodontal ligament (41), oral mucosa, or skin. An example of an autologous cell bank is one that stores umbilical cord stem cells (42). From a medical perspective, among the most valuable stem cells are those capable of neuronal differentiation, because these cells have the potential to be transformed into different cell morphologies in vitro, using lineage-specific induction factors; these include neuronal, adipogenic, chondrogenic, myogenic, and osteogenic cells (43, 44). It may be possible to use neuronal stem cells from adipose fat (43, 44) as part of regenerative medicine instead of bone marrow cells, possibly providing a less painful and less threatening alternative collection method. A company called MacroPore Biosurgery/Cytori Therapeutics Inc. is commercializing this approach, using a 1-hour process for human stem cell purification. Autologous stem cells have the fewest problems with immune rejection and pathogen transmission (45). Harvesting the patient's own cells makes them the least expensive to obtain and avoids legal and ethical concerns (46). However, in some cases suitable donor cells may not be available.

This concern applies to very ill or elderly persons. One potential disadvantage of harvesting cells from patients is that surgical operations might lead to postoperative sequelae, such as donor site infection (47). Autologous postnatal stem cells also must be isolated from mixed tissues and possibly expanded in number before they can be used. This takes time, so certain autologous regenerative medicine solutions may not be very quick. To accomplish endodontic regeneration, the most promising cells are autologous postnatal stem cells (48-51), because these appear to have the fewest disadvantages that would prevent them from being used clinically.

Allogenic cells originate from a donor of the same species (52). Examples of donor allogenic cells include blood cells used for a blood transfusion (53), bone marrow cells used for a bone marrow transplant (54), and donated egg cells used for in vitro transplantation (55). These donated cells are often stored in a cell bank, to be used by patients requiring them. In contrast to the application of donated cells, there are some ethical and legal constraints to the use of human cell lines to accomplish regenerative medicine (56). The use of preexisting cell lines and cell organ cultures removes the problems of harvesting cells from the patient and waiting weeks for replacement tissues to form in cell organ-tissue cultures (57). However, the most serious disadvantages of using preexisting cell lines from donors to treat patients are the risks of immune rejection and pathogen transmission (45). The use of donated allogenic cells, such as dermal fibroblasts from human foreskin, has been demonstrated to be immunologically safe and thus a viable choice for tissue engineering of skin for burn victims (58). The FDA has approved several companies producing skin for burn victims using donated dermal fibroblasts (59). The same technology may be applied to replace pulp tissues after root canal therapy, but it has not yet been evaluated and published.

Xenogenic cells are those isolated from individuals of another species. Pig tooth pulp cells have been transplanted into mice, and these have formed tooth crown structures (60, 61). This suggests it is feasible to accomplish the reverse therapy, eventually using donated animal pulp stem cells to create tooth tissues in humans. In particular, animal cells have been used quite extensively in experiments aimed at the construction of cardiovascular implants (62). The harvesting of cells from donor animals removes most of the legal and ethical issues associated with sourcing cells from other humans. However, many problems remain, such as the high potential for immune rejection and pathogen transmission from the donor animal to the human recipient (46). The future use of xenogenic stem cells is uncertain, and largely depends on the success of the other available stem cell therapies. If the results of allogenic and autologous pulp stem cell tissue regeneration are disappointing, then the use of xenogenic endodontic cells remains a viable option for developing an endodontic regeneration therapy.

Pulp Stem Cells

The dental pulp contains a population of stem cells, called pulp stem cells (63, 64) or, in the case of immature teeth, stem cells from human exfoliated deciduous teeth (SHED) (65, 66). Sometimes pulp stem cells are called odontoblastoid cells, because these cells appear to synthesize and secrete dentin matrix like the odontoblast cells they replace (67). After severe pulp damage or mechanical or caries exposure, the odontoblasts are often irreversibly injured beneath the wound site (68, 69). Odontoblasts are postmitotic terminally differentiated cells, and cannot proliferate to replace subjacent irreversibly injured odontoblasts (70). The source of the odontoblastoid cells that replace the odontoblasts and secrete reparative dentin bridges has proven to be controversial. Initially, the replacement of irreversibly injured odontoblasts by predetermined odontoblastoid cells that do not replicate their DNA after induction was suggested. It was proposed that the cells within the subodontoblast cell–rich layer or zone of Hohl adjacent to the odontoblasts (71) differentiate into odontoblastoids. However, the purpose of these cells appears to be limited to an odontoblast-supporting role, as the survival of these cells was linked to the survival of the odontoblasts and no proliferative or regenerative activity was observed (68, 69). The use of tritiated thymidine to study cellular division in the pulp by autoradiography after damage (72) revealed a peak in fibroblast activity close to the exposure site about 4 days after successful pulp capping of monkey teeth (73). An additional autoradiographic study of dentin bridge formation in monkey teeth, after calcium hydroxide direct pulp capping for up to 12 days (74), has revealed differences in the cellular labeling depending on the location of the wound site. Labeling of specific cells among the fibroblasts and perivascular cells shifted from low to high over time if the exposure was limited to the odontoblastic layer and the cell-free zone, whereas labeling changed from high to low if the exposure was deep into the pulpal tissue. More cells were labeled close to the reparative dentin bridge than in the pulp core. The autoradiographic findings did not show any labeling in the existing

