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REVIEW ARTICLE

Future prospects in endodontic regeneration - A review article

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Abstract

Regenerative medicine, aims to restore or establish the normal function of lost, diseased, damaged or aging cells, tissues and organs using a range of approaches; including cell-based and gene-based therapies, tissue engineering, and biomedical engineering. The future scope of regenerative endodontics may be increased to include the replacement of periapical tissues, periodontal ligaments, gingiva, and even tooth in-toto. The aim of this article is to review the prospects of endodontic regeneration.Future clinical research would likely focus on translating basic research findings into improved regenerative procedures, such as formation of cementum-like material on the dentinal walls that might lead to studies evaluating benefits of revascularization procedures for overall tooth resistance to fracture. Controlled differentiation into odontoblasts is an important area of research and amenable to tissue engineering concepts. The development of the delivery systems might permit structural reinforcement of the cervical area that might provide clinical opportunities to regenerate lost tooth structure, thereby permitting natural teeth to be retained. **Clinical significance:** Expanding the scope of regenerative medicine, researchers have also developed and applied regenerative procedures in oral soft and hard tissues.

Introduction

"Regenerative endodontics" is a branch of regenerative medicine and has been defined as biological procedures designed to replace damaged, diseased, or missing dental structures, including dentine and root as well as cells of the pulp-dentin complex, with living, viable tissues, preferably of the same origin, that restore the normal physiological functions of the pulp-dentin complex.[1]

Regenerative endodontic procedures use biologically based treatment modalities and pulpal cells. 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.[2]

Stem cells

The future of repair and regeneration depends on answers to the following questions. What is the nature of the stem cells that should be used to regenerate pulp tissue? A primary question must be asked as to what type of pulp-like 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? All stem cells in odontogenesis, with the exception of ameloblast progenitor cells, originate in the mesenchyme and are said to be of ectomesenchymal origin. When dental pulp stem cells (DPSCs) and stem cells from human exfoliated deciduous teeth (SHED) were compared, SHED cells showed higher potential to migrate and mineralize.[2]

Cell differentiation can lead to either adult progenitor or an odontoblast-like/osteoblast-like 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 formation of neurons. Therefore, is the use of multipotent progenitors or nonpotent cells, the cells of choice?[3,4]

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. Pulp

cells differentiate *in vitro* into odontoblast-like stem cells. The dentin formed, as previously mentioned and is tubular. Is there a possibility of dental pulp cells producing tubular dentin?[5,6] A recent study mixed pulp cells with hydroxyapatite (tricalcium phosphate powder) and generated a dentin-pulp like tissue.[7]

Bartouli and coworkers 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.[8]

Because repair and regeneration have different targets, the expectations of a particular therapy must be clear. Is regeneration of a non-mineralizing 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.[2] Caries may be the most common and dangerous of all types of injury, provoking adverse stimuli to the dental pulp. Many of the processes involved are thought to be the same as the initial pulp developmental processes occurring embryonically. 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.[9-11]

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.[12-15] 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. 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.[16-20]

Scaffolds

When stem cells are seeded on scaffolds, they are expected to attach, proliferate, and differentiate into new tissues that will eventually replace the scaffold. 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.[2,20-22]

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. 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 stem cells from apical papilla stem cells with apparent identification of NOTCH signaling expression.[23-26]

Although the number of scaffolds has increase questions remain that must be addressed.[2] 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. 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.[10,25-28]

Growth Factors and Signaling Pathways

A variety of growth factors have been identified and grouped into several classes. They include the following: Transforming growth factor (TGF)-α and TGF-β, bone morphogenetic proteins, fibroblast growth factors, hedgehog proteins, and tumor necrosis factors. 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. 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.[2,27]

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 revascularization the pulp tissue of avulsed or other traumatized teeth.[11]

Niches

Today, the ability of stem cell-based tissue engineering of teeth faces dilemmas of methods from development due to several differing conceptual issues. For example, where is the location and identity of odontogenic precursor cells that participate in reparative dentin formation?[12,13] Stem cells appear to have the ability for tissue repair and regeneration throughout life. 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.[26,27]

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? 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.[14]

Notch Signaling Proteins

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?

