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Regenerative Endodontics: Barriers and Strategies for Clinical Translation

Sahng G. Kim, DDS, MS^{1,2}, Jian Zhou, DDS, PhD¹, Ling Ye, DDS, PhD³, Shoko Cho, MD, PhD¹, Takahiro Suzuki, DDS, PhD¹, Susan Y. Fu, MD, PhD¹, Rujing Yang, MD¹, Xuedong Zhou, DDS, PhD³, and Jeremy J. Mao, DDS, PhD^{1,*}

¹Center for Craniofacial Regeneration, Columbia University

²Division of Endodontics, College of Dental Medicine, Columbia University 630 W. 168 St. – PH7E, New York, NY 10032

³Department of Endodontics, West China School of Stomatology Chengdu, China

SYNOPSIS

Despite a great deal of enthusiasm and effort, regenerative endodontics has encountered substantial challenges towards clinical translation. Recent adoption by the American Dental Association (ADA) of evoked pulp bleeding in immature permanent teeth is an important step for regenerative endodontics. However, there is no regenerative therapy for the majority of endodontic diseases. Simple recapitulation of cell therapy and tissue engineering strategies that are under development for other organ systems has not led to clinical translation in regeneration endodontics. Dental pulp stem cells may appear to be *a priori* choice for dental pulp regeneration. However, dental pulp stem cells may not be available in a patient who is in need of pulp regeneration. Even if dental pulp stem cells are available autologously or perhaps allogeneically,

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***Corresponding author.** Jeremy J. Mao, DDS, PhD, Professor and Zegarelli Endowed Chair, Director, Center for Craniofacial Regeneration, Columbia University, 630 W. 168 St. - PH7E, New York, NY 10032, Phone: 212-305-4475, jmiao@columbia.edu.
Sahng G. Kim, DDS, MS, Assistant Professor of Clinical Dental Medicine, Division of Endodontics, College of Dental Medicine, Columbia University, 630 W. 168 St. - PH7Stem #128, New York, NY 10032, sgk2114@columbia.edu
Jian Zhou, DDS, PhD, Center for Craniofacial Regeneration, Columbia University, 630 W. 168 St. - PH7E, New York, NY 10032, jz2422@columbia.edu
Ling Ye, DDS, Ph.D, Department of Endodontics, West China School of Stomatology, Sichuan University, 14#, 3rd section of Ren Min Nan Lu, Chengdu, Sichuan, China, 610041, yeling@scu.edu.cn
Shoko Cho, MD, PhD, Center for Craniofacial Regeneration, Columbia University, 630 W. 168 St. - PH7E, New York, NY 10032, sc3325@columbia.edu
Takahiro Suzuki, DDS, PhD, Center for Craniofacial Regeneration, Columbia University, 630 W. 168 St. - PH7E, New York, NY 10032, ts2490@columbia.edu
Susan Y. Fu, MD, PhD, Center for Craniofacial Regeneration, Columbia University, 630 W. 168 St. - PH7E, New York, NY 10032, fusions@gmail.com
Rujing Yang, MD, Center for Craniofacial Regeneration, Columbia University, 630 W. 168 St. - PH7E, New York, NY 10032, ry2164@columbi.edu
Xuedong Zhou, DDS, Ph.D, West China School of Stomatology, Sichuan University, 14#, 3rd section of Ren Min Nan Lu, Chengdu, Sichuan, China, 610041, zhoudx@scu.edu.cn

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Conflict of Interest

Columbia University is the owner of patents for several regenerative endodontic agents and methods on behalf of Dr. Jeremy Mao's laboratory.

one must address a multitude of scientific, regulatory and commercialization barriers, and unless these issues are resolved, transplantation of dental pulp stem cells will remain a scientific exercise, rather than a clinical reality. Recent work using novel biomaterial scaffolds and growth factors that orchestrate the homing of host endogenous cells represents a departure from traditional cell transplantation approaches and may accelerate clinical translation. Given the functions and scale of dental pulp and dentin, regenerative endodontics is poised to become one of the early biological solutions in regenerative dental medicine.

