# Human Dental Pulp Stem Cells: From Biology to Clinical Applications

RICCARDO D'AQUINO[1], ALFREDO DE ROSA[2], GREGORIO LAINO[2], FILIPPO CARUSO[2], LUIGI GUIDA[2], ROSARIO RULLO[2], VITTORIO CHECCHI[3], LUIGI LAINO[1], VIRGINIA TIRINO[1], AND GIANPAOLO PAPACCIO[1*]

[1]*Dipartimento di Medicina Sperimentale, Sezione di Istologia ed Embriologia, TESLab, Secondo Ateneo di Napoli, Napoli, Italy*
[2]*Dipartimento di Scienze Odontostomatologiche, Ortodontiche e Chirurgiche, Secondo Ateneo di Napoli, Napoli, Italy*
[3]*Dipartimento di Discipline Odontostomatologiche, Università degli Studi di Bologna, Alma Mater Studiorum, Bologna, Italy*

ABSTRACT    Dental pulp stem cells (DPSCs) can be found within the "cell rich zone" of dental pulp. Their embryonic origin, from neural crests, explains their multipotency. Up to now, two groups have studied these cells extensively, albeit with different results. One group claims that these cells produce a "dentin-like tissue", whereas the other research group has demonstrated that these cells are capable of producing bone, both in vitro and in vivo. In addition, it has been reported that these cells can be easily cryopreserved and stored for long periods of time and still retain their multipotency and bone-producing capacity. Moreover, recent attention has been focused on tissue engineering and on the properties of these cells: several scaffolds have been used to promote 3-D tissue formation and studies have demonstrated that DPSCs show good adherence and bone tissue formation on microconcavity surface textures. In addition, adult bone tissue with good vascularization has been obtained in grafts. These results enforce the notion that DPSCs can be used successfully for tissue engineering. *J. Exp. Zool. (Mol. Dev. Evol.) 310B, 2008.*    © 2008 Wiley-Liss, Inc.

---

**How to cite this article: D'aquino R, De Rosa A, Laino G, Caruso F, Guida L, Rullo R, Checchi V, Laino L, Tirino V, Papaccio G. 2008. Human dental pulp stem cells: from biology to clinical applications. J. Exp. Zool. (Mol. Dev. Evol.) 310B:[page range].**

---

## DENTAL PULP EMBRYOGENESIS

Embryonic cells migrate from the neural crests to reinforce head and neck mesenchyme strongly determining the development of this area of the human body. During the sixth week of embryogenesis, ectoderm covering the stomodeum begins to proliferate, giving rise to the dental laminae. Reciprocal interactions between ectoderm and mesoderm layers lead to placode formation. One of these thick, ovoid ectodermal structures develops into tooth germs, where cells, belonging to the neural crest, will differentiate into the dental germ, containing both dental papilla and follicle. Therefore, dental pulp is made of ecto-mesenchymal components, containing neural crest-derived cells, which display plasticity and multipotential capabilities (Sinanan et al., 2004).

Pulp is externally separated from dentin by odontoblasts and by Höhl's subodontoblastic cells, that are pre-odontoblasts (Goldberg and Smith, 2004). Adjacent to this layer the pulp is rich in collagen fibers and poor in cells. Then, another, more internal layer, contains progenitor cells and undifferentiated cells, some of which are

---

considered stem cells. (Jo et al., 2007). From this layer, undifferentiated cells migrate to various districts where they can differentiate under different stimuli and make new differentiated cells and tissues. The final, innermost layer is the core of the pulp; this area comprises the vascular plexus and nerves. Up to the more recent discoveries (Gronthos et al., 2000; D'Aquino et al., 2007), researchers hypothesized that Dental pulp stem cells (DPSCs) were present in this layer (Fitzgerald et al., 1990). Actually, only undifferentiated perivascular cells can be found in it.

The third molar tooth germ begins development around the sixth year of life. Until this time, embryonic tissues of dental lamina remain quiescent and undifferentiated within the jaw of the child. Although crown mineralization begins during the eighth year of life, often third molar roots are still incomplete at the age of 18. This means that the structure of those teeth is still immature at this age and a conspicuous pool of undifferentiated cells, resident within the "cell rich zone" of the dental germ pulp, are needed for development.

## STUDIES ON DPSCS

Several studies have been carried out to verify whether stem cells could become a source of stable differentiated cells, capable of inducing tissue formation: during the embryonic development these cells proliferate and differentiate to generate all tissues. Postnatal stem cells have an extraordinary plasticity. The individual cells, when expanded into colonies, retain their multilineage potential. Although their number is higher before the birth, there are several "loci" or "niches" inhabited by a significant number of stem cells within the adult human body (Laino et al., 2005). Among the adult tissues, dental pulp, the soft connective tissue entrapped within the dental crown, is an extremely rich site for stem cell collection: owing to its peculiar formation, the pulp chamber is a sort of "sealed niche" and may explain why it is possible to find a rather large number of stem cell there.

