Translational Opportunities in Stem Cell–based Endodontic Therapy: Where Are We and What Are We Missing?

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Abstract
Pulp regeneration is a biologic process occurring under specific circumstances. An endodontic treatment modality to accomplish pulp regeneration has emerged based on the response of undifferentiated cells that are often referred to as stem cells. The treatment itself is currently empirical based on clinicians’ thoughts and observations. The demonstration of a variety of dental stem cells initiated basic research detailing the properties and behavior of these cells. Attempts are made to bridge gaps in knowledge regarding treatment strategies by translating basic stem cell research into practice. However, neither the patient population likely to benefit from pulp regeneration nor most clinical parameters are well described. Classic topics in endodontics (eg, indication/clinical diagnosis, disinfection/irrigation, and root canal preparation/pretreatment) have to be revisited under the premises of pulp regeneration. Furthermore, new topics like the development of new diagnostic tools or new clinical success criteria will emerge, and the translational research itself will generate new insight into pulp regeneration mechanisms. (J Endod 2014;40:S82–S85)

Key Words
Endodontics, stem cell research, translational opportunities

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coot canal treatments are a very frequently performed component of dental practice worldwide and are often essential to retaining the natural dentition. Although progress has been made in the endodontic treatment technique, overall strategies and their success rates appear to be largely unchanged in the last 50 years (1–3). However, the isolation and characterization of various types of mesenchymal stem cells (MSCs) in dental tissues (4) may constitute a step toward a paradigm shift in the development of alternate, biologic treatment strategies. MSCs are cells capable of self-renewal and differentiation in vitro and in vivo into several tissues (eg, bone, cartilage, and adipose tissue) (5, 6). Basic science research into stem cells is a focus of current funding and publication efforts worldwide, but the history of a notion of stem cells is more than a century old (7). Maksimov described vascular stem cells in 1908, and the first relevant studies in embryonic stem cell research appeared in the 1950s (8).

It is not infrequent that basic research does not lead to clinical application in a timely fashion. That is why a systematic approach occurred in the United States to support translational research with the inception in 2006 of centers at various National Institutes of Health institutes dedicated to this endeavor (9). At least 2 journals (Translational Medicine and the Journal of Translational Medicine) are devoted to the topic; the hope is that medicine and dentistry will benefit from stem cell research translated to the clinic more broadly in the future (10).

Currently, clinical endodontics includes procedures that are based on the ability of stem cells to accomplish repair (eg, direct pulp capping, apexogenesis, apexification, and even pulpal regeneration). In this review, an attempt is made to critically assess the current status in pulp regeneration therapy with a specific focus on issues in which more research and translational research are needed (Table 1).

Review

Clinical Evidence: Case Reports versus Randomized Trials
Over the last decade, whole pulp regeneration (11), which may be defined as the reconstitution of viable tissue in a previously avascular space, has been shown in a number of published cases, beginning with the report by Iwaya et al (12) in 2001. Most of these reports describe patients with permanent teeth with a diagnosis of pulp necrosis. In addition to single cases, there are several case series (11, 13–15), 1 systematic retrospective analysis (16), and 1 case-control study (17). Collectively, from this material, there does not seem to be a consensus over the best clinical treatment details for this procedure nor is there a definitive label (18); should this be called pulp regeneration, revascularization, or revitalization?

In fact, the lack of prospective studies was identified as a need for action (10), but no such studies have emerged yet. To enable a larger phase 2 clinical trial, which may include 100–300 subjects, to render relevant data, efforts are needed that target gaps in knowledge in the current treatment paradigm. For example, initially, a triple antibiotic paste was used consisting of minocycline, ciprofloxacin, and metronidazole. This combination was advocated empirically (19) based on antimicrobial efficacy (20). It was subsequently found that this paste, although an effective antimicrobial agent, was associated with significant staining of the treated tooth; this would be an example in which translational research may have prevented negative outcomes.
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Pulp Regeneration—Translational Opportunities

**TABLE 1. Current Understanding and Translational Opportunities in Stem Cell–based Endodontic Treatment Modalities**

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**Indication for Pulp Regeneration**

At this point, only a limited patient population may benefit from regenerative procedures. As stated before, the main target group for regenerative endodontic therapy has been adolescents who present with pulpal necrosis and wide apical foramina. More specifically, most of these teeth are single-rooted anteriors (often with a history of trauma) or premolars (sometimes with dens evaginatus formation).