Keywords

regenerative; endodontics; pulp; dentin; regeneration; stem cells; tissue engineering

1. Introduction

Endodontics is a dental specialty that treats trauma and infections involving the dental pulp, dentin and periapical lesions. Each year, a total of ~16 million endodontic procedures are performed in the United States alone.¹ Root canal treatment (RCT) that involves the extirpation of the injured or infected dental pulp, and filling of the root canal and pulp chamber with bioinert materials is the most common endodontic treatment.² Success rates for endodontic therapies vary, depending on case selection, practitioner skills and availability of instruments and materials, etc. In general, current endodontic treatments are effective to eliminate pain and control infections.^{3,4} Therefore, why regenerative endodontics? Endodontic therapies, like many other dental treatments, are not without failure. Re-infections and tooth fractures are among some of the undesirable and frustrating complications for either the patient or the practitioner, and lead to additional lost work hours. Table 1 demonstrates causes and incidences of failure of common endodontic treatments, as well as how regenerative endodontics may address current endodontic failures.

In January 2011, the American Dental Association (ADA) adopted a new procedure code to allow practitioners to induce apical bleeding into the root canal in immature permanent teeth with necrotic pulps that have been extirpated.⁵ This is an important step by the endodontic community on its path to explore avenues of pulp and dentin regeneration. Endodontic treatment for immature permanent teeth with root apex not yet fully developed presents a unique clinical challenge. Delivery of conventional root canal therapy (RCT) in an injured or infected immature permanent tooth, while effective in removing infections and in managing symptoms, have a tendency to cause the arrest of root development. As meritorious as it is, the clinical consistency of this “induced bleeding” technique is lacking at this time. The endodontic community has, for decades, been searching for vital pulp therapies.⁶ Regenerative endodontics was proposed as an alternative to conventional endodontic therapies including RCT and others.⁷ In this review, we will discuss the challenges for translating current concepts in regenerative endodontics, and identify strategies to address existing barriers towards the development of clinical viable therapies.

2. Clinical Studies of Pulp Revascularization

Regenerative endodontics benefits from previous studies of pulp revascularization. The clinical rule of thumb has been that pulp revascularization may be accomplished in immature teeth with apical foramen > 1 mm in diameter.^{8,9} As early as 1966, Rule and Winter¹⁰ presented a case of continued root formation with apical closure of a non-vital immature mandibular premolar, following pulp bleeding. The root canal was mechanically instrumented and dressed with polyantibiotics, followed by absorbable iodoform placement. Nygaard-Ostby et al.¹¹ reported new connective tissue formation in root canals after complete pulpectomy and root filling short of the apical foramen. Nevin et al.¹² showed successful root maturation with a collagen-calcium phosphate gel introduced in an intruded maxillary lateral incisor with an immature root apex after root canal debridement. A case report by Iwaya et al.¹³ showed revascularization with apical closure and thickening of the root canals wall in a 13-year-old patient with a necrotic, immature, mandibular premolar secondary to a fractured dental tubercle. A similar case was reported by Banchs and Trope,¹⁴ in an 11-year-old with a necrotic, immature mandibular premolar following a fractured dental tubercle. The canal was irrigated with 5.25% sodium hypochlorite and Peridex without mechanical debridement. A triple antibiotic paste (minocycline, metronidazole and ciprofloxacin) was placed into the canal for one month and removed before bleeding was induced into root canal space with an endodontic explorer. Mineral trioxide aggregate (MTA) was placed over the blood clot ~3 mm below the CEJ followed by a bonded restoration. The tooth was responsive to cold at a 2-year follow-up and showed continued root maturation.¹⁴ There are additional case reports of pulp revascularization in necrotic, immature teeth that show continuous root maturation with dentinal wall thickening and a positive response to vitality tests.¹⁵⁻²² However, no randomized clinical trials have been performed on the efficacy of pulp revascularization and root development following induced pulp bleeding in immature permanent teeth.