odontoblast layer, or in a specific pulp location. This provided support for the theory that the progenitor stem cells for the odontoblastoid cells are resident undifferentiated mesenchymal cells (75). The origins of these cells may be related to the primary odontoblasts, because during tooth development, only the neural crest– derived cell population of the dental papilla is able to specifically respond to the basement membrane–mediated inductive signal for odontoblast differentiation (76, 77). The ability of both young and old teeth to respond to injury by induction of reparative dentinogenesis suggests that a small population of competent progenitor pulp stem cells may exist within the dental pulp throughout life. However, the debate on the nature of the precursor pulp stem cells giving rise to the odontoblastoid cells, as well as questions concerning the heterogeneity of the dental pulp population in adult teeth, remain to be resolved (78). Information on the mechanisms by which these cells are able to detect and respond to tooth injury is scarce, but this information will be valuable for use in developing tissue engineering and regenerative endodontic therapies.

One of the most significant obstacles to overcome in creating replacement pulp tissue for use in regenerative endodontics is to obtain progenitor pulp cells that will continually divide and produce cells or pulp tissues that can be implanted into root canal systems. Possibilities are the development of an autogenous human pulp stem cell line that is disease- and pathogen-free, and/or the development of a tissue biopsy transplantation technique using cells from the oral mucosa, as examples. The use of a human pulp stem cell line has the advantage that patients do not need to provide their own cells through a biopsy, and that pulp tissue constructs can be premade for quick implantation when they are needed. If a patient provides their own tissue to be used to create a pulp tissue construct, it is possible that the patient will have to wait some time until the cells have been purified and/or expanded in number. This latter point is based on the finding that many adult tissues contain only 1 to 4% stem cells (79), so purification is needed, and expansion of cell numbers would permit collection of smaller tissue biopsies. Alternatively, larger sources of autologous tissue might be required. The sourcing of stem cells to be used in endodontic, dental, and medical therapies is a significant limiting factor in the development of new therapies and should be a major research priority.

Stem Cell Identification

Stem cells can be identified and isolated from mixed cell populations by four commonly used techniques: (a) staining the cells with specific antibody markers and using a flow cytometer, in a process called fluorescent antibody cell sorting (FACS); (b) immunomagnetic bead selection; (c) immunohistochemical staining; and (d) physiological and histological criteria, including phenotype (appearance), chemotaxis, proliferation, differentiation, and mineralizing activity. FACS together with the protein marker CD34 is widely used to separate human stem cells expressing CD34 from peripheral blood, umbilical cord blood, and cell cultures (80). Different types of stem cells often express different proteins on their membranes and are therefore not identified by the same stem cell protein marker. The most studied dental stem cells are those of the dental pulp. Human pulp stem cells express von Willebrand factor CD146, alpha-smooth muscle actin, and 3G5 proteins (81). Human pulp stem cells also have a fibroblast phenoptype, with specific proliferation, differentiation, and mineralizing activity patterns (82).

Growth Factors

Growth factors are proteins that bind to receptors on the cell and induce cellular proliferation and/or differentiation (83). Many growth factors are quite versatile, stimulating cellular division in numerous cell types, while others are more cell specific (84). The names of individual growth factors often have little to do with their most important functions and exist because of the historical circumstances under which they arose. For example, fibroblast growth factor (FGF) was found in a cow brain extract by Gospadarowicz and colleagues (85) and tested in a bioassay which caused fibroblasts to proliferate. Currently, a variety of growth factors have been identified, with specific functions that can be used as part of stem cell and tissue engineering therapies (86–88). Many growth factors can be used to control stem cell activity, such as by increasing the rate of proliferation, inducing differentiation of the cells into another tissue type, or stimulating stem cells to synthesize and secrete mineralized matrix (89–91). A summary of the source, activity and usefulness of common growth factors is shown in Table 2.

