Regenerative Endodontics and Tissue Engineering

What the Future Holds?

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KEYWORDS

• Stem cells • Repair • Regeneration • Progenitor cells • Cell signaling
• Growth factors • Dental pulp • Dentin

KEY POINTS

- The work performed by researchers in regenerative endodontics and tissue engineering over the last decades has been superb; however, many questions remain to be answered. The basic biologic mechanisms must be elucidated that will allow the development of the dental pulp and dentin in situ.
- Development of stem cell lines are needed that are easily cultured, grown, maintained, and ready to be placed in a tooth together with a proper scaffold and the introduction of growth factors that allow "-like-tissue" formation.
- The need for controlled odontogenesis (dentin, pulp) that will continue to protect the tooth, now somewhat successful in animals, must become a normal and usual clinical therapy. Stress must be placed on the many questions that will lead to the design of effective, safe treatment options and therapies.

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INTRODUCTION

Regenerative endodontics is concerned with the development of biologically based treatment modalities that are used to replace diseased portions of the dental pulp or to allow complete formation of a dental pulp–like tissue that will act as the original dental pulp.[1] Today, the major effort in regenerative endodontics appears to use several types of stem cells placed on a scaffold inside the diseased root canal system of a tooth. With the addition of growth factors, externally or from dentin and/or remaining dental pulp, a pulplike tissue forms.[2] (Please see a review of tooth formation from embryonic tissues to a fully formed tooth by Tziatas and Kodonas.[3]) A form of regenerative endodontics began many years ago with the development of direct and indirect pulp-capping procedures. The need for a scaffold, vascular supply, growth factors, signaling mechanisms, migration of cells, and differentiation were not well known, nor were the actual events that occurred during the formation and regenerative processes known. Today's placement of a direct calcium hydroxide pulp cap leads to growth factor activation from surrounding hard tissues, inclusion of native stem cells from the remaining pulp tissue, and hard tissue formation (dentinlike hard tissue) that may also act as a scaffold and as a source for growth factors. The body of work in regenerative endodontics has grown exponentially, therefore this article is concerned with the future possibilities of an understanding of the processes and mechanisms to restore a vital, healthy tissue within a tooth in situ and include a review of the progress of laboratory studies that may lead to greater knowledge of the interactions at the cellular and molecular levels of tissue engineering.[4]

In a healthy tooth, the pulp/dentin complex undergoes dentin matrix formation with eventual mineralization. The dentin formed is in a physiologic process. The dentin formed is very similar to primary dentin, with a tubular structure that covers primary dentin with the dentin tubules being continuous between both primary and secondary dentin formations. In teeth that have been injured in some manner (caries, restorations, trauma), another type of dentin forms called tertiary dentin. This is a unique type of dentin that is not tubular in its formation but rather occurs as an atubular structure. There are 2 types of tertiary dentin: reactive and reparative.[5] Reactive dentin is formed by the remaining, original odontoblasts forming a matrix that becomes almost completely solid with no tubules; however, tunneling has been seen to occur in this form of dentin.[6] Reparative dentin is a matrix formed by new odontoblastlike cells that form precursor and stem cells found in the remaining vital pulp tissue. It also is formed without tubules.

Regenerative endodontic procedures use biologically based treatment modalities and pulpal cells.[7–9] The information available in regenerative studies to date, however, indicate that more must be learned about the interactions that occur between all cells, growth factors, proliferation and differentiation of cells, and the ability to use materials that will result in a well-formed, functioning tooth.[7,8]

PULP REPAIR AND REGENERATION

An excellent review by Goldberg[10] suggests that there are more questions than answers in the ability to effect pulp repair and pulp regeneration. The following is a summary of the questions he poses as to the future of pulp repair, pulp regeneration, and tissue engineering.

A distinction between endodontic repair and regeneration must be understood before one can understand the processes that occur in repair and regeneration. Repair indicates that healing occurs because the remaining damaged tissue is vital, original odontoblasts survive, and the pulp tissue can be restored to a normal-like form and

function. In the dental pulp, odontoblasts are reactivated, a dentin matrix is formed that becomes mineralized (reactionary dentin), and the pulp retains, for the most part, its biologic functions.