Torabinejad and Turman²³ reported a case of pulp revascularization using platelet-rich plasma (PRP) in a replanted, immature, necrotic maxillary premolar following accidental extraction. The necrotic pulp was removed with a barbed broach. The canal was irrigated with 5.25% sodium hypochlorite and medicated with a triple antibiotic paste for 22 days. PRP prepared from the patient's blood was injected into the canal space. The canal was sealed with Cavit and amalgam after MTA was placed over the PRP clot. At 5–6 month follow up, continued root maturation and root canal thickening was observed radiographically, along with a positive vitality test.

In summary, isolated clinical case reports have shown tangible results of pulp revascularization and some level of dentin/root development in immature, permanent teeth following pulp bleeding or PRP delivery, as a point-of-care approach. As clinically meritorious as these attempts are, treatment outcomes are anticipated to be variable, depending on a multitude of factors including the patient's intrinsic responses, the severity of disease, case selection and practitioner skills. There is a lack of randomized, prospective clinical trials without which it is virtually impossible to compare two or more therapies, or even to achieve a consistent outcome for a single therapy. Platelet-rich plasma (PRP) suffers from the shortcoming of drawing blood from the patient, and complexity of centrifuge and

purification. Again, practitioner variation is expected to affect the clinical outcome of PRP as a regenerative endodontics therapy. A clinically meritorious trial is currently underway to compare a triple antibiotic paste and induced bleeding into the root canal system with the standard treatment for immature necrotic teeth (MTA apexification).²⁴

Are there differences between pulp revascularization and pulp regeneration? In the endodontic literature, pulp revascularization is usually defined as re-introduction of vascularity in the root canal system.²⁵ Pulp regeneration, on the other hand, has not been precisely defined. Given that rigid definitions tend to restrict the development of new drugs, devices and therapies, it may be beneficial not to excessively debate the differences between pulp revascularization and pulp regeneration at this time. Synopses from several recent review articles on regenerative endodontics appear to indicate that pulp regeneration is the restoration of the pulp-dentin complex, which nonetheless is still somewhat vague.^{7,26–28} Our view is that pulp regeneration cannot take place without revascularization or angiogenesis, but pulp revascularization appears to indicate restoration of vascularity in the pulp but not necessarily the repopulation of odontoblasts that align on dentin surfaces. Although blood vessels are indispensable constituents of dental pulp, pulp regeneration is likely considered incomplete without an odontoblastic layer lining the dentin surface. Pulp regeneration may also be considered incomplete without nociceptive as well as sympathetic and parasympathetic nerve fibers, in addition to interstitial fibroblasts and perhaps most importantly, stem/progenitor cells that serve to replenish all pulp cells in the regenerated pulp when they undergo apoptosis and turnover. Thus, it may be helpful to conceptualize:

- Pulp revascularization = induction of angiogenesis in endodontically treated root canal.
- Pulp regeneration = pulp revascularization + restoration of functional odontoblasts and/or nerve fibers.

3. Regenerative Endodontics: Is it just a scientific exercise or can it become clinical treatments?

The American Association of Endodontists (AAE) deserves credit for its vision to enthusiastically endorse regenerative endodontics as one of the profession's future directions. However, ~16 years following the introduction of the concept of regenerative endodontics to the profession,²⁹ there is an element of palpable uncertainty and, perhaps frustration that regenerative endodontics remains a scientific exercise, rather than a clinical reality. This is demonstrated vividly by an acute and yet collegial exchange of Letters to the Editor of Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology. The initial letter was written by Professor Spångberg entitled "The Emperor's new cloth".³⁰ Professor Spångberg expressed concerns over the view that pulp re-vascularization, by evoked bleeding or triple antibiotic pastes, equates to pulp regeneration that is regarded as a paradigm shift in endodontics. The Spångberg letter was countered by a letter entitled "The wrong emperor" from Professor Kenneth Hargreaves and Dr. Alan Law, stating that pulp re-vascularization as in the classic endodontic literature has not utilized the principles of tissue engineering, namely stem cells, scaffolds and growth factors, and differs from recent

experimental studies of pulp regeneration.³¹ The matter is perhaps not as clear-cut as we may all have wished for, as evidenced by the following challenges.

