Paradigm Lost: A Perspective on the Design and Interpretation of Regenerative Endodontic Research

Kenneth M. Hargreaves, DDS, PhD, Anibal Diogenes, DDS, MS, PhD, and Fabricio B. Teixeira, DDS, MS, PhD

Abstract
Regenerative endodontic procedures are rapidly gaining the attention of clinicians and investigators alike. However, it is often challenging to understand various regenerative studies and to interpret their results. The present review addresses this problem by focusing on recent strategies for developing standardized clinical protocols, understanding the full spectrum of clinical and translational research and its relationship to selection of proper outcome measures, as well as reviewing the fundamental role of paradigms in designing and interpreting regenerative studies. (*J Endod* 2014;40:S65–S69)

Key Words
Mesenchymal stem cells, pulp biology, regenerative endodontics

Regenerative endodontic procedures are rapidly gaining the attention of clinicians and investigators alike. Several recent reviews (1–7), including articles published elsewhere in this symposium (8), provide important clinical and biological summaries of regenerative endodontic procedures. This review builds on this prior work by focusing on broader questions of developing standardized clinical protocols, understanding the spectrum of clinical and translational research and how it controls the selection of proper outcome measures, as well as emphasizing the role of paradigms in designing and interpreting regenerative studies.

Development of Standardized Regenerative Clinical Protocols

Many clinicians would agree that the modern era of regenerative endodontics was launched by the case report of Banchs and Trope (9) in 2004. This article prompted the subsequent publication of more than 150 regenerative endodontic cases (4). As recently summarized (4), a range of clinical protocols have been used to treat these cases, with varying irrigants, medicaments, clinical procedures, and follow-up times. This has led to the growing recognition of the need of developing a standardized clinical protocol for regenerative endodontic procedures. However, how can a standardized protocol be developed in the absence of randomized controlled clinical trials?

To address this issue, the American Association of Endodontists (AAE) formed a standing committee on regenerative endodontics in 2007. This committee meets regularly and has developed initiatives for forming an online clinical registry of regenerative cases and developing continuing education materials, new insurance treatment codes, and a standardized clinical protocol, with these materials available via the Internet (10). In developing guidelines for a standardized protocol, the AAE Regenerative Endodontics Committee followed a procedure similar to that used successfully for developing guidelines for prevention of infective endocarditis (11). Although the actual clinical procedures designed to prevent infective endocarditis or to deliver regenerative endodontic procedures are quite different, they share a similar lack of randomized controlled clinical trials and therefore are both based on lower levels of evidence. As illustrated in Table 1, standardized guidelines for both procedures have been developed by using an iterative process of interpreting relevant clinical and preclinical studies, evaluating the strength of the evidence, and forming consensus-driven recommended clinical protocols. This approach has been successfully used worldwide to promulgate guidelines for the prevention of infective endocarditis. Similarly, the most recent revision to the standardized regenerative endodontic protocol was published online in July 2013 and is available for global dissemination (12). Of course, unlike protocols to prevent bacterial endocarditis, regenerative endodontic procedures can be evaluated by randomized controlled trials, and future recommendations are likely to be based on much higher levels of evidence.

In contrast to developing evidence-based guidelines, one published report recommended that the current lower levels of evidence should restrict the use of regenerative procedures to only those cases when all other treatments are not suitable or have failed (15). As practicing clinician scientists, we do not agree with this viewpoint. The failure to provide treatment to children with immature teeth and pulpal necrosis would subject them to long-term functional and esthetic challenges, particularly because many of these cases are maxillary incisors. Moreover, alternative treatments such as extraction...
Pulp Regeneration—Translational Opportunities

and placement of dental implants are contraindicated in the child with a rapidly growing craniofacial skeleton. Finally, the continuous publication of an ever-increasing number of regenerative cases suggests that it is possible to save these teeth with satisfactory functional and esthetic outcomes (4). Just as with guidelines to prevent infective endocarditis, the structured development of standardized recommendations for regenerative endodontics provides an evidence-based approach that guides clinicians in providing necessary treatment. Of course, as the field evolves, it is likely that the guidelines will be revised on the basis of the outcomes of higher levels of evidence such as randomized clinical trials. From this perspective, it is important to realize that the premise of level of evidence is to apply the best available evidence in your practice and not to withhold treatment simply because the level of evidence is less than ideal.

Designing and Interpreting Regenerative Endodontic Studies

There is considerable debate on the ideal outcome of regenerative endodontic treatment. Is it complete histologic regeneration of the pulp-dentin complex? Is it continued root development? Is it lack of signs and symptoms of an infection? Is it the patient’s satisfaction with treatment? Although there are merits for each of these outcomes, the debate actually misses an important point; all of these outcomes are important, but they answer different questions.

