Stem Cells in Dental Practice: Perspectives in Conservative Pulp Therapies

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Stem cells are undifferentiated cells that have the capacity to self-renew. They have been discovered in many adult tissues, including teeth. The Dental Pulp Stem Cells are involved in dentinal repair by activation of growth factors, released after caries process and have the ability to regenerate the dentin-pulp-like complex. The molecular/cellular research raises the possibilities to grow new tissues and biological structures for clinical application, providing cells for therapies including cell transplantation and tissue engineering.

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INTRODUCTION

The stem cells are defined as clonogenic unspecialized cells capable of both self-renewal for long periods and multi-lineage differentiation, contributing to regenerate specific tissues. They can theoretically divide without limit to replenish other cells as long as the person is still alive.

In living organisms, stem cells are important for many reasons. In the 3 to 5 day old embryo, called the blastocyst, stem cells in developing tissues give rise to the multiple specialized cells types that make up the heart, lung, skin, and other tissues. Stem cells generate replacements for cells that are lost through normal wear, injury or disease. The specific conditions that allow stem cells to remain unspecialized and the signals inside and outside cells that trigger stem cells differentiation poses a challenge for the researcher.

There are two types of stem cells: the embryonic stem cells and the adult stem cells.

Embryonic stem cells are derived from embryos that are typically four or five days old called the blastocysts. These cells in the early embryo are pluripotent, i. e. they have the capacity to form all tissues. The cells of the inner cell mass of the blastocyst contribute to all tissues of the adult (Gilbert, 2000). Successful culture of stem cells from human embryos was reported for the first time in 1988 (Thompson, 1998). Stem cells could be exposed to specific combinations of growth and differentiation factors *in vitro*, which would induce their differentiation in desired directions. Different types of tissue could then be grown in culture and afterwards transplanted to patients. Also, pluripotent embryonic stem cells could be directly implanted into the patient's tissues, where they would then differentiate into specific cell types after encountering the appropriate niche. There is, in fact, some evidence that damage tissues may exert chemotactic influences on the stem cells and, thereby, stem cells might be specifically guided to sites where they are needed for tissue regeneration.

Adult stem cells are an undifferentiated cell that typically generates the cell types of the tissue in which they reside. They can renew themselves and the primary roles in a living organism are to maintain and repair the tissue in which they are found. Adult stem cells may also exhibit the ability to form specialized cell types of other tissues, which is known as transdifferentiation or plasticity. Scientists have found adult stem cells in many more tissues than they once thought possible. A number of experiments have suggested that certain adult stem cells types are pluripotent. Adult blood forming stem cells from bone marrow have been used in transplants for 30 years.

Certain kinds of adult stem cells seem to have the ability to differentiate into a number of different cell types, given the right conditions. Stem cells are thought to reside in a specific area of each tissue where they may remain quiescent for many years until they are activated by disease or tissue injury. The adult tissues reported to contain stem cells include brain, bone marrow, peripheral blood, blood vessels, skeletal muscle, skin and liver, and more recently, found in the dental pulp.

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Properties of dental pulp stem cells (DPSC)

Post-natal stem cells have been found in various tissues, including bone marrow, neural tissue, skin, retina and, more recently, in dental epithelium (Harada et al 1999; Fuchs and Segre 2000; Blau *et al*. 2001).

The bone marrow stromal stem cells (BMSCs), which have been defined as pluripotential adult stem cells, are a well known undifferentiated cell and have the potential to develop into osteoblasts, chondrocytes, adipocytes, myelossuportive fibrous stroma, and neural tissues (Freidenstein *et al*, 1978; Pittenger *et al*., 1999). They are characterized by their high proliferative capacity *ex vivo*, whereas maintaining their ability to differentiate into multiple stromal cell lineages. The tissue-specific differentiation of BMSCs seems to be dependent on their state of differentiation and commitment, and the microenvironment in which they are located.