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 NOTCH signaling proteins regulates stem cell behavior for tooth repair. NOTCH receptors are absent in adult rat pulp tissue; their expression was found to occur after pulp tissue injury. 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 odontoblast-like pulp cells.[15]

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. 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 two processes.[9]

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. 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.[27,28]

With respect to all these the innervation of the pulp plays critical role in the homeostasis of the dental pulp complex. Invasion of immune and inflammatory cells into sites of injury in the pulp is stimulated by sensory nerves. Sensory denervation results in rapid necrosis of the exposed pulp because of impaired blood flow, extravasation of immune cells. Reinnervation leads to recovery in the coronal dentin.[16]

Future Perspective of Endodontic Regeneration

For cell-based therapy, the source of cells is an issue. Dental stem cell supply is limited especially from autologous sources. Not every individual who needs the regeneration treatment has the cells readily available. Establishing dental stem cell banking may be a necessary step and further progress on establishing individualized induced pluripotent stem cells for dental tissue regeneration is imminent.[5]

The willingness of endodontists and other specialist dentists to accept training to deliver stem cell therapies and Regenerative Endodontic Procedures to their patients is unclear. The ethics of using stem cell therapies to accomplish dental treatment is controversial. Stem cells from exfoliated deciduous and extracted teeth can be saved and might provide supply for therapeutic interventions. A dentist office could become a stem cell bank for patients, who might require new bone, teeth or other oral tissues.[17]

In a questionnaire regarding REPs, threw up some interesting statistics. The result of the survey showed that most of the dentists (96.8%) agreed that regenerative therapy should be incorporated into dentistry, and the majority (51.6%) believed that it would take between 11 and 20 years for regenerative stem cell therapies to be used in dentistry, for the development of new tooth in laboratory. Only a few of the dentists (19.4%) indicated that they or their family members had used umbilical cord banking or another type of stem cell banking. Nevertheless, most dentists (93.5%) had the opinion that dental stem cell banking would be useful in regenerating dental tissues. Most of the dentists (77.4%) believed that the biggest obstacle to patient acceptance of regenerative dental treatments would be a higher cost of the treatment. Most dentists (96.8%) indicated that they would be willing to save teeth and dental tissue for regenerative dental treatment, and a majority (87.7%) thought that regenerative dental treatments would be a better treatment option than tooth implant replacement. About 87.1% of dentist agreed that stem cell and regenerative treatments should be tested on animals before clinical testing. Many dentists (58.1%)

were willing to deliver dental treatments involving embryonic stem cells sourced from a human fetus to their patients. In regard to the future of regenerative treatments, a majority of dentists (64.5%) believed that there is no risk in stem cell clinics delivering future dental treatments. A majority of the dentists (67.7%) were concerned about the health hazards associated with the use of stem cells as part of regenerative dentistry. The majority of dentists (87.1%) held the opinion that dental professional associations should regulate the use of REPs.

This survey concluded that dentists are supportive of using REPs in their dental practice and they are willing to undergo extra training and to buy new technology to provide new procedures. However, dentists also need more evidence for the effectiveness and safety of regenerative treatments before they REPs were recommended.[18]

Stem cells can be used for regenerative procedures in dentistry. Despite its scientific validity, cell transplantation it has encountered major difficulties in translation into clinical therapy. The therapeutic use of stem cell products derived from nonhuman species will be limited because of the risk of immunorejection. Allogeneic cell transplantation has concerns of potential immunorejection and contamination.

The cell cryopreservation/banking system suffers from the potential loss of cells and additional costs. Potential contamination during cell manipulation and the 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.[19]

Despite the initial promise, regenerative endodontics has encountered substantial barriers in clinical translation. DPSCs might seem to be a prior choice for dental pulp regeneration. However, DPSCs may not be available in patients who are in need of pulp/dentin regeneration therapy. Even if DPSCs are available autologously or allogeneically, one must address a multitude of scientific, regulatory and commercialization barriers; unless these issues are resolved, the transplantation of DPSCs for dental pulp regeneration 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 potential tumorigenesis. Excessive costs associated with this issue in addition to shipping, storage, handling issues, and regulatory difficulties, including unclear pathway and the general inability to ensure batch-to-batch consistency in cell quality, cast multidimensional questions for the practicality of cell transplantation. Biomaterial scaffolds are another area of innovation in regenerative endodontics. Several 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.

The outcome of the regenerative therapy is based on radiographic and histological evaluation in animals, whereas the case reports and case series emphasized the importance of follow-up, including recording symptoms, radiographs, and clinical tests. Due to the variability in recall, it is difficult to establish time frames in which practitioners may expect to see radiographic changes. This information is important because it can help in assessing if and when alternative treatments (i.e., apexification, traditional nonsurgical root canal therapy, or extraction) may be necessary.[19]

Conclusion

Future clinical research would likely focus on translating basic research findings into improved regenerative procedures, such as formation of cementum-like material on the dentinal walls that might lead to studies evaluating benefits of revascularization procedures for overall tooth resistance to fracture. Controlled differentiation into odontoblasts is an important area of research and amenable to tissue engineering concepts. The development of the delivery systems might permit structural reinforcement of the cervical area that might provide clinical opportunities to regenerate lost tooth structure, thereby permitting natural teeth to be retained.

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