The field of clinical and translational research has evolved considerably during the last 10 years (14–17), leading to a recognized model that depicts the entire spectrum of clinical and translational research (Fig. 1). This spectrum ranges from preclinical studies to human clinical research to clinical practice to population-based studies, and these domains have been characterized as T1, T2, T3, and T4 levels of research. These are not isolated silos of research; instead, knowledge and insight travel both ways across each of these domains. In general, T1 and T2 experiments are conducted with much more control over the experimental conditions than T3 and T4 studies, but with smaller sample sizes. In contrast, T3 and T4 studies generally incorporate much larger sample sizes collected under “real-life” conditions, but with data collected under less controlled experimental designs. For example, the study by Molander et al (18) on one-appointment and two-appointment procedures for nonsurgical root canal treatment is a classic T2-level study that was conducted under highly controlled experimental conditions (including microbial sampling), with well-defined outcome measures but a relatively small sample size (N = 101). In contrast, the study by Salehrabi and Rotstein (19) that used an insurance database on endodontic outcomes is a strong T3-level study collected under actual real-life (private practice) conditions, with an enormous sample size (N > 1.4 million) but using a relatively loosely defined clinical outcome (survival) that was collected under uncontrolled clinical conditions. It is important to realize that a T2 study is not inherently better or worse than a T3-level study. Instead, these studies have different purposes; a T2 study generates knowledge about the efficacy of clinical interventions applied under optimal highly reproducible conditions, whereas a T3 study provides important knowledge about how interventions work in real-life settings. Both levels of research are important as new knowledge is generated and applied to treating our patients. In addition, T4 research has major population-level or policy-level implications; one dental example is a cost-benefit analysis of water fluoridation on caries.

The organizational model depicted in Figure 1 has direct application to understanding why the “ideal” clinical outcome depends on the experimental question being asked. As a simple example, prior studies

| TABLE 1. Procedures for Developing Standardized Clinical Protocols |

<table>
<thead>
<tr>
<th>Domain</th>
<th>Process for developing standardized recommendations</th>
<th>Process for revising guidelines</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>Monitor literature for new findings that require revision to guidelines</td>
<td>Monitor literature for new findings that require revision to guidelines</td>
</tr>
<tr>
<td>T2</td>
<td>Monitor literature for new findings that require revision to guidelines</td>
<td>Monitor literature for new findings that require revision to guidelines</td>
</tr>
<tr>
<td>T3</td>
<td>Monitor literature for new findings that require revision to guidelines</td>
<td>Monitor literature for new findings that require revision to guidelines</td>
</tr>
<tr>
<td>T4</td>
<td>Monitor literature for new findings that require revision to guidelines</td>
<td>Monitor literature for new findings that require revision to guidelines</td>
</tr>
</tbody>
</table>

Figure 1. The spectrum of clinical and translational research. There are 4 broad domains of clinical and translational research (T1–T4). These domains serve to translate basic research knowledge into early testing for clinical efficacy or safety (T1), uses highly controlled experimental conditions (eg, a randomized clinical trial design) to evaluate clinical outcomes in patients (T2), studies how guidelines work in actual private practice settings (T3), and determines the impact of interventions on the health of populations (T4).
on mineral trioxide aggregate (MTA) have used preclinical methods (20), human clinical research (21), and private practice studies (22). However, the ideal outcome differs for each domain of research. Thus, if one is interested in studying MTA as a clinically useful endodontic root-end filling material (a T2 question), then the ideal outcomes are measures of clinical healing (21). On the other hand, if one is interested in the mechanism for MTA induction of hard tissue after pulp capping (a T1 question), then the ideal outcome may evaluate its effect on stem cell differentiation (20). The important point is that there is no single ideal outcome; instead, it depends on the question being asked and the associated T level of research (Fig. 1).

The importance of understanding the spectrum of clinical and translational research extends to designing and interpreting studies on regenerative endodontics (Fig. 2). It is critical to understand that the questions being asked in a T1 study differ from questions being asked in a T2 or T3 study (Fig. 2). Similarly, the ideal outcome in a T1 study differs from that in a T2 or T3 study. Thus, if one is interested in understanding whether the tissue formed is regeneratively determined hard tissue, then the study should be at a T1 level. Alternatively, if one is interested in clinically meaningful outcomes, then the pulp’s histologic phenotype is not as important as other outcomes such as tooth survival, healing of apical periodontitis, radiographic evidence of continued root development, or lack of symptoms (Fig. 2). Understanding that there is a spectrum of clinical and translational research (Fig. 1) and that the ideal outcome differs among the T-level domains (Fig. 2) allows the clinician and investigator to better design, interpret, and understand clinical regenerative research. In particular, clinicians can rely on T2 studies (eg, randomized controlled trials) to provide information on treatment outcomes under highly controlled conditions and can use T3-level studies to provide information on outcomes under real-life or private practice conditions. In summary, there is no optimal or singular clinical outcome; it depends on the question asked and the associated T level of research. If we are interested in performing regenerative procedures on patients, then histology is not the primary outcome measure; instead, healing of apical periodontitis, lack of signs or symptoms of infection, continued signs of root development, and possibly a return of responsiveness to pulp testing should be considered. Indeed, this philosophy is incorporated into the July 2013 revision of the AAE guidelines, which describe the primary goal of regenerative endodontic procedures as healing of apical periodontitis, the secondary goal as increased root wall thickness and/or length, and the tertiary goal as regaining positive response to pulp testing. Both the secondary and tertiary goals are defined as desirable but possibly not essential to determine clinical success.