3.1. Challenge #1 - What cells should be used for pulp/dentin regeneration?

Several studies have shown that transplantation of dental pulp or other stem cells can yield ectopic dental pulp-like tissues in tooth slices or fragments in vivo.^{32–36} Huang et al.³⁴ showed the formation of pulp-like tissue with dentin deposition in root fragments in mice by implanting stem/progenitor cells from the apical papilla and dental pulp. Cordeiro et al.³⁷ demonstrated the formation of vascular pulp-like tissue by implantation of a tooth slice with a gel that was seeded with stem cells from human exfoliated deciduous teeth (SHED), into the dorsum of immunodeficient mice. Cell delivery has certain advantages such as its ability to control the number of cells transplanted and potential utility of subpopulation of stem/progenitor cells. For example, isolated stem/progenitor cells can be sorted to select the “best” subpopulations, which are yet to be identified, for pulp regeneration. Iohara et al.^{35,38} showed that CD31[−]/CD146[−] or CD105⁺ side population (SP) cells from dental pulp had higher self-renewal capacity and differentiation potential compared to the parent, heterogeneous cells. These fractionated cells have been shown to have a greater potential of inducing the formation of nerves, vasculature and dentin in the root canal space.³³

Why should there be a question as to what cells are to be used for dental pulp regeneration? Many would say ‘dental pulp stem cells’. But consider this – for a patient in need of regenerative root canal therapy of a given tooth with the rest of the dentition completely healthy, what is the source of dental pulp stem cells? Wisdom teeth, if present, may be a theoretical source. However, the cost of the therapy is likely excessive by the time dental pulp stem cells are extracted from the wisdom teeth, processed in a scientific laboratory and shipped back to the practitioner, not to mention the risks of contamination, and acquisition of tumorigenesis upon cell culture, the lack of expertise of practitioners to handle cells, perceived poor consistency between patients, and difficulty with regulatory approval, etc. Cell therapy for medically incurable diseases, such as diabetes, Parkinson’s or spinal cord injuries, may well be acceptable at a high cost and some risks, but perhaps not for vital root canal therapy. Also, it is probable that the cost of ex vivo cell manipulation for the development of cell therapies of a medically incurable disease, such as spinal cord injuries, may be not that different from that of dental pulp regeneration. An important recent study showed that stem/progenitor cells can be isolated from inflamed pulp tissues.³⁹ Inflammation is known to stimulate the recruitment and/or differentiation of stem/progenitor cells. However, the risks of contamination and inconsistency are likely associated with the delivery of stem/progenitor cells that are isolated from infected pulp tissues, not to mention potential practitioner liabilities in case stem/progenitor cells from the patient’s infected pulp tissue fail to regenerate the pulp. Other stem cells including periodontal ligament, oral mucosa, dental follicle, apical papilla, bone marrow and adipose stem cells not only suffer from the same pitfalls as dental pulp stem cells as far as pulp regeneration is concerned, but also face additional uncertainty whether they can regenerate pulp tissues.

Despite its scientific validity, cell transplantation has encountered major difficulties in translation into a clinical therapy in regenerative endodontics. The therapeutic use of stem

cell products derived from a nonhuman species will be limited because of the risk of immunorejection from nonhuman animal cells. Allogeneic cell transplantation has concerns of potential immunorejection and contamination. Cell cryopreservation/banking system suffers from potential loss of cells and additional costs. Potential contamination during cell manipulation, and additional costs of shipping and storage are additional barriers of cell transplantation. Few practitioners today know how to handle a vial of cells. In case a few cells, among thousands or millions of cells that are transplanted, acquire oncogenes during *ex vivo* cell processing, a practitioner, company or hospital would likely be held liable, not to mention a tragic clinical outcome.