A recent study (21) attempted to estimate the need for current regenerative treatment by the number of cases in the United States that were billed according to insurance codes for apexogenesis or apexification. The total number of root canal treatments in the United States in the year 2003 was about 22 million; the number of apexification/apexogenesis treatments was only 0.1% of the total (22). Hence, the conclusion was made that in order to make regenerative therapy more meaningful an extension to other patient groups is desirable (21). Accordingly, more epidemiologic research is clearly required to identify such a patient pool that can benefit from different approaches to pulp regeneration (Table 1).

In order to extend the spectrum of regenerative treatment to a more fiscally meaningful level, cases with smaller apical dimensions (ie, mature apical foramina) have to be included. Similarly, multirooted teeth, with their more complicated anatomy and related issues in disinfection, should be considered for possible pulp regeneration. There currently appears to be a very limited number of cases showing the potential for regenerating molar pulps (23, 24); it is expected that outcomes are more variable for this tooth group compared with anteriors and single-rooted premolars.

**Root Development and Revascularization**

Data from the trauma literature (25, 26) suggested that apical diameters of a 1-mm diameter and larger are favorable for complete revascularization. However, it was shown recently for regeneration cases in beagle dogs that smaller sizes may allow for some pulp regeneration in specific conditions (27). This is a 1 area in which translational research is expected to produce important data, specifically to determine which are the most conducive conditions for angiogenesis. Vasculatization of experimentally implanted tooth slices was enhanced by topical vascular endothelial growth factor (28); it is likely, given the appropriate cues, that stem cells themselves differentiate into functional endothelium.

Possible avenues to move forward in basic research are to model different simulated canal sizes or use mediators involved with angiogenesis or mediators that promote stem cell migration. These basic research questions are currently addressed, and translation to the clinic is expected to occur in due course.

**Treatment Modalities**

The second readily identifiable opportunity for translational research in pulp regeneration based on stem cell biology is the overall treatment modality (Table 1). Most building blocks of the clinical practice of pulp regeneration are empirical and not directly driven by basic science research. This situation is an example of not using the paradigm of basic research followed by translation and then clinical research; rather, pulp regeneration was described by clinical observation many years ago by Nygaard Osby and Hjortdal (29).

Systematically, 2 distinctly different strategies exist involving stem cells for the repair and/or regeneration of damaged tissues (30): first, the acellular approach with in situ stimulation of stem cells and modulation of their activity (31) and, second, the cellular approach consisting of ex vivo cell culture and the use of stem cells in tissue engineering (10). However, the current clinical practice for the treatment modalities cited earlier does not involve such a cellular approach.

The former strategy (32) relies primarily on providing tissue conditions and a scaffold. Then, locally present cells or cells attracted from distant sites are believed to populate the defect. In contrast, a cell-mediated strategy (33) requires harvesting and expanding stem cells in culture. These cells, perhaps seeded onto a carrier, are placed into the empty space to be filled with pulp tissue. Those favoring a cell-mediated approach argue that the geometry (eg, the size of the space to be filled with regenerated pulp) may reduce the chance for success for cell-free pulp regeneration therapy (34) but concede that some regeneration does take place under the current (cell-free) regeneration strategy.

**Antimicrobial Strategies**

In order to move regenerative therapy forward, it is essential to validate a number of subelements of treatment strategies. For example, cell culture–based research has indicated that triple antibiotic paste, although antimicrobial effective, may be highly toxic to stem cells of the apical papilla (35). Indeed, according to those authors, calcium hydroxide may be more favorable for stem cell survival compared with dual and triple antibiotic paste, which, in turn, supports earlier clinical reports that successfully used calcium hydroxide dressing for pulp regeneration (13, 15).

Similarly, the irrigant of choice is debated; sodium hypochlorite appears to be needed to disinfect, and chlorhexidine is recommended by some authors (18, 36). However, systematic research indicated that chlorhexidine is toxic to stem cells (37), and, therefore, this irrigant should not be used in regenerative therapy.

By inference, the use of EDTA was recommended as an irrigant after disinfection had been accomplished; this was based on basic research findings by Smith et al (38) that EDTA liberates transforming growth factor beta stored in radicular wall dentin. In turn, this mediator promotes the differentiation of dental pulp stem cells into odontoblast-like cells and subsequent hard tissue deposition. This clearly is another example in which more translational research may confirm the validity of an approach in clinical practice.