Impact of Paradigms in Designing and Interpreting Regenerative Endodontic Research

In 1962, Thomas Kuhn published his landmark book, The Structure of Scientific Revolutions (23). He argued that science is not simply a linear accumulation of data and theories, a process that he called normal science. Instead, he proposed that there were long periods of normal science interrupted by a scientific revolution in thinking that led to a paradigm shift and another new period of normal science. For example, the Ptolemy model of the solar system views the Earth as the center of the cosmos, with the planets, the Sun, and stars orbiting around it. However, over time, improvements in instruments (eg, telescopes and watches) led to an increasing number of experimentally observed inconsistencies not predicted by this model. These inconsistencies eventually led to a scientific revolution, culminating in the alternative Copernican model of the solar system, with the Earth orbiting around the Sun (23). Although 2 scientists might each observe the Sun rising at dawn, the one with a Ptolemy model would interpret this event as evidence that the Sun quickly orbits the Earth every day, whereas the scientist holding the Copernican paradigm would interpret the sunrise as evidence of Earth’s 24-hour rotation. This historical example illustrates the fundamental point that a prevailing paradigm provides an intellectual framework that strongly influences how scientists design and interpret their experiments.

We believe that the concept of paradigms also holds for research on regenerative endodontics. Two prevailing paradigms have been advanced in the regenerative endodontic literature. The first is

Figure 2. Effect of T-level research domain on the optimal question and outcome measure for regenerative endodontic research. Each T level of research addresses a different type of question, and the ideal outcome depends on the T-level of research and the specific question being addressed. For example, a T3-level study on regenerative endodontics in a private practice setting would not be expected to provide outcomes on the histology of the tissue in the root canal system. Each T-level study has different ideal outcome measures. AP, apical periodontitis.
revascularization, a model derived from the recognized role of the blood clot in wound healing (24, 25) and later promulgated in the trauma literature. In the revascularization paradigm, an immature tooth with pulpal necrosis would be disinfected, followed by overinstrumentation beyond the apex to allow intracanal bleeding. Subsequent tissue ingrowth would be interpreted as due to the formation of a blood clot leading to normal wound healing. The revascularization paradigm does correctly predict the observation of tissue ingrowth in these cases. However, the historical finding that odontoblasts could not be detected in this tissue (24, 25) effectively stopped further research in the field. Essentially, the blood clot/ wound healing paradigm is insufficient to guide the design of experiments that identify the conditions required to regenerate the pulp-dentin complex.

An alternative paradigm is regenerative endodontics (26). This model is based on the concepts of tissue engineering (27) and, importantly, requires clinical protocols that do not damage stem cells, growth factors, or scaffolds (28–31). A recent T1-level study provides evidence for this paradigm with the demonstration of the delivery of mesenchymal stem cells into the root canal systems of immature teeth with pulpal necrosis (32). Similar to the revascularization model, the regeneration paradigm also correctly predicts the observation of tissue ingrowth in these cases. However, it differs by providing the conceptual framework to guide future experiments that evaluate the impact of scaffolds, growth factors, irritants, medicaments, dentin, and microbes on the differentiation and proliferation of stem cells in the root canal space. Indeed, an examination of articles recently published in this field demonstrates an increasing number of studies focused exactly on these experimental variables (28–30, 33–43). From a revascularization viewpoint, these studies make little sense because they will not influence how a blood clot triggers wound healing. However, from a regenerative viewpoint, these studies are precisely focused on defining the conditions required for regeneration of a functional pulp-dentin complex.

To misquote the 17th century English poet John Milton, our field has recently experienced a “paradigm lost,” with the loss of the revascularization paradigm and the emergence of a regenerative paradigm. Understanding the importance of paradigm shifts and, in particular, the key concept that one’s intellectual framework, or paradigm, controls how scientists design and interpret their experiments provides an overarching perspective for understanding the design and interpretation of regenerative endodontic research.

Acknowledgments
Supported in part by R34 DE20864 and by NCATS UL1TR001120.
The authors deny any conflicts of interest related to this study.

References