The similarity between bone marrow stromal stem cells and dental pulp stem cells in the gene expression profiles have already been shown (SHI *et al*. 2001). Dental pulp stem cells (DPSC), like osteoblasts, express bone markers such as bone sialoprotein, alkaline phosphatase, type I collagen, and osteocalcin (Kuo *et al*, 1992; Tsukamoto *et al*, 1992; Nakashima *et al*, 1994; Butler *et al*, 1997; Shiba *et al*, 1998; Buurma *et al*, 1999; Buchaille *et al*., 2000). Previous reports have demonstrated that various bone formation regulators, including TGF ß (transforming growth factor ß) and others cytokinas, are members that regulated their differentiation (Shiba *et al*, 1998; Onishi *et al*, 1999).

Research has been made in order to compare the proliferative and differentiation potential between BMSCs and DPSCs cells. The *in vitro* experiments of colony-forming efficiency and cell proliferation from dental pulp tissue and bone marrow indicated that the incidence of colony-forming cells is more clonogenic in dental pulp than in bone marrow (Gronthos *et al*, 2000).

According to Gronthos *et al*, (2002), the DPSCs have the ability to regenerate the dentin-pulp-like complex. Following *in vivo* transplantation, they were capable of forming ectopic dentin and associated pulp tissue, representing a novel adult stem cell population that possesses the properties of high proliferative potential, the capacity of self-renewal, and multi-lineage differentiation. Analysis of the dentin matrix formed by single-colony-derived strains demonstrated a mineralized dentin matrix, containing organized collagen fibers, similar to that formed by multi-colony-derived DPSCs. Although, DPSCs of single-colony-derived strains differ each other with respect to their rate of odontogenesis, showing that DPSCs possess stem-cell-like qualities, including self-renewal capacity and multilineage differentiation.

The ethical aspects related to the utilization of embryonic stem cells in the tissue engineering and cells therapies have risen to the search for auxiliary resources of high quality of stem cells. The transition from primary teeth to adult permanent teeth is a unique and physiologic process in which the development and eruption of permanent teeth is coordinated with the resorption of the roots of primary teeth. Recently, a population of high quality human stem cells was found in the exfoliated human primary teeth (Miura *et al*, 2003). The stem cells from human exfoliated primary teeth (SHED) were identified to form part of a highly proliferative, clonogenic cells capable of differentiating into a variety of cell types including neural cells, adipocytes, and odontoblasts. In comparison with BMSCs and DPSCs, SHED showed a significantly higher proliferation rate. The SHEDs have the osteoinductive capacity *in vivo*, but failed to reconstitute a dentin-pulp-like complex, perhaps in order to have more immature characteristics than other post-natal stem-cell population (Miura *et al*., 2003).

The human post-natal stem cells from accessible resources, like the ones derived from exfoliated primary teeth, can constitute in a potential clinical application, providing cells for stem cell therapies including cell transplantation and tissue engineering.

The application of molecular biology and DPSC in conservative pulp therapies

Strong evidence suggest the presence of resting progenitor or stem cells in dental pulp (Gronthos *et al*, 2000, 2002; Shi and Gronthos, 2003; Miura *et al*., 2003). Reparative dentin synthesis is a complex biological process that requires the presence of progenitor cells for their proliferation, migration, recruitment and activation at the injury site. Little is known about the activation and migration of these cells in response to injury.

Dentinal repair in post-natal organism occurs through the activity of specialized cells, called odontoblasts, which are thought to be maintained by an as yet undefined precursor population associated with pulp tissue. In restorative dentistry, moderate carious lesions stimulate the secretory activity of the odontoblasts to elaborate reparative dentin (Tziafas, 1995; Smith *et al*, 1995) while, deep cavity preparation or severe carious lesions may lead to partial destruction of the odontoblastic layer. These conditions attracted cells to the injury site and differentiated into odontoblast-like cells that can replace the necrotic odontoblasts and secrete a reparative dentin matrix (about *et al*., 2000).

Third human molars after deep cavity preparation involving pulpal exposure demonstrated that perivascular progenitor cells can proliferate in response to odontoblasts injury (Téclès *et al*, 2005).