During the sixth week of embryogenesis, the ectoderm covering the stomodeum begins to proliferate, giving rise to the dental laminae. Ectoderm–mesoderm interactions then lead to placode formation. One of these ovoidal ectodermal structures develops into tooth germs, where neural crest cells differentiate into the dental organ, dental papilla and dental follicle. Therefore, dental pulp is made of both ectodermic and mesenchymal components, containing neural crest cells that display plasticity and multipotential capability. These adult stem cells have been called DPSCs, when found in permanent teeth, and SHEDs (Stem Cells from Human Exfoliated Deciduous), when found in deciduous teeth. DPSCs have been isolated for the first time in 2000 by Gronthos et al.; these cells exhibited a differentiation potential for odontoblastic, adipogenic and neural cytotypes. The same group isolated SHEDs from deciduous teeth and compared DPSCs with bone marrow stem cells (BMSCs) (Miura et al., 2003). They reported a superimposable ability of these cells to form calcified tissue although different lineages were observed: DPSCs seemed to undergo odontoblastic differentiation, whereas BMSCs became osteoblasts after loading on HA-TCP scaffold. The same authors reported that SHEDs were different from DPSCs, affirming that they were "more immature": this probably because they were able to differentiate into a variety of cell types, to an extent greater than than DPSCs. Actually, they demonstrated that SHEDs were able to differentiate into a variety of cell types. They called some of these cytotypes "osteoblast-like" and "odontoblast-like" cells; because of their immaturity (Miura et al., 2003). The main commitment of these cells seemed to be the formation of a mineralized tissue (Luisi et al., 2007; Wei et al., 2007), similar to dentin, (Kitagawa et al., 2007), as shown by in vivo transplantation of these cells into immunodeficient mice (Miura et al., 2003). These cells are involved in the development of several but different hard tissues, including crown and root dentin, cementum and alveolar bone; a role in root reabsorption of deciduous teeth has been hypothesized for DPSCs (Yildirim et al., 2008). In vitro DPSCs have been shown to produce sporadic but densely calcified nodules (Gronthos et al., 2000). In addition, the same group (Gronthos et al., 2002) found that these cells are capable of forming ectopic mineralized tissue, similar to dentin, but only when grafted in vivo or when placed on the surface of human dentin in vivo (Batouli et al., 2003) and when exposed to tooth germ conditioned medium (Yu et al., 2006) or similar differentiation factors (Tonomura et al., 2007). These studies, performed in deciduous and permanent teeth, were done without specifically selecting stem cells (Gronthos et al., 2000, 2002; Batouli et al., 2003), which under specific stimuli, differentiate into several cell types, including neurons and adipocytes. Sporadic dense nodules

were found to be formed in vitro, but they underwent mineralization and bone or dentin-like formation only when grafted in vivo. These data, regarding a multipotential differentiative ability of those cells were further confirmed (Iohara et al., 2006; Srisawasdi and Pavasant, 2007) although the main commitment remains to form mineralized tissues, as evidenced in several papers (Ueno et al., 2006; Otaki et al., 2007; Hosoya et al., 2007); however, this is what usually happens during dental tissues development. (Bosshardt, 2005).

Moreover, other researchers have investigated another aspect of DPDCs, i.e. their putative immunosuppressive activity (Pierdomenico et al., 2005). This would be fascinating, if confirmed.

## STROMAL BONE-PRODUCING DPSCS AND STROMAL BONE-PRODUCING SHEDS: MAIN PROPERTIES AND PERFORMANCIES

Laino et al. (2005) isolated a selected subpopulation of DPSC called Stromal Bone Producing Dental Pulp Stem Cells (SBP-DPSCs), multipotential cells that could give rise to a variety of cell types and tissues including adipocytes, neural cell progenitors and myotubes (Laino et al., 2005, 2006; Papaccio et al., 2006). Previous experiments demonstrated that stem cells, isolated from the pulp of human exfoliated deciduous teeth and expanded in vitro, showed a~9% positivity for STRO-1, considered an early marker of mesenchymal stem cells (Gronthos et al., 2002). This antibody identified a cell surface antigen expressed by the osteogenic fraction of stromal precursors in human bone marrow as well as in erythroid precursors. In particular, the STRO-1 antigen is extremely important in selecting dental pulp cells (Yang et al., 2007a,b). SBP-DPSCs, representing roughly 10% of dental pulp cells display their multipotency (Laino et al., 2005,

2006); in fact they differentiate into smooth muscle cells, adipocytes, neurons and osteoblasts. The latter is also substantiated by the RUNX-2 expression, a transcription factor essential for inducing osteoblast differentiation.

