

Pulp tissue from primary teeth: new source of stem cells

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ABSTRACT

SHED (stem cells from human exfoliated deciduous teeth) represent a population of postnatal stem cells capable of extensive proliferation and multipotential differentiation. Primary teeth may be an ideal source of postnatal stem cells to regenerate tooth structures and bone, and possibly to treat neural tissue injury or degenerative diseases. SHED are highly proliferative cells derived from an accessible tissue source, and therefore hold potential for providing enough cells for clinical applications. In this review, we describe the current knowledge about dental pulp stem cells and discuss tissue engineering approaches that use SHED to replace irreversibly inflamed or necrotic pulps with a healthy and functionally competent tissue that is capable of forming new dentin.

Key words: Tissue regeneration. Dental pulp. Tissue engineering. Endodontics.

INTRODUCTION

The history of research on adult stem cells began about 40 years ago. In the sixties, researchers discovered that bone marrow contains at least two kinds of stem cells. One population, called hematopoietic stem cells, forms all the types of blood cells in the body. A second population, called bone marrow stromal cells, was discovered few years later. Stromal cells are a mixed population of progenitor cells that generate bone, cartilage, fat, and fibrous connective tissue^{2,4-7,16,43,45,48,52,57}. Today, stem cell biology is one of the most fascinating areas of sciences. However, like many other expanding fields of scientific inquiry, research on stem cells raises scientific questions as rapidly as it generates new discoveries.

Postnatal stem cells have been isolated from a variety of tissues including bone marrow, brain, skin, hair follicles, skeletal muscle and dental pulp. These cells are thought to possess great therapeutic potential for repairing damaged and/or defective tissues^{3-5,8,9,12-15,17,19,20,24,29,35,37,44,58}. It has been shown

that stem cells from human exfoliated deciduous teeth (SHED) represent a population of postnatal stem cells capable of extensive proliferation and multipotential differentiation. The transition from deciduous to permanent teeth is a very unique and dynamic process in which the development and eruption of permanent teeth synchronize with the resorption of the roots of deciduous teeth. Exfoliated deciduous tooth is similar in some ways to an umbilical cord, containing stem cells that may offer a unique postnatal stem cell source for potential clinical applications^{1,10,39,56}. It has been demonstrated that stem cells are present within the pulp tissue not only of deciduous teeth, but also in permanent teeth (dental pulp stem cells – DPSC), and in the periodontal ligament (periodontal ligament stem cells – PDLSC)^{2,3,7,13,16,19,20,26,28,40,44,51}. This manuscript is designed to serve as a comprehensive synthesis of our current knowledge of stem cells from human exfoliated deciduous teeth and to describe the current efforts on tissue engineering with the use of SHED in order to replace irreversibly inflamed or necrotic pulps due to dental caries by a healthy

and functionally competent tissue.

Stem cells

Stem cell is a broad term used to describe a wide variety of cells from varying sources. Stem cells can be divided into two categories – embryonic and adult. Embryonic stem cells are totipotent cells capable of differentiation into virtually any cell type, as well as being propagated indefinitely in an undifferentiated state^{1,3,5,8,10,14,16-18,24,25,27,28,31-34,38,39,47,49,50,54}. Adult stem cells are not totipotent, and they can be further classified depending on their origin and differentiation potential^{3,14,19,26,31,35}. The use of embryonic stem cells generates several ethical concerns regarding the consumption of blastocysts. This makes postnatal stem cells a more feasible approach for translation into clinical practice.

Stem cells fascinate us from both theoretical and practical viewpoints. Their defining characteristics – extensive proliferative potential and ability to give rise to one or more differentiated cell types – are more common in early mammalian embryos. However, embryonic cells lose these properties as differentiation ensues and growth-promoting signals decline. By adulthood, the few remaining stem cells are dispersed and virtually invisible, but the surviving adult stem cells achieve something remarkable: they can operate as “steady state” – that is, they can generate on average one replacement stem cell and one tissue-specific cell at each division with no apparent limit (Figure 1).

Stem cells differ from other kinds of cells in the body. All stem cells, regardless of their source, have three general properties: they are unspecialized; they are capable of continuous self-renewal; and they can give rise to specialized cell types.

Stem cells are unspecialized. One of the fundamental properties of a stem cell is that it does not have any tissue-specific structures that allow it to perform specialized functions. A stem cell cannot work with its neighbors to pump blood through the body (like a heart muscle cell); it cannot carry molecules of oxygen through the bloodstream (like a nerve cell). However, unspecialized stem cells can give rise to specialized cells, including heart muscle cells, blood cells, or nerve cells^{1,7,9,17,24,30}.

