

## Regenerative Endodontics: Part 1

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### Introduction

The dental pulp is of mesenchymal origin. It is a complex tissue comprised of nerves, connective tissue fibers, odontoblasts, fibroblasts, immunocompetent cells, stem cells, vascular tissue, ground substance, interstitial fluid, and other cellular components. The close relationship between odontoblasts and dentin is often referred to as the dentin-pulp complex. The function of pulp as sensory tissue is well known. However, pulp tissue also has regenerative potential equally important in its protective role. It has a lifelong ability to produce new dentin in response to trauma and disease (1). Once the functional properties of the pulp are restored, continued dentin formation is possible. A vital pulp also protects against apical periodontitis (2).

Preservation of the natural dentition remains a primary objective in endodontic practice. When the pulp is diseased or necessitates removal for restorative reasons, it can be replaced with an artificial filling material. Esthetics, form and function are not primarily dependent upon a vital pulp after root maturation, but there are disadvantages to conventional root canal treatment. In teeth with completely formed apices, obturating a root canal with gutta percha meets nearly all of Grossman's proposed characteristics of an ideal root canal filling material except being impervious to moisture or fluid and nonporous (3). Today, it is known that gutta percha does not provide a barrier against the microorganisms implicated in apical disease (4). In an immature root, thin dentinal walls are susceptible to fracture and may render the tooth non-restorable. Apexification with calcium hydroxide was popular, but it is now known that long-term and short-term (30 days) use could weaken the dentin of mature human, permanent teeth, making them more prone to fracture (5,6). Innovative dental materials in combination with regenerative medicine have renewed an interest in preserving dental pulp in order to retain the natural dentition.

Treating the diseased or traumatized immature tooth remains a challenge. Preservation of a partially vital pulp and revascularization or regeneration of the pulp can offer many advantages over traditional root canal therapy (7-9). An exciting protocol exists that may allow for maturation of the root even in the presence of apical disease (10).

The aim of this clinical update is twofold. Part 1 will present an overview of regenerative medicine, stem cell terminology and the application of these concepts to endodontics. The principles that govern the preservation and repair of immature teeth are the same ideas that promise regrowth of a pulp or natural tooth. Part 2 explains a current clinical procedure for managing diseased or traumatized pulps of permanent teeth with immature root apices and reviews the current literature

regarding this procedure and that of implanting engineered pulp into root canal systems.

### Regenerative Medicine and Dentistry

Tissue engineering is a rapidly developing interdisciplinary field likely to revolutionize health care and the quality of life for millions of patients (11). Specifically, regenerative medicine uses a combination of cells, engineering materials, and biochemical factors to improve or replace biological functions. There are endless opportunities for research in tissue engineering, including the biomechanical aspects of design and the supporting informatics, ranging from gene sequencing to data mining tools (11). Following, is a brief review of some basic concepts which are essential to understanding the laboratory research of complex regenerative endodontic procedures (REPs). Familiarization with the terminology and concepts are equally important for appreciating the rationale for the clinical approach taken in modern pulp therapy protocols.

#### 1. Cell Source

Embryonic stem cell - The first such cell was derived from an early mouse embryo, first discovered in 1981. It is now defined as a "primitive (undifferentiated) cell derived from a 5-day preimplantation embryo that is capable of dividing without differentiating for a prolonged period in culture, and is known to develop into cells and tissues of the three primary germ layers" (11).

Human embryonic stem cell (hESC) - a type of stem cell derived from the inner cell mass (ICM) of the blastocyst of a 4-5 day embryo. These human embryos are typically derived from eggs donated for research purposes by consenting adults and fertilized in vitro. The cells are abundant, easily grown in culture and are pluripotent, making them capable of becoming any tissue type in the body (11).

Adult (or somatic) stem cell - an undifferentiated cell found in differentiated tissue that can renew and differentiate (with limitations) to form the specialized cell types of tissue from which it originated. Examples include blood cells becoming neurons, liver cells that can be made to produce insulin, hematopoietic stem cells that can develop into heart muscle and dental apical papilla cells to produce pulp, dentin and cementum. These cells are few in number and difficult to isolate. In contrast to the widely publicized hESCs, these cells have been studied in dentistry. Specifically, five potential sources have been identified: human exfoliated deciduous teeth (SHED), dental pulp stem cells (DPSC), stem cells from apical papilla (SCAP), periodontal ligament stem cells (PDLSC) and dental follicle precursor cells (DFPC) (12).

## 2. Scaffolds

Scaffolds are necessary for cell differentiation and growth. Ideally, the scaffold would be of appropriate morphology, absorbable over time and possess optimal modulus of elasticity and porosity. A favorable porosity permits cell migration, adhesion and proliferation. The physical structure of a scaffold can be injectable or solid for implantation. Most injectable scaffolds are hydrogels. Hydrogels are polymers which are flexible and absorbent due to their high water content. Hydrogels can have undesirable nanoscale porosity. However, an FDA-approved injectable polymer poly(lactic-co-glycolic) acid scaffold with in situ pore formation has had promising research results (13). Examples of solid scaffolds are collagen, polymer and calcium phosphate. Of the solid-type scaffolds, the polymer and collagen types show promise for their ability to support cell adhesion, proliferation and differentiation (14-17). Lately, platelet-rich-plasma (PRP) has been suggested to satisfy the ideal properties of a scaffold. It is autologous, easy to prepare, and forms a 3-D fibrin matrix. However, research to assess its place in regenerative endodontic applications still needs to be completed.

## 3. Signals

Signaling molecules or morphogens are extracellularly secreted proteins governing interactions between epithelial-mesenchymal tissues. These molecules stimulate cell proliferation and direct cell differentiation (18). Examples of morphogens are bone morphogenetic proteins (BMP's/BMP genes), fibroblast growth factors (FGF), wingless and int-related proteins (Wnts), hedgehog proteins (Hhs) and tumor necrotic factor (TNF). It is likely the combination of cell and signal determines the end product. For example, the signal will establish whether cells from the same source of stem cells will differentiate into an odontoblast or some other cell type.

## Directions in Research

Researchers do not yet fully appreciate the genetic and molecular controls of processes that are key to developing new strategies for therapy in regenerative medicine. Much effort is directed at understanding the *signals* that turn specific genes on and off to influence cell differentiation. Questions still needing to be answered include: 1. Why can embryonic stem cells proliferate for a year or more in the lab without differentiating, but most adult stem cells cannot, and 2. What are the factors that normally regulate stem cell proliferation and self-renewal (11).

## Clinical Implications

As research questions in stem cell technology are answered, the possibilities for using regenerative techniques in Endodontics and other surgical dental specialties are limited only by the imagination. Present ways in which regenerative treatments show promise are in pulp therapy for immature teeth, replanting regenerated pulps into root canals and growing new teeth. All are further explored in Part 2 of this clinical update.

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