These cells, selected for c-kit, CD34 and STRO-1 positivity (Fig. 1), were found to be able to produce an autologous woven bone tissue in vitro (Fig. 2). Head and neck hard tissues of the body have, other than a mesodermal origin, a neural crest source, and this has been demonstrated by the expression of the c-kit antigen, which is usually expressed in neural crest-derived cells, such as melanocyte precursors. The latter is compatible with the presence of c-kit expressing cells in the area of developing teeth. Therefore, CD34 and c-kit co-expression was used to isolate a population of stromal stem cells of neural crest origin.

## PASSAGES AND SENESCENCE OF DPSCS

DPSCs can survive for long periods and can be passaged several times. It is possible to obtain more than 80 passages without clear signs of
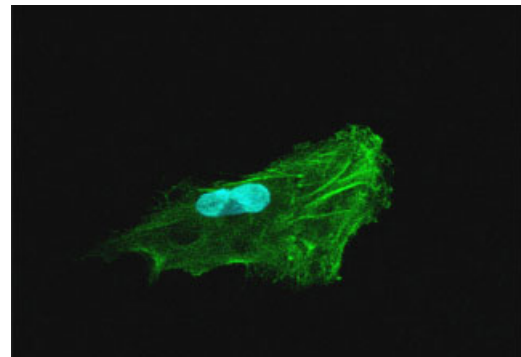


Fig. 1. Cytoarchitecture of a dental pulp stem cell. Cells, selected for c-kit$^+$, CD34$^+$ and STRO-1$^+$ were observed under a confocal microscopy. The green fluorescence stains the cell cytoskeleton (revealed by phalloidin); DAPI stains the nucleus. Original magnification $\times 400$.
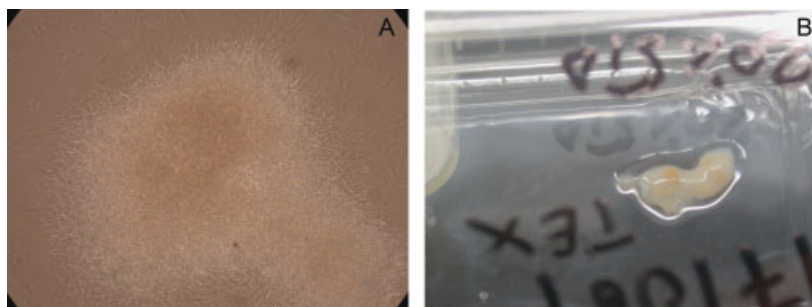


Fig. 2. Living autologous bone nodules. (**A**) Calcified nodule within cultured dental pulp stem cells after 40 days. Original magnification $\times 100$. (**B**) Living autologous bone (LAB) chip obtained after 60 days of culture of dental pulp stem cells.

senescence (Laino et al., 2005, 2006). Remarkably, after several passages, DPSCs still exhibit plasticity and capacity for nodule formation and are capable of forming bone chips in vitro. Osteoblast-derived cells produce a large-scale woven bone, which was observed in at least 100 25 cm² flasks. They can lose their capability to form woven bone chips when detached from their substrates, because they lose cell to cell contacts, which are of primary relevance for extracellular matrix secretion (D'Aquino et al., 2007).

## IN VIVO STUDIES

The woven bone tissue generated in vitro by SBP-DPSCs, called living autologous bone (LAB), is remodeled into a lamellar bone when transplanted in vivo (Laino et al., 2005, 2006; d'Aquino et al., 2007). Actually, after transplantation in vivo, the tissue is remodeled to form a lamellar bone through co-differentiation of SBP-DPSC into osteoblasts and endotheliocytes (d'Aquino et al., 2007). In fact, SBP-DPSCs produce bone but not dentin, as shown by mRNA transcript that express all markers of bone including osteocalcin, Runx-2, collagen I, but not dentin sialo phospoho protein (DSPP), which is specific for dentin, by the high expression of alkaline phosphatase (Laino et al., 2005, 2006) and by in vivo histomorphometry (d'Aquino et al., 2007). In the latter paper it was shown that SBP-DPSCs change their antigenic surface expression during differentiation. Moreover, after in vivo transplantation, a complete integration of vessels within bone chips takes place, leading to the formation of a vascularized bone tissue (Fig. 3).