If regenerative endodontics is to have a significant effect on clinical practice, it must primarily focus on providing effective therapies for regenerating functioning pulp tissue and, ideally, restoring lost dentinal structure. Toward this aim, increased understanding of the biological processes mediating tissue repair has allowed some investigators to mimic or supplement tooth reparative responses. Dentin contains many proteins capable of stimulating tissue responses. Demineralization of the dental tissues can lead to the release of growth factors following the application of cavity etching agents, restorative materials, and even caries (92). Indeed, it is likely that much of the therapeutic effect of calcium hydroxide may be because of its extraction of growth factors from the dentin matrix (93). Once released, these growth factors may play key roles in signaling many of the events of tertiary dentinogenesis, a response of pulp-dentin repair (94, 95).

Growth factors, especially those of the transforming growth factor-beta (TGF_{β}) family, are important in cellular signaling for odontoblast differentiation and stimulation of dentin matrix secretion. These growth factors are secreted by odontoblasts and deposited within the dentin matrix (96), where they remain protected in an active form through interaction with other components of the dentin matrix (97). The addition of purified dentin protein fractions has stimulated an increase in tertiary dentin matrix secretion (98).

Another important family of growth factors in tooth development (99) and regeneration (100) consists of the bone morphogenic proteins (BMPs). Recombinant human BMP2 stimulates differentiation of adult pulp stem cells into an odontoblastoid morphology in culture (101–103). The similar effects of TGF B1-3 and BMP7 have been demonstrated in cultured tooth slices (104, 105). Recombinant BMP-2, -4, and -7 induce formation of reparative dentin in vivo (106–108). The application of recombinant human insulin-like growth factor-1 together with collagen has been found to induce complete dentin bridging and tubular dentin formation (109). This indicates the potential of adding growth factors before pulp capping, or incorporating them into restorative and endodontic materials to stimulate dentin and pulp regeneration. In the longer term, growth factors will likely be used in conjunction with postnatal stem cells to accomplish the tissue engineering replacement of diseased tooth pulp.

An Overview of Potential Technologies for Regenerative Endodontics

We have identified several major areas of research that might have application in the development of regenerative endodontic techniques. 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 cell printing, and (g) gene delivery. These regenerative endodontic techniques are based on the basic tissue engineering principles already described and include specific consideration of cells, growth factors, and scaffolds.

TABLE 2. The source, activity and usefulness of common growth factors

Abbreviation	Factor	Primary Source	Activity	Usefulness
BMP	Bone morphogenetic proteins	Bone matrix	BMP induces differentiation of osteoblasts and mineralization of bone	BMP is used to make stem cells synthesize and secrete mineral matrix
CSF	Colony stimulating factor	A wide range of cells	CSFs are cytokines that stimulate the proliferation of specific pluripotent bone stem cells	CSF can be used to increase stem cell numbers
EGF	Epidermal growth factor	Submaxillary glands	EGF promotes proliferation of mesenchymal, glial and epithelial cells	EGF can be used to increase stem cell numbers
FGF	Fibroblast growth factor	A wide range of cells	FGF promotes proliferation of many cells	FGF can be used to increase stem cell numbers
IGF	Insulin-like growth factor-I or II	I - liver II–variety of cells	IGF promotes proliferation of many cell types	IGF can be used to increase stem cell numbers
IL	Interleukins IL-1 to IL-13	Leukocytes	IL are cytokines which stimulate the humoral and cellular immune responses	Promotes inflammatory cell activity
PDGF	Platelet-derived growth factor	Platelets, endothelial cells, placenta	PDGF promotes proliferation of connective tissue, glial and smooth muscle cells	PDGF can be used to increase stem cell numbers
TGF-α	Transforming growth factor-alpha	Macrophages, brain cells, and keratinocytes	TGF-α may be important for normal wound healing	Induces epithelial and tissue structure development
TGF-β	Transforming growth factor-beta	Dentin matrix, activated TH_1 cells (T-helper) and natural killer (NK) cells	TGF-β is anti-inflammatory, promotes wound healing, inhibits macrophage and lymphocyte proliferation	TGF-β1 is present in dentin matrix and has been used to promote mineralization of pulp tissue
NGF	Nerve growth factor	A protein secreted by a neuron's target tissue	NGF is critical for the survival and maintenance of sympathetic and sensory neurons.	Promotes neuron outgrowth and neural cell survival