As an alternative to cell transplantation, Kim et al.⁴⁰ showed the regeneration of the pulp-dentin complex by cell homing. Root canal therapy was performed in clinically extracted human teeth including pulp extirpation and instrumentation, with the only exception of root canal filling, followed by autoclaving of the teeth to remove any biological or organic components. Instead of gutta percha, growth factors in a collagen scaffold were placed in surgically treated root canals of the human teeth that were autoclaved to remove any biological or organic components. Following 3- to 6-wk *in vivo* implantation in the dorsum of rats, dental pulp-like tissue formed in the entire length of root canals with vascular, odontoblastic-like and neural components⁴⁰. This was the first study to demonstrate regeneration of dental pulp-like tissues by the homing of host endogenous cells and without cell transplantation. Growth factors selected for pulp-dentin regeneration in this study include basic fibroblast growth factors (bFGFs), vascular endothelial growth factors (VEGFs), platelet-derived growth factors (PDGFs), nerve growth factors (NGFs) and bone morphogenetic protein-7 (BMP7). bFGF was selected for chemotaxis and angiogenesis; VEGF for chemotaxis, mitogenesis, and angiogenesis; PDGF for angiogenesis; NGF for survival and growth of nerve fibers; and BMP-7 for mineralized tissue formation. Cell homing was originally defined as migration of hematopoietic stem cells from bone marrow to the periphery, and ultimately to stem cell niches. We extended this concept to describe the recruitment of host endogenous cells, likely including stem/progenitor cells, and subsequent tissue formation.⁴¹ A major advantage of cell homing is to regenerate by host endogenous cells, and the elimination of all *ex vivo* cell processing steps that are necessarily associated with cell transplantation.

Certain signaling molecules serve as bioactive cues that orchestrate the regenerative process. Signaling molecules include growth factors, cytokines, chemical compounds or hormones. Among them, PDGF, VEGF, bFGF, BMPs and NGF have been tested in dental pulp regeneration.⁴⁰ Their effects have been extensively discussed in 'Interactions and effects of growth factors on dental pulp stem cells' in this issue of the *Dental Clinics of North America*. Controlled release of signaling molecules may be necessary to address rapid diffusion and enzymatic breakdown of peptides and proteins.^{41,42} For example, control-released FGF2 from gelatin hydrogels promoted the formation of dentin-particles in amputated rat molar pulp, whereas adsorption of FGF-2 in collagen sponges induced reparative dentin formation.⁴³ In an effort to develop scaffolds with controlled release of growth factors, PLGA-based fibrous scaffold have been developed to demonstrate the release of BMP-2 in a linear pattern over a period of about 4 weeks.^{44,45} Table 2 below

compares the pros and cons of cell transplantation and cell homing approaches in dental pulp regeneration.

3.2. Challenge #2 - What scaffolding material should be used in pulp/dentin regeneration?

Scaffolds are likely indispensable for pulp/dentin regeneration and provide structural support for cells, either transplanted or endogenously homed while they synthesize tissues.⁴⁶ Scaffolds can be either native or synthetic materials.⁴⁶ An ideal scaffold should promote cell attachment and provide a conducive environment for pulp or dentin regeneration.