Dentin matrix can be considered a reservoir of growth factors, since growth factors such as transforming growth factors (TGF ß), bone morphogenic protein (BMP), fibroblast growth factor (FGF) and insulin-like growth factor (IGF) are secreted by functional odontoblasts and pulp fibroblasts (Finkelman *et al*., 1990; Ruch *et al*, 1995). These factors are released after dentin demineralization by caries process and seem to be involved in the proliferation and differentiation of pulp cells, providing chemotactic signals to recruit progenitor pulp cells at injury site and to initiate tissue repair (Martin, 1997; D'Souza *et al*, 1998).

The deposition of tertiary dentin matrix is an occurrence that can be observed in the indirect pulp capping (IPC). In this procedure, non-remineralizable tissue is removed and a thin layer of caries is left at the deepest site of the cavity avoiding the possibility of pulp exposure (Mc Donald and Avery, 1994; AAPD, 2001). It is believed that IPC cause a mild grade injury, and the odontoblasts and other pulpal cells are stimulated or up-regulated to secrete a reactionary type of tertiary dentin matrix. In this case, reparative dentinogenesis needs recruitment of progenitor cells from the pulp population, their differentiation into odontoblast-like cells and then stimulation of matrix secretion. Growth factors provide the molecular signal for this induction of odontoblast differentiation and the subsequent stimulation of matrix secretion by these cells.

The dental pulp has a naturally high vascularized tissue and conservative pulp procedures like direct pulp capping and pulpotomy result in the injury of blood vessels. The injured endothelial cells

release chemotactic factors 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 (Martin, 1997; Tedder *et al*, 1995). It was suggested that the endothelial injury is involved in the recruitment of odontoblast-like cells at the injury site (Mathieu *et al*, 2005). This information raises the speculations about the use of stem cells and signal molecules in conservative pulp therapies and in trauma to teeth, with incomplete root formation, leading to a more biological approach.

Future studies must be focused on the molecular and cellular events that play an important role in tooth regeneration, physiology/embryology, treatment related events and therapies related with stem-cells, contributing to a better clinical dental practice.