Regeneration indicates that the pulp is completely necrotic (complete degradation) and a tissue (pulplike) must be formed that may function as the original tissue. Questions arise as to how the resultant cells and tissue react in relation to the original tissue. Studies have demonstrated that a completely necrotic pulp, combined with a periapical lesion and incomplete root formation, is the usual clinical finding. Initial treatment is undertaken to remove the infection and heal the lesion. This is followed by formation of the tooth root, creation of new odontoblastlike cells functioning as a pulplike tissue.[1,11–15] In these cases, the new pulplike tissue continues to form a hard dentinlike tissue, generally without tubules closing the root canal space in what appears to be an event that occurs rapidly.[11] The events occurring in these teeth may cause the need for further therapeutic interventions, including root canal or surgical root end therapy. The mechanisms for these events are not fully understood, hence the term "-like tissue." Can the processes be controlled to produce a more natural tissue reaction that occurs over many years without closing down the root canal space? Ideally, the process should mimic the development of secondary dentin formation, which is physiologic in nature and occurs in an uninvolved pulp as a natural part of the aging process.

The future of repair and regeneration depends on answers to the questions posed in the Goldberg[10] article. What is the nature of the stem cells that should be used to regenerate pulp tissue? This is of great importance, as researchers appear to have isolated several different stem cell lines, which, in itself generates several other questions. Greater attention to the analysis of the biologic properties of dental tissue–derived mesenchymal stem cells using both in vitro and in vivo systems is necessary. A recent study concluded that both dental pulp stem cells (DPSCs) and stem cells from the apical papilla (SCAPs) could differentiate into odontoblastlike cells with the potential to migrate and mineralize leading to 3-dimensional dentinlike structures; however, SCAPs had a higher population capacity and proliferation rate compared with DPSCs. This may be an advantage for dental tissue repair and regeneration from the standpoint of cryopreservation of the cells in large quantities and a high mineralization rate may shorten the process.[15]

STEM CELLS

All stem cells in odontogenesis, with the exception of ameloblast progenitor cells, originate in the mesenchyme and are said to be of ectomesenchymal origin. DPSCs are isolated from the dental pulp and can regenerate into new stem cell lines that can differentiate into other cell lines. As the developmental ability of these cells in vitro is limited, they are more useful in in vivo studies, as more complex tissues arise. For example, dentin/pulplike tissues arise from DPSCs, such as dentinlike and pulplike tissues.[15–19]

SHEDS are stem cells from exfoliated, human, deciduous teeth, which are a readily accessible source of adult stem cells from impacted third molars. In vivo, removal of these teeth led to collection of multipotent stem cells having the potential to differentiate into odontoblastlike cells,[20] neurons,[21] and osteoinductive cells.[22–25] Periodontal ligament stem cells (PDLSCs) can form cementum and periodontal ligament and, when transplanted into mice, bone and cementum structures were seen.[26–29] Dental follicle stem cells (DFSCs) are collected from the follicles that surround developing third molars. These cells have a major role in the genesis of cementum and cementoblastlike cells.[30]

SCAPs are harvested from the apex of a developing tooth. The papilla is a precursor of the dental pulp. As in other stem cells, SCAPs express early mesenchymal surface markers.[16,31–34] A reading of the quoted articles will show that the previously listed references demonstrate that, in many instances, the studies compare one type of stem cell to another or several others. These stem cells are proliferative with characteristic markers such as Stro-1, CD146/MUC18, and CD44 (see articles by Sedgley and colleagues and Law and colleagues elsewhere in this issue). This leads to questions as to which markers should be recognized that will allow collection and development of a cell line that can be maintained and colonized and introduced into a tooth as an in vivo treatment option. A primary question must be asked as to what type of pulplike tissue should be the result of implantation?

Is it possible to obtain a functional, nonmineralized pulp that is vascularized and innervated as the original tissue would be? Or is the aim to develop a pulp tissue that would induce an increased amount of mineralization that could serve as a substitute for root canal therapy? Cell differentiation can lead to either adult progenitor or an odontoblastlike/osteoblastlike cell, which is divergent from other results obtained.