Stem cells are capable of self-renewal. Unlike muscle cells, blood cells, or nerve cells, which do not normally replicate themselves, stem cells may replicate many times. A starting population of stem cells that proliferates for several months in the laboratory can yield millions of cells. If the resulting cells continue to be unspecialized, like the parent stem cells, the cells are said to be capable of long-term self-renewal.

Stem cells can give rise to specialized cells. When unspecialized stem cells give rise to specialized cells, the process is called differentiation. The internal

signals are controlled by proteins that regulate gene expression and are called transcriptional factors. The external signals for cell differentiation include molecules secreted by other cells, physical contact with neighboring cells, and molecules from the local microenvironment.

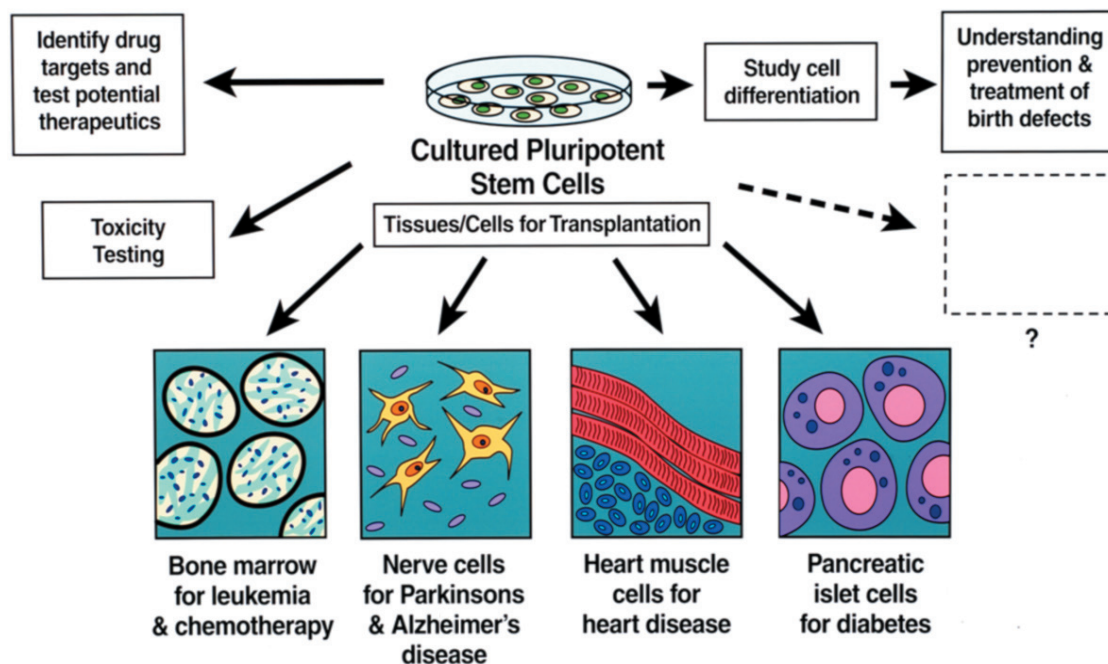
Dental caries and pulp response

Dental caries is the most common chronic disease affecting children in several countries. Despite advances in oral health, dental and oral diseases continue to plague children. Factors contributing to an oral health decline include lack of access to care, inadequate availability of preventive measures and lack of knowledge regarding the importance of oral health. Oral tissue infections and associated nutritional deficits can lead to imbalances in oral flora populations, eventually contributing to compromised overall health and reduced quality of children's life^{6,7}. Over the last decades, biomedical research has focused primarily on the understanding of the mechanisms of biological functions in health and disease. For example, our understanding of the mechanisms of dental caries has advanced tremendously⁵. Dentists are faced every day with the task of restoring tooth structure lost during the progression of caries lesions. The development of materials that allow for strong and stable bonding to tooth structure, and the development of esthetic materials that are resistant to wear and degradation in the oral environment, has significantly enhanced the long-term clinical outcomes of restorative procedures. Nevertheless, dental clinicians know that no material available today can mimic all the physical, mechanical and esthetic properties of enamel and dentin^{5,42}.