During their differentiating process, SBP-DPSCs were observed to change their surface antigen
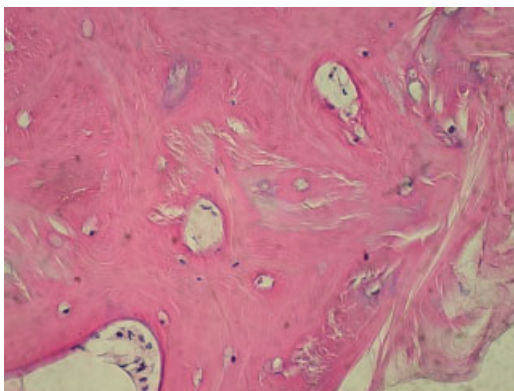


Fig. 3. In vivo transplantation results. Figure showing a bone sample stained with hematoxylin–eosin staining obtained after in vivo transplantation of LAB. Original magnification ×100.

expression, as they differentiated. At day 40, starting from a common Flk-1$^+$/STRO-1$^+$/CD44$^+$ progenitor, stem cells start to differentiate in two cytotypes: about 70% of them became Flk-1$^+$/STRO-1$^-$/CD44$^+$/ RUNX-2$^+$ osteogenic progenitor cells, whereas the remaining 30% became Flk-1$^+$/STRO-1$^+$/CD44$^+$/ CD54$^+$ endothelial cells. Interestingly, these cells were always negative for DSPP, a marker of dentin, demonstrating that the hard tissue they produce is bone and not dentin: the production of dentin actually needs a pool of factors present in dental papilla (Lesot et al., 2001; Yuasa et al., 2004).

This observation indicates that vasculogenesis takes place in vitro within the newly synthesized tissue. The formation of vessels explains the vitality of bone chips when transplanted in immunosuppressed rats.

## CO-DIFFERENTIATION AND VESSEL FORMATION

Transplantation results obtained with SBP-DPSCs are of extreme interest for the development of novel therapies. After transplantation in immunosuppressed rats, both woven chips and stem cells challenged with a scaffold become adult bone (d'Aquino et al., 2007). In addition, the dimensions of the obtained bone are the same as the grafted chips or the scaffolds (Trubiani et al., 2003). In particular, complete Haver's channels, containing blood vessels, and surrounded by bone arranged in a lamellar configuration have been obtained. This is the first demonstration of a complete bone obtained from stem cells.

During the ossification process, SBP-DPSCs give rise to both osteoblasts and endotheliocytes, leading to the formation of an adult bone tissue after in vivo transplantation. The presence of vessels and their complete integration with host (D'Aquino et al., 2007), other than being the first demonstration of a complete tissue growth from stem cells, is of great importance for therapy. This is a model of synergic differentiation, whose key aspect is the expression of flk-1, which is pivotal for the coupling of osteogenesis and vasculogenesis. This also represents an interesting aspect for development and a complete and efficient three-dimensional tissue reconstruction therapy.

## CRYOPRESERVATION OF DPSCS

Cryopreservation of cells and tissue, mainly of the reproductive system, has been significantly improved recently, but to date prevailingly hematopoietic stem cells have been cryopreserved and

then successfully utilized for transplantation. Moreover, to date there are no reports on the ability of either stem cells or already differentiated cells to re-start proliferation, differentiation and new tissue formation for therapeutic use.

After long-term cryopreservation (2 years), osteoblasts differentiated from SBP-DPSCs, are still capable of quickly re-starting proliferation and the production of mineralized matrix, in a manner similar to what we have already demonstrated for fresh cells (Laino et al., 2005, 2006; Papaccio, 2006). The differences in percentages regarding STRO-1 and flk-1 with respect to the percentages observed for the other stem cell antigens are owing to the fact that we performed multi-parametric cell sorting using both morphological and antigenic criteria, and sorted first for CD117 and CD34 together and then sequentially for STRO-1 and flk-1 antigens, before and after cryopreservation. Thus, pre-endothelial cells, such as pericytes positive for both CD117 and CD34, could have altered the overall percentages. These cells would be responsible for the differentiation of endothelium, which occurs in parallel with osteoblast differentiation, as demonstrated in the embryo during the ossification process. Moreover, both osteoblasts and endotheliocytes express the VEGF-2 receptor (flk-1).

Furthermore, after thawing, no apoptotic death was observed, and cells retained their differentiation multipotency, all of which are of interest when assessing the suitability of stem cells for use after cryopreservation. Moreover, osteoblasts produced a large-scale woven bone, which was observed in at least 100 25 cm$^2$ flasks. Samples of this bone, when transplanted into immunosuppressed rats, were remodeled into lamellar bone, further demonstrating their vitality.

Ultrastructurally, osteoblasts were cuboidal in shape, forming a layer along the border of the extracellular matrix, as observed in vivo during osteogenesis. These differentiated cells contained an extremely diffuse RER as well as matrix membrane vesicles, containing crystal-like structures. These ultrastructural observations confirmed that cells were unaltered.