The question of using multipotent stem cells remains unsettled, especially when attempting to regenerate pulpal tissue. The cells necessary are present in the pulp and can be associated with odontoblast and osteoblast cells, endothelial cells, and, later, formation of neurons. Therefore, is the use of multipotent progenitors or nonpotent cells, the cells of choice?[15,19]

In the future, it may be possible to minimally invade and isolate suitable stem cells, have them undergo differentiation in vitro, and combine and develop them into tooth structures.[35] Pulp cells differentiate in vitro into odontoblastlike stem cells. The dentin formed, as previously mentioned, is atubular. Is there a possibility of dental pulp cells producing tubular dentin?[36] A recent study mixed pulp cells with a hydroxyapatite (tricalcium phosphate powder) and generated a dentin-pulplike tissue.[17] Bartouli and coworkers[37] transplanted tubular dentin on the surface of dentin-pulp slices and generated increased amounts of tubular dentin; however, the origin of the progenitor cells giving rise to new odontoblasts (tubular dentin) and the signaling pathways in cell differentiation have not been clearly identified and remain a matter of debate.[36]

Greater knowledge related to the location and identity of odontogenic precursor cells that participate in reparative dentin formation is required. Implant experiments have begun to identify genuine progenitor cell markers and molecular signal pathways that allow stem cell recruitment. Implantation experiments using pulp-derived precursor cell lines have started to provide evidence that, in the absence of carriers or biomolecules, exogenous stem cells have the capacity to promote efficient tooth repair.[38]

Because repair and regeneration have different targets, the expectations of a particular therapy must be clear. Is regeneration of a nonmineralizing pulp the proper goal or is generation of a tissue that may become a completely mineralized root canal system the proper treatment option? Each aim uses specific tools that are valid for bioengineering treatment modalities.[10]

Caries may be the most common and dangerous of all types of injury, provoking adverse stimuli to the dental pulp. Understanding caries management (see article by Chogle and colleagues elsewhere in this issue for a review of the pulpal response to caries) has led to improved understanding of mineralization of teeth, further leading to therapies necessary to restore the biologic behavior of the entire pulp-dentin complex.[39]

The pulp-dentin complex is protective in nature, as its main roles are to manufacture dentin matrices and to restart dentinogenesis to protect the new pulplike tissue from injury or insult. Many of the processes involved are thought to be the same as the initial pulp developmental processes occurring embryonically.[40] Because the onset of injury

in the dental pulp may be a result of caries, markers of inflammation are different, depending on the depth of the inflammatory process of the lesion.[41]

Still somewhat unclear is how inflammation may overwhelm and cause degeneration in the pulp, as opposed to its role in the regeneration of that tissue. To understand the treatment prognosis, understanding the balance between infection and inflammation is necessary, together with an understanding of proinflammatory and anti-inflammatory mediators and how they relate to the innate and adaptive immune systems.

Many studies have reported that several populations of stem cells in and around the tooth pulp are able to be used to repair or regenerate the pulp/dentin complex. These populations of cells include DPSCs,[17,18,20] SCAPs,[19,32,34] PDLSCs,[27,29] and mesenchymal stem cells.[33,42] SHEDs are human pulp cells of the dental follicle collected from impacted third molars.[20,43] These cells may have the dual ability to repair (heal), regenerate a particular tissue, or differentiate in a manner that causes a change in the ability of these cells to form original tissue. As previously mentioned, odontoblasts normally secrete a tubular dentin (both primary and secondary) as a normal physiologic function throughout life that maintains the tubular structure in both dentins. However, insults to the pulp may cause newly formed odontoblastlike cells to form an atubular (tertiary) dentin that is not tubular and not physiologic. Rather it is formed as a result of the pulplike tissue reacting as a defender of that tissue. To be able to use these cell lines clinically, translational research in the future will require both researchers and skilled clinicians who can develop new and novel therapies that can eventually be tested and used in clinical environments to answer these questions.