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 overinstrumentation (110–112). 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 (113–115) and increases revascularization of avulsed and necrotic teeth (116, 117), 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 fibers or growth factors (118). 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 (119). This finding suggests that revascularization of necrotic pulps with fully formed (closed) apices might require instrumentation of the tooth apex to approximately 1 to 2 mm in apical diameter to allow systemic bleeding into root canal systems. Clearly, 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 (110). Another important point is that younger adult patients generally have a greater capacity for healing (120).

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. First, 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 (121). Second, enlargement of the apical foramen is necessary to promote vascularizaton 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 (122–126).

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 (127). 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 (4). However, there are several disadvantages to a delivery method of injecting cells. First, the cells may have low survival rates. Second, the cells might migrate to different locations within the body (128), 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. In general, scaffolds, cells, and bioactive signaling molecules are needed to induce stem cell differentiation into a dental tissue type (129). 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 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 (130). 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 (131). 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 (132). 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 (133, 134). So far, growing dental pulp cells on collagens I and III has not proved to be successful (135), 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 μm from the maximum oxygen diffusion distance from a capillary blood supply are at risk of anoxia and necrosis (136). 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 (137). A scaffold should contain growth factors to aid stem cell proliferation and differentiation, leading to improved and faster tissue development (138). Growth factors were described in the previous section. The scaffold may also contain nutrients promoting cell survival and growth (139), 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 (140). In addition, the scaffold may exert essential mechanical and biological functions needed by replacement tissue (141). In pulp-exposed teeth, dentin chips have been found to stimulate reparative dentin bridge formation (142). Dentin chips may provide a matrix for pulp stem cell attachment (143) and also be a reservoir of growth factors (144). 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. Biodegradability is essential, since scaffolds need to be absorbed by the surrounding tissues without the necessity of surgical removal (145). 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 (146). 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 (147).

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 (148). 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 (149). 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 (150). 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 (151). 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 (152, 153), 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 (154). 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 (155, 156). 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 (157). 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 (158). 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 (159).

Three-Dimensional Cell Printing

The final approach for creating replacement pulp tissue may be to create it using a three-dimensional cell printing technique (160). In theory, an ink-jet-like device is used to dispense layers of cells suspended in a hydrogel (161) to recreate the structure of the tooth pulp tissue. The three-dimensional cell printing technique can be used to precisely position cells (162), 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 (163).

Gene Therapy

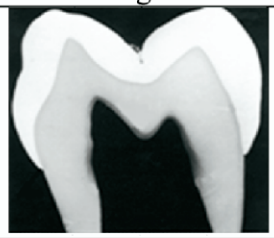
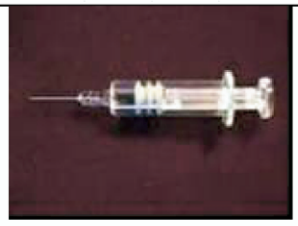
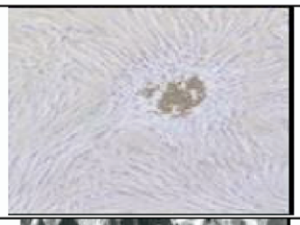
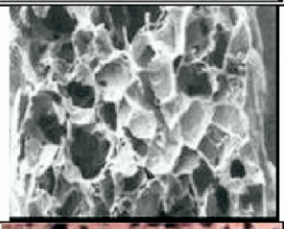
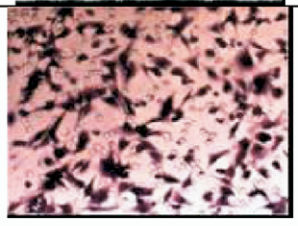
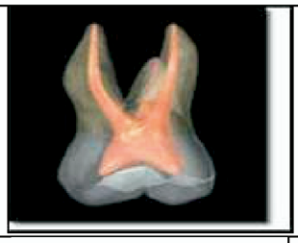
The year 2003 marked a major milestone in the realm of genetics and molecular biology. That year marked the 50th anniversary of the discovery of the double-helical structure of DNA by Watson and Crick. On April 14, 2003, 20 sequencing centers in five different countries declared the human genome project complete. This milestone will make possible new medical treatments involving gene therapy (164). All human cells contain a 1-m strand of DNA containing 3 billion base pairs, with the sole exception of nonnucleated 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 (165). 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 (166). 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 (167, 168). Nonviral gene delivery systems include plasmids, peptides, gene guns, DNA-ligand complexes, electroporation, sonoporation, and cationic liposomes (169, 170). 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 (4). 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 (171). 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 (P.M.) 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 (167). 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 (172). 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. The advantages and disadvantages of these development issues for regenerative endodontic techniques are summarized in Table 3. Gene therapy is a relatively new field, and evidence is lacking to demonstrate that this therapy has the potential to rescue necrotic pulp. At this time, the potential benefits and disadvantages are largely theoretical.