A study was performed on cryopreserved tissue samples of minced periodontal ligament (Seo et al., 2005). Conversely, another group (Papaccio et al., 2006) obtained completely negative results cryopreserving whole pulps. This is probably owing to the fact that dental pulp is a loose connective or, more appropriately a ''mucous connective'' tissue with high water content. In any case,

cryopreservation of whole dental pulp does lead to safe recovery (Zhang et al., 2006a). These features and abilities make these cells attractive for therapeutic three-dimensional tissue reconstruction, with the potential of tailoring storage and recovery to the needs of the patient.

## BONE TISSUE ENGINEERING STUDIES

DPSCs showed differentiation profiles similar to those showed during bone differentiation (Hwang et al., 2008) and this event make them very interesting as a model to study osteogenesis (Liu et al., 2007) and the relationship with scaffolds (Zhang et al., 2006a,b).

SBP-DPSCs, when undergoing differentiation into pre-osteoblasts, deposit an extracellular matrix that becomes a calcified woven bone tissue called LAB (Laino et al., 2005). Calcein staining positivity, in addition to the other markers, strongly confirmed the presence of calcium deposits within this tissue, and stresses the effectiveness of the mineralization process. In addition, ALP activity increases significantly in parallel to cell differentiation. In addition, it has been shown that there are no differences regarding expansion rate, number of calcification centers and LAB nodules obtained per well of cells selected from younger (up to 29 years) and older (30–45 years) subjects (Laino et al., 2005).

Interestingly, positivity for CD44, seen in inflammatory processes of the pulp (Pisterna and Siragusa, 2007), osteocalcin and RUNX-2, evidences that cells were differentiating toward the osteoblastic lineage. The involvement of Runx-related gene in dental pulp mineralization processes has been intensely investigated (Zheng et al., 2007). Strong expression of RUNX-2, a transcription factor essential for osteoblast differentiation, is of primary importance, because it is closely related to the promotion of ossification; in addition, targeted disruption of RUNX-2 results in complete lack of woven bone formation by osteoblasts. Other than forming woven bone in vitro it has been demonstrated that this tissue undergoes remodeling, when transplanted in immunocompromised rats, and becomes a lamellar bone with entrapped osteocytes (Laino et al., 2006; D'Aquino et al., 2007). The latter confirms that SHEDs differentiate in osteoblasts and then osteocytes, differently to what has been previously reported (Miura et al., 2003) but similarly to DPSCs (Laino et al., 2005). Actually, it has been speculated that SHEDs possess the ability to differentiate in

functional odontoblast-like cells and that unlike DPSCs, they did not directly differentiate in osteoblasts (Miura et al., 2003). These authors other than using different methods and markers to select stem cells, which may lead to different results, needed the use of an osteoinductive template for in vivo transplants (Zhang et al., 2006a,b). The findings of Laino et al. (2006) disagree because the cells that they selected are probably different as they differentiate into osteoblasts and produce a woven or fibrous bone, which, without the need of osteoinductive templates, after in vivo transplantation, is remodeled into a lamellar bone. Therefore, these cells appear to be good candidates for bone tissue reconstruction protocols and bone regeneration models, because of good cellular morphology and high BMP-2 and VEGF secretion (Graziano et al., 2007). The role played by BMP2 is crucial and this protein has been hypothesized as biological tool in gene therapy of dentin regeneration (Nakashima et al., 2006). The concave texturing of the substrate elicits cytoarchitectural responses and adaptation in which the cells appear to favor intimate contacts with the secondary microconcavities and cellular polarization in human tissues (Zhang et al., 2003). Such behavior is accompanied by increased release of BMP-2 and VEGF into the culture medium and by higher ALP activity. It is likely that increased release of potent factors such as BMP-2 and VEGF and the higher ALP activity could have significant biological ramifications. By their proven involvement and potency in bone formation and angiogenesis, these factors and enzymatic activity may influence the responses and developmental program of stromal-derived cells via autocrine mechanisms and it is also influenced by surrounding cells via paracrine pathways. In this likely scenario, increased levels of BMP-2 and VEGF could be responsible for the greater amounts of bone tissue they observed in vitro (Graziano et al., 2008) and after transplantation of the colonized microconcavity-rich scaffold.

Angiogenesis could become itself a main goal for the clinical application of DPSCs, owing to the importance that angiogenetic factors (Tran-Hung et al., 2007) have in the native tissue (Grando Mattuella et al., 2007a,b); here, the biological events seem to be supported by nonstem population such as fibroblasts (Tran-Hung et al., 2006).

Today in vitro bone regeneration studies are limited by the main difficulty to obtain a cytotype capable of forming a complete tissue and not only a monolayer or cells surrounded by a mineralized matrix. Owing to their high proliferation rate and efficiency in producing bone chips, DPSCs seem to be the best candidates to study bone formation with respect to BMSCs, whose efficiency is limited by the fact that they differentiate into osteoblasts and produce small calcified nodule, but not chips of bone tissue.