SCAFFOLDS

A scaffold is thought of as a 3-dimensional construct or support substance used for several tissue engineering applications. When stem cells are seeded on scaffolds, they are expected to attach, proliferate, and differentiate into new tissues that will eventually replace the scaffold. Scaffolds should be biocompatible, not elicit an inflammatory response or be cytotoxic, support cell organization and vascularization, allow new regenerated tissue to form, be sterilizable, and be stable while maintaining mechanical form and strength. They should have an inductive ability with added growth factors and morphogens for a more rapid cell attachment, proliferation, migration, and differentiation into a specific tissue.[44]

The choice of a scaffold is critical in tissue regeneration. Most scaffolds are organic in nature and used to provide surfaces on which cells may adhere, grow, and organize.[44] Scaffolds chosen for laboratory studies are diverse, including natural or synthetic polymers, extracellular matrices (EMCs), self-assembling systems, hydrogels, and bioactive ceramics. Recently, a synthetic polymer polycaprolactone was successful in growing increasing numbers of SCAP stem cells with apparent identification of NOTCH signaling expression.[44]

Although the number of scaffolds has increased (see the excellent review by Sakai and colleagues[45]), questions remain that must be addressed. For example, are scaffolds able to support various kinds of stem cells or are they stem cell–specific? Are stem cells able to be seeded with like results on more than one scaffold? What are the limitations of the use of one or another scaffold that may be natural or synthetic scaffolds? The use of a self-assembling peptide system that allows a “bottom-up” approach of generating EMC materials, offering high control at the molecular level, will be a major step forward in constructing future scaffolds.[44] The peptide system is referred to as a tunable matrix with several features that possibly allow scaffolds to be designed, as different requirements are needed to regenerate a tissue.[4]

NICHES

Today, the ability of stem cell–based tissue engineering of teeth faces dilemmas of methods from development owing to several differing conceptual issues. For example, where is the location and identity of odontogenic precursor cells that participate in reparative dentin formation?[38,46,47]

Stem cells appear to have the ability for tissue repair and regeneration throughout life. They may react by their ability to cause the self-renewal repair of a particular tissue by *differentiation* of the cells into other tissues and are influenced by a microenvironment called the stem cell niche. A niche determines how dental pulp cells regulate and participate in tissue maintenance, repair, and regeneration. Niches are located around the body in various tissues. The niches exist in specific anatomic locations where they house stem cells (few in number) where the cells can be renewed. These niches in their specific locations within the dental pulp and dentin regulate how stem cells participate in tissue repair and regeneration. Specific signals from precise areas (see section on NOTCH proteins) in the niche permit stem cells to maintain vitality and to change their ultimate fate and number.[48,49] When signaled to do so, they travel to the site of injury.[50] The signaling proteins functioning in these processes have been studied but more research is needed to determine the mechanisms that allow stem cells from a particular niche to increase in number and migrate to the area of injury. The stem cell niche may be thought of as an interactive structural unit that facilitates cell-fate decisions. Molecular cross-talk events, together with the signaling of specific molecules, occur in the right place at the right time. In other words, anatomic organization coordinates stem cell function in time and space. Both positive and negative signals are integrated with intercellular pathways that share the process with other signaling proteins and growth factors.[51]

Questions arise as to the environment of the niche surrounding the stem cells. Does that environment maintain stem cell lineage specificity? Are postnatal stem cells capable of converting from one type of cell into another, as they may do naturally in the body?[52] A stem cell niche is a group of cells in special tissue locations that maintain stem cells. Niches are variable, containing different cell types depending on the need of its environment. The niche may be thought of as an anchor for a particular stem cell that generates extrinsic factors that control stem cell numbers and their fate. Signaling molecules, such as bone morphogenic proteins (BMPs) and NOTCH molecules, regulate stem cell behavior, such as self-renewal and fate of that group of stem cells.[53]

NOTCH SIGNALING PROTEINS

NOTCH proteins are important regulators of stem cells in the cell's ability to function properly. They have the capacity to induce proliferation or differentiation in stem cells. For example, injuries to the dental pulp may lead to death of odontoblasts (apoptosis). This triggers the activation of pulp stem cells, leading to their proliferation, migration to the area of injury, and differentiation into another type of odontoblastlike cell to replace the apoptotic odontoblasts.[54]

When injury occurs to the dental pulp stem cells, large numbers leave their niche and travel to the areas of the injuries. NOTCH signaling proteins initiate migration of DPSCs through signaling mechanisms to the site of injury leading to proliferation and differentiation into odontoblastlike cells. This results in the formation of a reparativelike dentin matrix with eventual mineralization. The question still unanswered is that, although the niches contain only a few cells, what signaling molecules are responsible for the almost immediate increase in numbers of cells that are activated, proliferate and differentiate, and migrate to aid the pulp in its ability to be repaired?