Natural polymers include natural extracellular matrix components such as collagen and fibronectin, typically biocompatible and biodegradable. Zhang et al.⁴⁷ used a collagen sponge, a porous ceramic and a fibrous titanium mesh seeded with dental pulp stem cells. Mineralized deposits were observed with the expression of dentin sialoprotein after cells were cultured in osteogenic medium for 4 wks. Upon the implantation of cell-seeded scaffolds subcutaneously in nude mice for 6 or 12 wks, dentin sialophosphoprotein was expressed in all scaffolds, but mainly connective tissues were observed to be regenerated except for in ceramic group, where some calcification was observed.⁴⁷

Hyaluronic acid (HA) sponge as a natural polymer has been used in dental pulp regeneration. Inuyama et al.⁴⁸ investigated the effect of HA sponge on odontoblastic cell lines (KN-3) *in vitro* and on amputated dental pulp of rat molar *in vivo*. *In vitro*, KN-3 cells attached to HA and collagen sponges. Expression of IL-6 and TNF- α in KN-3 cell-seeded HA sponge were almost equivalent to those in collagen sponge, and the numbers of granulated leukocytes migrated into HA sponge were significantly lower than those into collagen sponge.

Chitosan, chemically similar to cellulose, has been investigated for the use as a scaffold for dental pulp regeneration.⁴⁹ Chitosan monomers (D-glucosamine hydrochloride) were tested to investigate the cell metabolism and wound healing in *in vitro* and *in vivo* experiments. The expression of alkaline phosphatase (ALP) activity increased significantly in 3 days of culture of the chitosan monomer group. Bone morphogenetic protein-2 activity also increased after 7 days of osteoblast culture. IL-8 synthesis was suppressed by the chitosan monomer in dental pulp cells. *In vivo*, direct pulp capping with chitosan monomer in rats showed that chitosan monomer induced minimal inflammatory cell infiltration after 1 day, and promoted proliferation of pulp fibroblasts after 3 days, induced mineralization by odontoblastic cells after 5 and 7 days.⁴⁹

A number of additional synthetic polymers such as poly(lactic) acid (PLA), poly(l-lactic) acid (PLLA), poly(glycolic) acid (PGA), poly(D,L-lactide-co-glycolide) (PLGA), and poly(ϵ -caprolactone) (PCL) have been suggested as potential scaffolds for pulp regeneration. The synthetic polymers are non-toxic, biodegradable and allow precise manipulation of the physicochemical properties such as mechanical stiffness, degradation rate, porosity and microstructure.⁵⁰

PLA and PLLA have been used as synthetic scaffolds in *in vivo* studies for pulp regeneration. Cordeiro et al.¹⁷ used PLA scaffolds seeded with SHED in a tooth slice

implantation model. The subcutaneously implanted tooth slices with SHED-seeded PLA scaffolds into immunodeficient mice have been demonstrated to induce differentiation into odontoblast-like cells and endothelial-like cells in newly regenerated tissue based on the expression of dentin sialoprotein and B-galactosidase staining, respectively and facilitate the regeneration of pulp-like tissue. Similar experiments were carried out by Sakai et al.,⁵¹ showing SHED-seeded PLLA scaffolds promoted dental pulp cell differentiation into endothelial cells and odontoblasts.

PLGA has been widely used as a synthetic polymer scaffold in tissue engineering^{52,53} and recently used in an *in vivo* pulp regeneration study. Huang et al.¹¹ used PLGA scaffolds seeded with stem/progenitor cells from apical papilla and dental pulp stem cells in a tooth slice implantation model. It was demonstrated that dentin-like tissue along the wall of root canals and pulp-like tissue could be regenerated after 3–4 months of subcutaneous implantation of teeth with cell-seeded PLGA scaffolds into immunocompromised mice.

A few studies have investigated the performance of nanofiber scaffolds for regeneration of the dentin-pulp complex. In a study by Yang et al.,⁵⁴ electrospun nanofibers consisting of PCL/gelatin with or without nano-hydroxyapatite (nHA) which were seeded with rat dental pulp stem cells (DPSCs) and tested in *in vitro* and *in vivo* experiments. Nanofiber scaffolds supported cell proliferation and odontoblastic differentiation on the basis of DNA content, ALP activity and osteocalcin expression. Subcutaneous implantation of the cell-seeded nanoscaffolds showed mineralized tissue formation without inflammatory response, suggesting a potential use for pulp capping. Wang et al.^{55,56} also showed that nanofibers fabricated from PLLA polymers could enhance attachment, proliferation and odontoblastic differentiation of human dental pulp stem/progenitor cells *in vivo* and *in vitro*.