Scaffold's structure and its influence upon cells are a breaking point in bone tissue engineering. To study the relationship between biomaterials and stem cells during their osteogenic differentiation process more specifically in bone tissue building, we need that cells would be able to actively proliferate, differentiate and produce a bone tissue as better as they can. Therefore, SBP-DPSCs may be a good standard to study the ossification process on substrates suitable for clinical application in bone reconstruction (Graziano et al., 2007, 2008). In fact, their ability to produce LAB already in vitro is of great interest to further analyze the effects of scaffolds, which are mediated by cell interactions with substrates. Interestingly, these cells show a lifespan and an high and long-lasting osteogenic capacity (Papaccio et al., 2006). These findings have been further substantiated by the results obtained challenging the surface texturing with SBP-DPSCs in a three-dimensional cell culture system. In fact, data obtained from engineered tissues, made on different scaffolds in a roller apparatus for 30 days (Graziano et al., 2008) clearly demonstrate that a new-formed bone tissue is obtained and that their different thickness strictly depends on the growing surface and, in particular, on the specific texture that has been adopted. Actually, the obtained new bone is of considerable thickness when the scaffold is made of a micro concave surface; it is of a lesser thickness in the case of a smooth surface and it is almost absent in the case of a convex surface, confirming all the results obtained with plane cultures. At the end of the rotating period (30 days) on convex surfaces, instead of building a bone tissue, cells were found at the bottom of the scaffold. Moreover, concave texturing induces an earlier and quantitatively more enhanced bone differentiation: in fact, it has been observed that BAP expression occurs earlier; cells adopt a polygonal shape with filopodia-like and lamellipodia-like extensions (cells can be regarded as spreading and differentiating), and nuclear polarity is observed, an index of secretion, cell activity and matrix formation (Graziano et al., 2008). These observations open the way to a more extensive application of tissue engineering of craniofacial tissues (Mao et al., 2006).

In conclusion: (1) dental pulp is a remarkable site of stem cells; (2) collecting stem cells from dental pulp is a noninvasive practice that can be performed in the adult during life and in the young after surgical extraction of wisdom teeth, a common surgical practice; (3) tissue sacrifice is very low when collecting dental pulp stem cells; (4) several cytotypes can be obtained from dental pulp stem cells owing to their multipotency; (5) transplantation of new-formed bone tissue obtained from dental pulp stem cells leads to the formation of vascularized adult bone and integration between the graft and the surrounding host blood supply; (6) dental pulp stem cells can be cryopreserved and stored for long periods; (7) dental pulp is ideal for tissue engineering and for clinical use in several pathologies requiring bone tissue growth and repair. In addition, tooth extraction is a clinical/therapeutical need. If bone marrow is the site of first choice for hematopoietic stem cell collection, dental pulp must be considered one of the major sites for mesenchymal cell collection. The good results obtained up to now reinforce this thought.

## LITERATURE CITED

Batouli S, Miura M, Brahim J, Tsutsui TW, Fisher LW, Gronthos S, Robey PG, Shi S. 2003. Comparison of stem-cell-mediated osteogenesis and dentinogenesis. J Dent Res 82:976–981.

Bosshardt DD. 2005. Are cementoblasts a subpopulation of osteoblasts or a unique phenotype? J Dent Res 84:390–406.

D'Aquino R, Graziano A, Sampaolesi M, Laino G, Pirozzi G, De Rosa A, Papaccio G. 2007. Human postnatal dental pulp cells co-differentiate into osteoblasts and endotheliocytes: a pivotal synergy leading to adult bone tissue formation. Cell Death Differ 14:1162–1171.

Fitzgerald M, Chiego Jr DJ, Heys DR. 1990. Autoradiographic analysis of odontoblast replacement following pulp exposure in primate teeth. Arch Oral Biol 35:707–715.

Goldberg M, Smith AJ. 2004. Cells and extracellular matrices of dentin and pulp: a biological basis for repair and tissue engineering. Crit Rev Oral Biol Med 15:13–27.

Grando Mattuella L, de Figueiredo JA, Nör JE, de Araujo FB, Fossati AC. 2007a. Vascular endothelial growth factor receptor-2 expression in the pulp of human primary and young permanent teeth. J Endod 33:1408–1412.

Grando Mattuella L, Westphalen Bento L, de Figueiredo JA, Nör JE, de Araujo FB, Fossati AC. 2007b. Vascular endothelial growth factor and its relationship with the dental pulp. J Endod 33:524–530. Review.