The NOTCH proteins (1–4) are large, transmembrane receptors controlling cell fate decisions and the formation of cell compartments during embryonic development.[55,56] Ligands bind to the NOTCH receptors, triggering enzymatic activities. A complex is formed that activates the transcription of target genes that maintain cells in a proliferative/undifferentiated state. The expression of NOTCH receptors and ligands have been detected in developing dental pulp and teeth during injury and repair.[57] NOTCH proteins are involved in cell-cell signaling through direct cell-to-cell contact where one cell possesses a transmembrane receptor and the other cell possesses a membrane-bound ligand.[57]

The NOTCH family of signaling proteins causes an asymmetric stem cell division in the stem cells that ensures stem cell renewal. This allows the daughter cells to differentiate, leading to repair and regeneration of tissues. Signaling from NOTCH proteins plays a key role in the fate of the cell in determination and maintenance of stem cells. Its activation or inhibition can regulate the cell's fate.

In sum, when dental pulp is injured, stem cells are recruited from the niches where they reside to the areas of the injury. Odontoblasts survive, leading to repair, or die and are replaced by new odontoblastlike cells causing regeneration of the pulplike tissue.[43] Other molecules (BMPs, submembers of the transforming growth factor beta [TGF-β] family of growth factors) are released from dentin and play an important role in pulp healing.[58,59] Increased amounts of TGF-β are released during the death of the original cells, which leads to reparative dentin formation. When viewed microscopically, however, the areas thought to be niches appear as normal pulp tissue. More studies are needed to answer the previously mentioned questions, which will lead to the exact growth factor or combinations of growth factors that will mimic the reaction of repair mechanisms and allow the tooth to develop normally.

The above indicates that NOTCH signaling in their role of regulating stem cell behavior may be important for tooth repair. NOTCH receptors are absent in adult rat pulp tissue; their expression was found to occur after pulp tissue injury.[23,60] These studies also suggest that NOTCH signaling may act as a negative molecule in stem cell differentiation. The future of the full extent of NOTCH signaling abilities plus other signaling proteins that may be present are not fully known, which indicates that their ability in repair processing and participation in healing is not fully understood. Finally, it has not been demonstrated that NOTCH-positive stem cells participate in the repair process and leading to differentiation into odontoblastlike pulp cells.[10]

VASCULARIZATION

The understanding of the mechanisms that underlie dental pulp angiogenic responses still are not completely understood. Revascularization is critical for the development of new therapies necessary to regulate the dental pulp. New therapeutic methodology could be used for the regulation and expression of angiogenic factors, such as vascular endothelial growth factor and fibroblast growth factor 2 to revascularize the pulp tissue of avulsed or other traumatized teeth.[61] In cases of anterior tooth avulsion or intrusion and extrusion in a young child, displacement of the tooth may lead to apical blood vessel and nerve rupture. In these cases, the teeth remain with open apices and large pulp chambers favoring repair and regenerative treatment modalities occur that may involve stem cell therapy.[62,63]

GROWTH FACTORS AND SIGNALING PATHWAYS

Growth factors are peptide molecules that transmit signals to control cell behavior and activity. They act through interaction with specific receptors located on the surfaces of

cells. A variety of growth factors have been identified and grouped into several classes. They include the following: TGF-α and TGF-β,[64] BMPs, fibroblast growth factors, Hedgehog proteins, and tumor necrosis factors.[65] Growth factors are responsible for signaling many of the events in tooth morphogenesis and response of the dental pulp to caries, microorganisms, and other noxious stimuli. Several studies have found that growth factors are present in the matrix of tooth dentin.[5,11,64,66]

Although studies have been performed, the results have yet to be used in a manner that allows regeneration and repair while not decreasing the volume of pulp tissue. Because the formation of secondary dentin is thought to be physiologic and occurs throughout life, the growth factors must be used in a manner that allows normal processes to continue as would occur in a virgin tooth with no restoration or caries or other stimuli that would increase the chance of narrowing and limiting natural processes in the dental pulp.