The field of tooth regeneration is one of the ultimate goals of restoring the loss of natural teeth. The ability to obtain and manipulate postnatal tissue easily from individuals to generate biological replacement tooth materials, such as dentin, enamel, and periodontal ligament, or, even better, to replace complete teeth of predetermined size and shape, is extremely valuable^{1,8,10}. Small amounts of reparative dentin can be induced to form in response to subtle tooth injury and cementum also exhibits limited regenerative capabilities. In contrast, enamel exhibits no regenerative capacity because progenitor dental epithelial cells that form enamel lose this ability well before tooth eruption^{8,9}. The high susceptibility of teeth to damage, combined with non-regenerative nature of dental tissues, emphasizes the need for regenerative tooth therapies in children and adults^{4,10,30,41,55}.

In pathological conditions, such as mild carious dentin lesions, odontoblastic activity is stimulated to produce reactionary dentin. Severe carious lesions or deep cavity preparation may lead to local

The Promise of Stem Cell Research



Source: National Institute of Health. The promise of stem cell research [figure on the internet]. Bethesda: NIH; 2009 [cited 2010 May 03]. Available from: <http://alumnimagazine.uconn.edu/sprg2007/feature2.html>

Figure 1- Diagram depicting the expected clinical applications of some stem cell research

odontoblastic death. However, undifferentiated cells attracted to the injury site can differentiate into odontoblast-like cells and secrete a reparative dentin matrix. Several lines of evidence suggest the presence of progenitor or postnatal stem cells in the pulp capable of differentiation into odontoblast-like cells and secretion of reparative dentin *in vitro*^{11,12}. Odontoblast cells are post-mitotic and are responsible for the secretion of primary dentin. Indeed, reparative dentin synthesis is a complex biological process. It requires the presence of progenitor cells, their proliferation, migration, recruitment and activation at the injury site to differentiate into odontoblast-like cells secreting the hard protective reparative dentin. The dental pulp is a highly vascularized tissue and pulp cavity preparation results in a subsequent injury to the pulp tissue including the blood vessels. It is recognized that injured endothelial cells release chemotactic factors and signaling molecules to initiate the inflammatory process and express adhesion molecules necessary for the recruitment of inflammatory and progenitor cells for initiating the healing process^{14,15}. The presence of inflammation, which will be exacerbated by bacterial infection, is well recognized as a moderator of regeneration and will probably inhibit regenerative processes as long as it is maintained^{16,18}.

The concept of migration of stem cells to the site of injury for differentiation into a new generation of odontoblast-like cells is very important

event for cell recruitment during regeneration when the vitality of the primary odontoblasts is compromised^{17,19,20}. It is unclear whether this reflects a direct effect on the stem cells for regeneration or the molecular signaling processes responsible for their differentiation. Nevertheless, the presence of inflammation will compromise stem cell recruitment and differentiation during regeneration^{22,23}. An understanding that local angiogenesis is a physiologic event during healing at all wound sites, including the dental pulp with a remarkable importance of nutrition during the healing process, and it may also increase the opportunities for perivascular stem cells recruitment during regeneration²⁶.

Dental pulp-derived cells are potentially a useful alternative for cell replacement in the treatment of tissues known to contain neural stem cells. It has been demonstrated the first successful induction of neural differentiation of rat dental pulp-derived cells, particularly into a glial cell lineage. Successful *in vitro* and *in vivo* differentiation of neural stem cells obtained from bone marrow into neurons and/or oligodendrocytes has been also reported^{38,39,48}. The authors concluded that the existence of neural stem cells in tissues other than the central nervous system may represent a significant step toward providing more diverse and multiple sources of stem cells for regenerative medicine³⁸.

The understanding of pulp biology has improved significantly in recent years. However, it is still

not possible to state with certainty which cell populations and which specific molecular signaling pathways predominate during dental regeneration. If pulpal stem cells are to be optimally harnessed for dental regeneration, then strategies are required to ensure their effective recruitment at sites of injury. Whilst such recruitment does occur during natural regeneration, it is a somewhat haphazard process lacking in control. Directed recruitment of these cells might be achieved through local application of enriched cell populations, either by harvesting cells from non-autologous teeth or autologous shed deciduous primary teeth.

Dental pulp tissue engineering with the use of SHED

Although conventional restorative materials, such as amalgam, composite resin and glass ionomer cement, have been proven effective in the maintenance of teeth in the oral cavity, they clearly present a limited lifespan, thus requiring replacement over time. Additionally, a significant percentage of treated teeth undergo pulp inflammation and necrosis, requiring further endodontic treatment and prosthetic reconstruction or tooth extraction. Therefore, the development of new techniques capable of regenerating lost tooth structure would benefit the population significantly¹³.