Graziano A, d'Aquino R, Cusella-de Angelis MG, Laino G, Piattelli A, Pacifici M, De Rosa A, Papaccio G. 2007. Concave pit-containing scaffold surfaces improve stem cell-derived osteoblast performance and lead to significant bone tissue formation. PLoS ONE 2:e496.

Graziano A, d'Aquino R, Cusella-De Angelis MG, De Francesco F, Giordano A, Laino G, Piattelli A, Traini T, De Rosa A, Papaccio G. 2008. Scaffold's surface geometry significantly affects human stem cell bone tissue engineering. J Cell Physiol 214:166–172.

Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. 2000. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci USA 97:13625–13630.

Gronthos S, Brahim J, Li W, Fisher LW, Cherman N, Boyde A, DenBesten P, Robey PG, Shi S. 2002. Stem cell properties of human dental pulp stem cells. J Dent Res 81:531–535.

Hosoya A, Nakamura H, Ninomiya T, Hoshi K, Yoshiba K, Yoshiba N, Takahashi M, Okabe T, Sahara N, Yamada H, Kasahara E, Ozawa H. 2007. Hard tissue formation in subcutaneously transplanted rat dental pulp. J Dent Res 86:469–474.

Hwang YC, Hwang IN, Oh WM, Park JC, Lee DS, Son HH. 2008. Influence of TGF-beta1 on the expression of BSP, DSP, TGF-beta1 receptor I and Smad proteins during reparative dentinogenesis. J Mol Histol 39:153–160.

Iohara K, Zheng L, Ito M, Tomokiyo A, Matsushita K, Nakashima M. 2006. Side population cells isolated from porcine dental pulp tissue with self-renewal and multipotency for dentinogenesis, chondrogenesis, adipogenesis, and neurogenesis. Stem Cells 24:2493–2503.

Jo YY, Lee HJ, Kook SY, Choung HW, Park JY, Chung JH, Choung YH, Kim ES, Yang HC, Choung PH. 2007. Isolation and characterization of postnatal stem cells from human dental tissues. Tissue Eng 13:767–773.

Kitagawa M, Ueda H, Iizuka S, Sakamoto K, Oka H, Kudo Y, Ogawa I, Miyauchi M, Tahara H, Takata T. 2007. Immortalization and characterization of human dental pulp cells with odontoblastic differentiation. Arch Oral Biol 52:727–731.

Laino G, d'Aquino R, Graziano A, Lanza V, Carinci F, Pirozzi G, Naro F, Papaccio G. 2005. Dental pulp stem cells can be detected in aged humans: an useful source for living autologous fibrous bone tissue (LAB). J Bone Miner Res 20:1394–1402.

Laino G, Graziano A, d'Aquino R, Pirozzi G, Lanza V, Valiante S, De Rosa A, Naro F, Vivarelli E, Papaccio G. 2006. An approachable human adult stem cell source for hard-tissue engineering. J Cell Physiol 206:693–701.

Lesot H, Lisi S, Peterkova R, Peterka M, Mitolo V, Ruch JV. 2001. Epigenetic signals during odontoblast differentiation. Adv Dent Res 15:8–13.

Liu J, Jin T, Chang S, Ritchie HH, Smith AJ, Clarkson BH. 2007. Matrix and TGF- beta-related gene expression during human dental pulp stem cell (DPSC) mineralization. In Vitro Cell Dev Biol Anim 43:120–128.

Luisi SB, Barbachan JJ, Chies JA, Filho MS. 2007. Behavior of human dental pulp cells exposed to transforming growth factor-beta1 and acidic fibroblast growth factor in culture. J Endod 33:833–835.

Mao JJ, Giannobile WV, Helms JA, Hollister SJ, Krebsbach PH, Longaker MT, Shi S. 2006. Craniofacial tissue engineering by stem cells. J Dent Res 85:966–979. Review.

Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, Shi S. 2003. SHED: stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci USA 100:5807–5812.

Nakashima M, Iohara K, Zheng L. 2006. Gene therapy for dentin regeneration with bone morphogenetic proteins. Curr Gene Ther 6:551–560. Review.

Otaki S, Ueshima S, Shiraishi K, Sugiyama K, Hamada S, Yorimoto M, Matsuo O. 2007. Mesenchymal progenitor cells in adult human dental pulp and their ability to form bone

when transplanted into immunocompromised mice. Cell Biol Int 31:1191–1197.

Papaccio G, Graziano A, d'Aquino R, Graziano MF, Pirozzi G, Menditti D, De Rosa A, Carinci F, Laino G. 2006. Long-term cryopreservation of dental pulp stem cells (SBP-DPSCs) and their differentiated osteoblasts: a cell source for tissue repair. J Cell Physiol 208:319–325.