A Call to Action: Research Priorities for Developing Regenerative Endodontic Techniques

The following represents an initial framework to identify major research priorities in developing regenerative endodontic techniques.

TABLE 3. Developmental approaches for regenerative endodontic techniques

Technique	Image	Advantages	Disadvantages
Root-canal revascularization: open up tooth apex to 1 mm to allow bleeding into root canals		✓ Lowest risk of immune rejection ✓ Lowest risk of pathogen transmission	➢ Minimal case reports published to date ➢ Potential risk of necrosis if tissue becomes reinfected
Stem cell therapy: autologous or allogenic stem or cells are delivered to teeth via injectable matrix		✓ Quick, ✓ Easy delivery ✓ Least painful ✓ Cells are easy to harvest	➢ Low cell survival ➢ Cells do not produce new functioning pulp ➢ High risk of complications
Pulp implant: pulp tissue is grown in the laboratory in sheets and implanted surgically		✓ Sheets of cells are easy to grow ✓ More stable than an injection of dissociated cells	➢ Sheets lack vascularity so only small constructs are possible ➢ Must be engineered to fit root canal precisely
Scaffold implant: pulp cells are seeded onto a 3-D scaffold made of polymers and surgically implanted		✓ Structure supports cell organization ✓ Some materials may promote vascularization	➢ Low cell survival after implantation ➢ Must be engineered to fit root canal precisely
3-D cell printing: ink-jet-like device dispenses layers of cells in a hydrogel which is surgically implanted		✓ Multiple cell types can be precisely positioned	➢ Must be engineered to fit root canal precisely ➢ Early-stage research has yet to prove functional in vivo
Injectable scaffolds: polymerizable hydrogels, alone or containing cell suspension are delivered by injection		✓ Easy delivery ✓ May promote regeneration by providing substitute for extracellular matrix	➢ Limited control over tissue formation ➢ Low cell survival ➢ Early-stage research has yet to prove functional in vivo
Gene therapy: mineralizing genes are transfected into the vital pulp cells of necrotic and symptomatic teeth		✓ May avoid cleaning and shaping root canals ✓May avoid the need to implant stem cells	➢ Most cells in a necrotic tooth are already dead ➢ Difficult to control ➢ Risk of health hazards ➢ Not approved by the FDA