4. Conclusions

Despite initial promise, regenerative endodontics has encountered substantial barriers in clinical translation. Dental pulp stem cells may appear to be *a priori* choice for dental pulp regeneration. However, dental pulp stem cells may not be available in a patient who is in need of pulp/dentin regeneration therapy. Even if dental pulp stem cells are available autologously or perhaps allogeneically, one must address a multitude of scientific, regulatory and commercialization barriers, and unless these issues are resolved, transplantation of dental pulp stem cells will remain a scientific exercise, rather than a clinical reality. These barriers include cell isolation, *ex vivo* manipulation with the potential for changing cell phenotype, and safety issues including immunorejection, potential contamination, pathogen transmission and tumorigenesis. Excessive costs associated with all of these in addition to shipping, storage, handling issues, and regulatory issues including unclear pathway and general inability to ensure batch-to-batch consistency in cell quality cast multi-dimensional questions for the practicality of this approach. Cell homing offers an alternative to cell transplantation for dental pulp/dentin regeneration. The release strategies of signaling molecules that orchestrate cell homing needs to be further defined. Biomaterial scaffolds are another area of innovation in regenerative endodontics. A number of natural and synthetic polymers have shown positive results *in vivo*. Preclinical animal models and

randomized clinical trials that test novel therapies are indispensable for translating regenerative technologies into clinical therapies.

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Key Points

1. Despite a great deal of enthusiasm and effort, regenerative endodontics has encountered substantial challenges towards clinical translation. Recent adoption by the American Dental Association (ADA) of evoked pulp bleeding in immature permanent teeth is an important step for regenerative endodontics. However, there is no regenerative therapy for the majority of endodontic diseases.
2. Simple recapitulation of cell therapy and tissue engineering strategies that are under development for other organ systems has not led to clinical translation in regeneration endodontics. Dental pulp stem cells may appear to be *a priori* choice for dental pulp regeneration. However, dental pulp stem cells may not be available in a patient who is in need of pulp regeneration.
3. Even if dental pulp stem cells are available autologously or perhaps allogeneically, one must address a multitude of scientific, regulatory and commercialization barriers, and unless these issues are resolved, transplantation of dental pulp stem cells will remain a scientific exercise, rather than a clinical reality.
4. Recent work using novel biomaterial scaffolds and growth factors that orchestrate the homing of host endogenous cells represents a departure from traditional cell transplantation approaches and may accelerate clinical translation. Given the functions and scale of dental pulp and dentin, regenerative endodontics is poised to become one of the early biological solutions in regenerative dental medicine.

Table 1

Causes and incidence of failure of nonsurgical endodontic treatments and benefits of regenerative endodontic therapy.

Therapy	Causes	Incidence of failure	Regenerative therapy
Primary root canal treatment	Persistent infection	15%–32% ³	Immunological defense
	Tooth/Root fracture		Restored homeostasis
	Restoration failure		Functional pulp-dentin complex
Secondary root canal treatment	Persistent infection	23% ⁴	Immunological defense
	Tooth/Root fracture		Restored vascular supply
	Restoration failure		Option to deliver antibiotics

Table 2

Comparison of cell transplantation and cell homing approaches for regenerative endodontics

	Cell transplantation	Cell homing
Benefits	Ability to control cell number	No cell isolation
	Availability of a cell source in periapical defects	No immunorejection
	Utility of subpopulations of stem/progenitor cells	No <i>ex vivo</i> cell processing steps
Barriers	Cell isolation from patients required	Shortage of cells for regeneration in periapical defects
	Immunorejection	
	Contamination during cell manipulation, transplantation, and storage	
	Tumorigenesis	
	High cost for commercialization and manufacturing stem cell products	