REFERENCES

1. Gilbert SF. Developmental Biology, ed 6. Sunderland, Sinauer.; 314-318. 2000
2. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. Science.; 282:1145-47. 1998
3. Harada H, Kettunen P, Jung HS, Mustonen T, Wang YA, Thesleff I. Localization of putative stem cells in dental epithelium and their association with Notch and FGF signaling. J Cell Biol.; 147:105-120. 1999
4. Fuchs E, Segre JA. Stem cells: a new lease on life. Cell,; 100:143-155, 2000.
5. Blau HM, Brazelton TR, Weimann JM. The envolving concept of a stem cell: entity or function? Cell.; 105:829-841. 2001
6. Friedenstein AJ, Ivanov-Smolenski AA, Chajlakan RK, Gorskaya UF, Kuralesova AI, Latzinik NW, Gerasimow UW. Exp. Hematol.; 6: 440-44. 1978
7. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Science,; 284:143-47. 1999
8. Shi S, Gehron RP, Gronthos S. Comparison of gene expression profiles for human, dental pulp and bone marrow stromal stem cells by cDNA microarray analysis. Bone. 29:532-9. 2001
9. Kuo MY, Lan WH, Lin SK, Tsai KS, Hahn LJ. Collagen gene expression in human dental pulp cell cultures. Arch Oral Biol; 37:945-952. 1992
10. Tsukamoto Y, Fukutani S, Shin-Ike T, Kubota T, Sato S, Suzuki Y, et al. Mineralized nodule formation by cultures of human dental pulp-derived fibroblasts. Arch oral Biol; 37:1045-1055. 1992
11. Nakashima M, Nagasawa H, Yamada Y, Reddi AH. Regulatory role of transforming growth factor-beta, bone morphogenetic protein-2, and protein-4 on gene expression of extracellular matrix proteins and differentiation of dental pulp cells. Dev Biol; 162:18-28. 1994
12. Butler WT, Ritchie HH, Bronckers AL. Extracellular matrix proteins of dentine. Ciba Found Symp; 205:107-115. 1997
13. Shiba H, Fujita T, Doi N, Nakamura S, Nakanishi K, Takemoto T, et al. Differential effects of various growth factors and cytokines on the syn theses of DNA, type I collagen, laminin, fribronectin, osteonectin/ secreted protein, acidic and rich in cysteine (SPARC), and alkaline phosphatase by human pulp cells in culture. J Cell Physiol; 174:194-205. 1998
14. Buurma B, Gu K, Rutherford RB. Transplantation of human pulpal and gingival fibroblasts attached to synthetic scaffolds. Eur J Oral Sci; 107:282-9. 1999
15. Buchaille R, Couble ML, Magloire H, Bleicher F. Expression of the small leucine-rich proteoglycan osteoadherin/osteomodulin in human dental pulp and developing rat teeth. Bone; 27:256-70. 2000
16. Onishi T, Kinoshita S, Shintani S, Sobue S, Oshima T. Stimulation of proliferation and differentiation of dog dental pulp cells in serum-free culture medium by insulin-like growth factor. Arch Oral Biol; 44:361-371. 1999
17. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. PNAS; 97:13625-30. 2000
18. Gronthos S, Brahim J, Li W, Fisher LW, Cherman N, Boyde A, et al. Stem cell properties of human dental pulp stem pulp stem cell. J Dent Res. 8: 531-5. 2002
19. Miura M, Gronthos S, Mingrui Z, Lu B, Fisher LW, Robey PG, Shi S. SHED: stem cells from human exfoliated deciduous teeth. PNAS, 10:5807-5812. 2003
20. Tziafas D. Basic mechanisms of cytodifferentiation and dentinogenesis during pulp repair. Int J Dev Biol.; 39:281-90. 1995
21. Smith AJ, Cass IDYM, Perry H, Begue-Kirn C, Ruch CV, Lesot H. Reactionary dentinogenesis. Int J Dv Biol.; 39:273-80. 1995
22. About I, Bottero MJ, De Denato P, Camps J, Franquin JC, Mitsiadis TA. Human dentin production in vitro. Exp Cell Res.; 258:33-41. 2000
23. Téclès O, Laurent P, Zygouritsas S, Burger, AS, Camps J, Dejou J, About I. Activation of human dental pulp progenitor/stem cells in response to odontoblast injury. Arch Oral Biol.; 50:103-8. 2005
24. Finkelman RD, Mohan S, Jennings JC, Taylor AK, Jepsen S, Baylink DJ. Quantification of growth factors IGF-I, SGF/IGF-II and TGF-beta in human dentin. J Bone Miner Res.; 5:717-22. 1990
25. Ruch JV, Lesot H, Bègue-Kirn C. Odontoblast differentiation. Int J Dev Biol. 39:51-68. 1995
26. Martin P. Wound healing; aiming for perfect skin regeneration. Science. 276:75-81. 1997
27. D'Souza RN, Cavender A, Sood R, Tarnuzzer R, Dichinson D, Roberts A, et al. Dental abnormalities in mice lacking funtional TGF-1. Int J Dev Biol. 23:119-31. 1998
28. McDonald RE, Avery DR. Treatment of deep caries, vital pulp expo sure, and pulpless teeth. In: RE McDonald, DR Avery, eds. Dentistry for the child and adolescent. 6a ed. Philadelphia: CV Mosby Co :428-454. 1994
29. American Academy of Pediatric Dentistry. Reference Manual: Guidelines for pulp therapy for primary and Young permanent teeth. Pediatr Dent.; 67:70. 2001
30. Tedder TF, Steeber DA, Chen A, Engel P. The selectins; vascular adhesion molecules. Faseb J 9:866-73. 1995
31. Mathieu S, Battari AE, Dejou J, About I. Role of injured endothelial cells in the recruitment of human pulp cells. Arch Oral Biol. 50:109-113. 2005