Nowadays, tissue engineering, which corresponds to a multidisciplinary science that brings together biology, engineering and clinical sciences with developing new tissues and organs⁴², constitutes a promise as a new method to repair congenital and/or diseased teeth^{36,57}. Particularly, the main goal of dental pulp tissue engineering is to replace the inflamed or necrotic pulp by a healthy and functional tissue, capable of forming new dentin. This science is based on principles in which undifferentiated cells placed into biocompatible scaffolds respond to specific signals that induce their proliferation, migration and differentiation into specialized cell lineages.

Common tissue engineering involves the seeding of appropriate cells in biodegradable scaffolds built with desirable mechanical features and the stimulation of cell growth and differentiation *in vitro*. When this set is implanted *in vivo*, it may undergo remodeling and maturation into a complete functional tissue^{36,57}. Therefore, dental pulp tissue engineering may be the first step toward dentin regeneration in necrotic teeth, as well as an alternative to conventional endodontic treatment²¹, with the advantage of restoring tooth vitality. Hence, the identification of appropriate cells, the development of conductive scaffolds and the understanding of morphogenetic signaling required to induce cells to regenerate lost tissues

are mandatory^{42,43,53,59}.

Stem cells can be induced to differentiate into a specific phenotype through the manipulation of cell culture conditions. It is possible to control or restrict available differentiation pathways and selectively generate enriched cultures with a particular phenotype. These manipulations include cell stimuli with specific cytokines, growth factors, amino acids, other proteins and active ions, and co-culture with a specific cell type or tissue⁴⁶.

In vivo evidence for pulp cells being capable of generating a dentin-pulp-like complex have been demonstrated by Gronthos, et al.¹⁸ (2000) and Miura, et al.³⁹ (2003) who transplanted DPSC and SHED, respectively, mixed with hydroxyapatite/tricalcium phosphate into immunocompromised mice. Within 6 weeks, a dentin-like structure lined with odontoblast-like cells that surrounded a pulp-like interstitial tissue and had their process penetrating into tubular structures was observed. Moreover, collagen fibers oriented perpendicularly to the odontoblast layer within the dentin, which is characteristic of primary dentin. Dentin-like material formed in the implants had a mineralized globular aspect, compatible with human dentin structure. In addition, odontoblast-like cells and the remaining pulp cells were from the donor^{18,39}. When DPSC were seeded onto human dentin surface and implanted into immunocompromised mice, scattered reparative dentin-like structure was deposited on the dentin surface⁴.

Cordeiro, et al.¹¹ (2008) evaluated morphologic characteristics of the tissue formed when SHED seeded in biodegradable scaffolds prepared within human tooth slices were transplanted into immunodeficient mice. The resulting tissue presented architecture and cellularity that closely resemble those of a physiologic dental pulp. Ultrastructural analysis with transmission electron microscopy and immunohistochemistry for dentin sialoprotein suggested that SHED differentiated into odontoblast-like cells *in vivo*. Notably, SHED also differentiated into endothelial-like cells, as demonstrated by B-galactosidase staining of cells lining the walls of blood-containing vessels in tissues engineered with SHED stably transduced with LacZ.

Using the same experimental model, Sakai, et al.⁴⁸ (2010) tested the hypothesis that SHED differentiate into functional odontoblasts and endothelial cells. SHED differentiated into functional odontoblasts that generated new dentin, as determined by tetracycline staining and confocal microscopy. These cells also differentiated into vascular endothelial cells, as determined by B-galactosidase staining of LacZ-tagged SHED. *In vitro*, vascular endothelial growth factor (VEGF) induced SHED to express VEGFR2, CD31, and VE-Cadherin (markers of endothelium) and organize

into capillary-like sprouts. VEGF induced ERK and AKT phosphorylation (indicative of differentiation), while inhibiting phosphorylation of STAT3 (indicative of stemness).

We are at an exciting point of a new era of restorative dentistry harnessing the biological activity of the dental tissues to facilitate wound healing and tissue regeneration. There is still much to learn of the nature, potentiality and behavior of dental stem cells, but the opportunities for their exploitation in dental tissue regeneration are immense and will lead to significant benefits for the management of the effects of dental disease⁴⁴.

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