Pierdomenico L, Bonsi L, Calvitti M, Rondelli D, Arpinati M, Chirumbolo G, Becchetti E, Marchionni C, Alviano F, Fossati V, Staffolani N, Franchina M, Grossi A, Bagnara GP. 2005. Multipotent mesenchymal stem cells with immunosuppressive activity can be easily isolated from dental pulp. Transplantation 80:836–842.

Pisterna GV, Siragusa M. 2007. CD44 Presence in inflamed pulp tissue. J Endod 33:1203–1207.

Seo BM, Miura M, Sonoyama W, Coppe C, Stanyon R, Shi S. 2005. Recovery of stem cells from cryopreserved periodontal ligament. J Dent Res 84:907–912.

Sinanan AC, Hunt NP, Lewis MP. 2004. Human adult craniofacial muscle-derived cells: neural-cell adhesion-molecule (NCAM; CD56)-expressing cells appear to contain multipotential stem cells. Biotechnol Appl Biochem 40:25–34.

Srisawasdi S, Pavasant P. 2007. Different roles of dexamethasone on transforming growth factor-beta1-induced fibronectin and nerve growth factor expression in dental pulp cells. J Endod 33:1057–1060.

Tonomura A, Sumita Y, Ando Y, Iejima D, Kagami H, Honda MJ, Ueda M. 2007. Differential inducibility of human and porcine dental pulp-derived cells into odontoblasts. Connect Tissue Res 48:229–238.

Tran-Hung L, Mathieu S, About I. 2006. Role of human pulp fibroblasts in angiogenesis. J Dent Res 85:819–823.

Tran-Hung L, Laurent P, Camps J, About I. 2007. Quantification of angiogenic growth factors released by human dental cells after injury. Arch Oral Biol 53:9–13.

Trubiani O, Tripodi D, Delle Fratte T, Caputi S, Di Primio R. 2003. Human dental pulp vasculogenesis evaluated by CD34 antigen expression and morphological arrangement. J Dent Res 82:742–747.

Ueno A, Yamashita K, Miyoshi K, Horiguchi T, Ruspita I, Abe K, Noma T. 2006. Soluble matrix from osteoblastic cells induces mineralization by dental pulp cells. J Med Invest 53:297–302.

Wei X, Ling J, Wu L, Liu L, Xiao Y. 2007. Expression of mineralization markers in dental pulp cells. J Endod 33:703–708.

Yang X, van den Dolder J, Walboomers X., Zhang W, Bian Z, Fan, Jansen JA. 2007a. The odontogenic potential of STRO-1 sorted rat dental pulp stem cells in vitro. J Tissue Eng Regen Med 1:66–73.

Yang X, van der Kraan PM, van den Dolder J, Walboomers XF, Bian Z, Fan M, Jansen JA. 2007b. STRO-1 selected rat dental pulp stem cells transfected with adenoviral-mediated human bone morphogenetic protein 2 gene show enhanced odontogenic differentiation. Tissue Eng 13:2803–2812.

Yildirim S, Yapar M, Sermet U, Sener K, Kubar A. 2008. The role of dental pulp cells in resorption of deciduous teeth. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 105:113–120.

Yu J, Deng Z, Shi J, Zhai H, Nie X, Zhuang H, Li Y, Jin Y. 2006. Differentiation of dental pulp stem cells into regular-shaped dentin-pulp complex induced by tooth germ cell conditioned medium. Tissue Eng 12:3097–3105.

Yuasa K, Fukumotom S, Kamasakim Y, Yamadam A, Fukumotom E, Kanaokam K, Saitom K, Harada Hm, Arikawa-Hirasawam E, Miyagoe-Suzukim Y, Takedam S, Okamotom K, Kato Y, Fujiwara T. 2004. Laminin alpha2 is essential for odontoblast differentiation regulating dentinsialoprotein expression. J Biol Chem 279: 10286–10292.

Zhang J, Niu C, Ye L, Huang H, He X, Tong WG, Ross J, Haug J, Johnson T, Feng JQ, Harris S, Wiedemann LM, Mishina Y, Li L. 2003. Identification of the haematopoietic stem cell niche and control of the niche size. Nature 425:836–840.

Zhang W, Walboomers X, van Kuppevelt TH, Daamen WF, Bian Z, Jansen JA. 2006a. The performance of human dental pulp stem cells on different three-dimensional scaffold materials. Biomaterials 27: 5658–5668.

Zhang W, Walboomers XF, Shi S, Fan M, Jansen JA. 2006b. Multilineage differentiation potential of stem cells derived from human dental pulp after cryopreservation. Tissue Eng 12:2813–2823.

Zheng L, Iohara K, Ishikawa M, Into T, Takano-Yamamoto T, Matsushita K, Nakashima M. 2007. Runx3 negatively regulates Osterix expression in dental pulp cells. Biochem J 405:69–75.