**Clinical Outcomes: Expectations and Reality**

The third larger opportunity for such translational research is associated with the main desired outcome—continuous root development (Table 1). This outcome is considered important, specifically for root strengthening to the extent that teeth with regenerated pulps...
become more fracture resistant. Indeed, systematic assessment based on radiographs of pulp regeneration cases has shown both increased radicular wall thickness and root lengthening (16). Moreover, specific experimental conditions have produced dentinlike deposits with regular tubulelike structures (39). However, histology from available human specimens has failed to show regular dentin deposition (40, 41) similar to earlier findings in dogs (42).

More importantly, even considering dentin deposition from a regenerated pulp, it is not clear if the fracture resistance of teeth with regenerated pulps is higher. Fracture mechanics of teeth is driven by fatigue over time (Fig. 1) as much as catastrophic loading; in fact, much is still to be learned about the conditions under which dentin fracture propagate (43). Indeed, basic research into the correlation of dentin quality and distribution with fracture toughness is lacking; this type of research when translated to the clinic is likely to impact endodontic treatment in general. However, there are ongoing efforts outside dentistry to enhance the mechanical properties of regenerated hard tissue (eg, by modifying scaffolds) (44).

One may argue that strengthening will not occur coronal to the level of the restoration, which is typically below the cementoenamel junction (18, 19). This leaves the pericervical dentin unchanged during regenerative therapy and may leave these teeth vulnerable for additional fractures either by impact or fatigue (43) similar to earlier findings in dogs (42).

Interestingly, cross-sectional data from trauma cases suggest that younger patients (<11 years) are 8 times more likely to refracture a traumatized tooth, and almost 50% of patients with trauma experience another trauma in the same area (45). This finding makes a case for a multidisciplinary approach to trauma (46). It appears to be reasonable to follow a similar multidisciplinary and translational approach in pulp regeneration.

Research Outcomes: Pulp Tests and Beyond

To test the overall benefit of pulp regeneration, outcome criteria have to be defined; these criteria need to go beyond the mere radiographic demonstration of hard tissue deposition (16). One way to address this dilemma is the inclusion of a quality of life criterion, similar to what has been proposed earlier for nonsurgical endodontics (47). One may argue that an outcome such as 20-year survival of an anterior tooth after trauma is excellent even with the end result of a vertical fracture (Fig. 1).

One additional criterion that has been used is the demonstration of a positive response to electric pulp testing. Several case reports have indicated such a vital response (19); however, it appears unlikely that there will be a positive response to cold or electrical stimuli in all cases.

On the other hand, the development of a pulp test, not on the basis of physical stimuli but rather molecular mediators, could be an unintended and very important avenue for future basic and translational research. The first steps in this direction have already been made (48) and are likely to extend the spectrum of stem cell–based endodontics into a not necessarily expected direction—vital pulp therapy. At the same time, one has to be aware of the potential of biomarkers to fail (49).

Vital pulp therapy, whenever successful, serves the same goals as pulp regeneration, in fact perhaps better. This is more evident when one questions why pulp regeneration is beneficial. Beyond the not-yet proven potential to strengthen an immature root, this would be conserving an immunologically active “root canal filling” that combats leakage better than any existing filling material. Consequently, the boundaries of what can be expected from vital pulp therapy have been explored (50).

One of the possible unintended consequences of basic research in this area can be the development of better disinfection methods to provide conditions that favor stem cell differentiation. Such methods would, in turn, benefit conventional nonsurgical endodontics. Another perhaps even more important consequence is the opportunity to do more basic research into stem cell behavior. Recently, using dental stem cells as a molecular laboratory, researchers identified a protein associated with bone and tooth mineralization in a hereditary form of hypophosphatemic rickets (51). This example of basic research may be seen as spawned by the availability of dental stem cells (4); similar research considering a broader approach to outcomes beyond hard tissue deposition may make endodontics more meaningful in the context of our patients’ well-being. Certainly, streamlined translational research (52) has the potential to create new treatment paradigms in endodontic therapy.

Conclusions

The current status of stem cell–based endodontic therapy (ie, pulp regeneration) is characterized by an empirical approach. At the same time, basic research into dental stem cells is well documented. Only very recently efforts are emerging to bridge this gap with translational research. A realistic set of expected outcomes and clearly defined research streams are needed to address present gaps in knowledge. Specific opportunities exist in this arena that have the potential to create meaningful changes in endodontic therapy in the near and distant future.
future, perhaps with several unexpected consequences (eg, new diagnostic tests and outcome measures).

Acknowledgments

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References