INFLAMMATION-REGENERATION

Although much is known about the mechanisms of both inflammation and regeneration, a failing in most studies occurs because there is a tendency to consider these entities separately rather than together.[67] Future studies should concentrate on both at the same time as they both occur, one step at a time (continuous until repair occurs). Inflammatory processes are seen as being antagonistic to these same processes that indicate that regeneration is occurring. Direct data have now emerged indicating that there is a relationship between the 2 processes.[41] The first (inflammation) results in tissue breakdown, whereas the latter develops regenerative (new tissue formation) actions. No doubt, increased inflammation may impede regeneration; however, if the inflammatory response is low grade, it may promote regenerative mechanisms that may include angiogenic stem cell processes. The data, however, are somewhat ambivalent, as greater levels of inflammation are studied alone without comparing the data that occur with regenerative effects. Therefore, it is necessary in the future not to separate the processes but attempt to study both at the same time. In the future, proper animal studies are necessary to demonstrate that these processes are fully described before clinical studies are undertaken. The limiting factor in both processes is the location of the dental pulp. Dentin surrounds the dental pulp and, although an inflammatory response to incipient caries may either regenerate or become a scar, the pulp tissue will be reduced in volume and other forms of dentin will occur that narrow the pulp tissue space. Studies need to be performed that develop suitable materials that will be able to reach the dental pulp through dentin tubules to regenerate original tissue without limiting the root canal system space.

SUMMARY

Tissue regeneration and engineering is the most challenging part of a tissue repair/regeneration program. The dental pulp is very small in relation to other human body tissues. Therefore, the idea of regenerated dental pulp tissue in a tooth has become a common thought among dental researchers. The regenerated tissue must contain the following attributes: it should be vascularized, contain similar cell density and architecture of the EMC, give rise to new odontoblasts lining dentin surfaces, and produce new dentin matrices that become mineralized and be innervated.[42,68]

The aspect of dentin-pulp tissue engineering is of great interest, with a large number of studies performed over the past several years. However, the science is still not able to allow clinical procedures to be performed routinely in animals or in humans. There are no clinical studies that can be routinely performed in an effort that will lead to

dentin-pulp repair and regeneration.[50] Manufacturers must be able to produce materials, both biologic and synthetic, that are reliable and safe. Expanded cell populations must consider the possibility of genetic instability. The hope of research to date rests on the ability that the use of naturally occurring cells at the site of injury may lessen side-effect risks. Better understanding of the dentin-pulp complex biology will lead to an exciting era of the development of cell-based approaches.[50]

Other challenges have become apparent as a result of the large number of studies involving stem cells, growth factors, and so forth in repair and regeneration and tissue engineering. An interesting aspect of these challenges involves the necessity of revascularization, which, although not ignored, has not been overly stressed in the science needed to repair/regenerate dental pulp tissue. Most procedures concerned with revascularization are conducted using a young group of individuals, 12 to 15 years old, with fully developed teeth whose pulp tissue contains high stem cell populations. The various components, stem cells, scaffolds, and growth factors, together with establishing an adequate vascular supply, can become optimized and integrated to produce, repair, and regenerate the dentin-pulp complex on a regular basis; however, there have been few, if any studies on adults. A survey of endodontists in 2009 found 96% of respondents believed that regenerative treatment modalities would become a normal, therapeutic option for teeth that otherwise would be removed.[69] Although supportive of research, endodontists foresaw the need for increased funding, increased clinical trials, and development of new therapies.

Many teeth are not treated because of the fear that they cannot be restored. Endodontics now has a better than 90% or higher rate of success.[70–74] Many endodontic specialists report success of 95% or better, especially when survival is considered with success and failure. More patients are treated by placement of implant-restored crowns rather than treated with root canal procedures on teeth that lend themselves to successful endodontic therapy. Therefore, there is a need for clinical specialties in dentistry to agree to a formula or creation of definitive standards that allow endodontists to successfully treat teeth or re-treat failures, allow prosthodontists to develop materials that prevent microleakage, and allow periodontists the ability to place implants when necessary. The development of regenerative endodontics may make those other procedures not needed (extraction and implant replacement), especially with the expectation that both hard (dentin, enamel) and soft (innervated and vascularized dental pulp) tissues would become a normal and successful procedure.

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