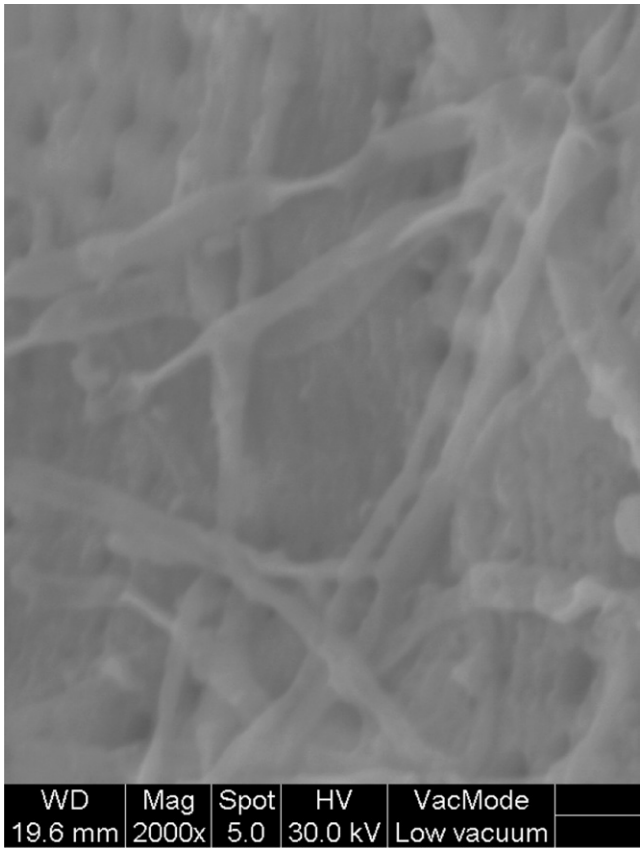


Figure 2. Stem cell obturation of root canals after root dentin irrigation with 6% NaOCl and 17% EDTA. Periodontal stem cells were seeded onto dentin matrix for 1 week in a tissue-culture incubator in the absence of a scaffold. This treatment mimics the use of oral mucosal cells for pulp tissue replacement after cleaning and shaping (unpublished).

They are not listed in order of priority, but rather in the approximate sequence that they might be applied in a particular case.

Improved Methods to Disinfect and Shape Root Canal Systems

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 (173), and it is generally recognized that the long-term prognosis of direct pulp capping infected tissue is poor and not recommended (174). 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. One of us (P.M.) has completed several unpublished investigations of the interactions between periodontal stem cells and the dentin surface. Our initial unpublished results show that relatively few periodontal stem cells will naturally attach and grow on cleaned and shaped root canal systems, as can be seen in Fig. 2, and even fewer cells attach to a dentin smear layer. To successfully attach and adhere to root canal dentin, the stem cells must be supported within a polymer or hydrogel scaffold. Furthermore, we have observed that pulp stem cells, periodontal stem cells, and fibroblasts do not adhere and grow in infected root canal systems; the presence of infection renders the treatment unsuccessful (unpublished observations). This indicates that 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.

Substantial numbers of bacterial species have been identified as inhabitants of the oral cavity. However, because of bacterial interactions, nutrient availability and low oxygen potentials in root canal systems, the numbers of bacterial species present in endodontic infections are restricted (175). These selective conditions lead to the predominance of facultative and strictly anaerobic microorganisms that survive and multiply, causing infections that stimulate local bone resorption (176). Disinfection is one of the main objectives of root canal preparation. 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 (177). Its excellent properties of tissue dissolution and antimicrobial activity make it the irrigant of choice for the treatment of teeth with pulp necrosis, even though it has several undesirable characteristics, such as tissue toxicity at high concentrations and so forth (178, 179). Moreover, sodium hypochlorite does not totally clean the surfaces of the root canal systems (180). Chlorhexidine gluconate has been studied for its various properties, antimicrobial activity (181, 182), and biocompatibility (182), with the objective of evaluating it as an alternative to sodium hypochlorite (183, 184). Disinfection of bacteria is clinically important, particularly *Enterococcus faecalis*, because it has been isolated from infected root canal systems and appears more frequently in cases of revisional endodontic treatment (185, 186).

Regenerative endodontics would benefit from a new generation of irrigants that are as effective as current irrigants, but are nonhazardous to patient tissues. One such irrigation solution under development is based on a plant extract by us (P.M.), which suggests that there are many natural compounds (187) able to clean and disinfect root canal systems, with greatly reduced risk of tissue toxicity. This is an important area of research, because development of the ideal disinfectant, irrigant, and chelating agent would benefit patients and the profession.

Smear Layer Removal

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. The smear layer is a 1- to 5-μm-thick layer (188) of denatured cutting debris produced on instrumented cavity surfaces, and is composed of dentin, odontoblastic processes, nonspecific inorganic contaminants, and microorganisms (189, 190). The removal of the smear layer from the instrumented root canal walls is becoming less controversial in clinical practice (191). Its removal provides better sealing of the endodontic filling material to dentin, and avoids the leakage of microorganisms into oral tissues (192). Chemical chelating agents are used to remove the smear layer from root canal walls, most commonly a 17% solution of ethylenediaminetetraacetic acid (EDTA) that is applied as a final flush (193). Several other solutions have been investigated for removing smear layers,

including doxycycline, a tetracycline congener (194); citric acid (195); and, most recently, MTAD (196). MTAD is an aqueous solution of 3% doxycycline, 4.25% citric acid, and 0.5% polysorbate 80 detergent (197). This biocompatible intracanal irrigant (198) is commercially available as a two-part set that is mixed on demand (BioPure MTAD, DentsplyTulsa, Tulsa, OK). In this product, doxycycline hyclate is used instead of its free base, doxycycline monohydrate, to increase the water solubility of this broad spectrum antibiotic (199). MTAD has been reported to be effective in removing endodontic smear layers (200), eliminating microbes that are resistant to conventional endodontic irrigants and dressings (201), and providing sustained antimicrobial activity through the affinity of doxycycline to bind to dental hard tissues (202, 203). However, its interaction with regenerating pulpal tissue is unknown.

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, which involves enlarging the tooth apex to approximately 1 to 2 mm to allow bleeding into root canals and generation of vital tissue 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. Each of these techniques to regenerate pulp tissue will have advantages and limitations that still have to be defined through basic science and clinical research.

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 (204). 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. This tissue construct may involve an injectable slurry of [hydrogel + cells + X (growth factors, etc)] or [hydrogel + X (growth factors, etc.)], then this two-layer method would be fairly easy to accomplish. Moreover, 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. Both these delivery methods reduce the need for an autogenous pulp stem cell population that will not be readily available to endodontists, because the teeth requiring treatment are presumably infected and necrotic. This proposed delivery method would help avoid the potential for immune and infection issues surrounding the use of an allogenic pulp stem cell line. Of course, these alternative methods must be investigated using preclinical in vitro studies, usage studies in animals, and, eventually, clinical trials.

TABLE 4. Summary of the barriers to be addressed to permit the introduction of regenerative endodontics

Disinfection and shaping of root canals in a fashion to permit regenerative endodontics.
Chemomechanical debridement — cleaning and shaping root canals
Irrigants — 6% sodium hypochlorite and 2% chlorhexidine gluconate and alternatives
Medicaments — Calcium hydroxide, triple antibiotics, MTAD, and alternatives
Creation of replacement pulp-dentin tissues
Pulp revascularization by apex instrumentation
Stem cells; allogenic, autologous, xenogenic, umbilical cord sources
Growth factors; BMP-2, -4, -7; TGF-B1,-B2,-B3 among others
Gene therapy; identification of mineralizing genes
Tissue engineering; cell culture, scaffolds, hydrogels
Delivery of replacement pulp-dentin tissues
Surgical implantation methods
Injection site
Dental restorative materials
Improve the quality of sealing between restorative materials and dentin
Ensure long-term sealing to prevent recurrent pulpitis
Measuring appropriate clinical outcomes
Vascular blood flow
Mineralizing odontoblastoid cells
Intact afferent innervations
Lack of signs or symptoms

Measuring Appropriate Clinical Outcomes

Once a tissue engineered pulp has been implanted, it is not ethical to remove functioning tissues to conduct a histological analysis. Therefore, it will not be possible to histologically investigate mineralizing odontoblastoid cell functioning or nerve innervation. Clinicians will have to rely on the noninvasive tests in use today, such as laser Doppler blood flowmetry in teeth (205); pulp testing involving heat, cold, and electricity (206); and lack of signs or symptoms. Magnetic resonance imaging (MRI) has shown the potential to distinguish between vital and nonvital tooth pulps (207), but MRI machines are very expensive and must be greatly reduced in price to become widespread. The ideal clinical outcome is a nonsymptomatic tooth that never needs retreatment, but nonsubjective vitality assessment methods are essential to validate that regenerative endodontic techniques are truly effective. A summary of the challenges to the introduction of regenerative endodontics are shown in Table 4.

Conclusions

The clinical success rates of endodontic treatments can exceed 90% (208–210). 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.

Several developmental issues have been described to accomplish endodontic regeneration. Each one of the regenerative techniques has advantages and disadvantages, and some of the techniques are hypothetical, or at an early stage of development. The available case reports of pulp revascularization were generally reported on young patients (with high stem cell populations) and teeth with open apices. However, for regenerative endodontic procedures to be widely available and predictable, endodontists will have to depend on tissue engineering therapies to regenerate pulp dentin tissue. The proposed therapies involving stem cells, growth factors, and tissue engineering all require pulp re-

vascularization, in itself an enormous challenge. One of the most challenging aspects of developing a regenerative endodontic therapy is to understand how the various component procedures can be optimized and integrated to produce the outcome of a regenerated pulp-dentin complex. The future development of regenerative endodontic procedures will require a comprehensive research program directed at each of these components and their application to our patients. The authors believe that regenerative endodontics is an inevitable therapy, and they call for action from scientists, funding agencies, and the endodontic profession to pool resources to hasten its development. The unleashed potential of regenerative endodontics may benefit millions of patients each year.

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