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Dental Pulp Stem Cells: A Promising Tool for Bone Regeneration

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Abstract Human tissues are different in term of regenerative properties. Stem cells are a promising tool for tissue regeneration, thanks to their particular characteristics of proliferation, differentiation and plasticity. Several "loci" or "niches" within the adult human body are colonized by a significant number of stem cells. However, access to these potential collection sites often is a limiting point. The interaction with biomaterials is a further point that needs to be considered for the therapeutic use of stem cells. Dental pulp stem cells (DPSCs) have been demonstrated to answer all of these issues: access to the collection site of these cells is easy and produces very low morbidity; extraction of stem cells from pulp tissue is highly efficiency; they have an extensive differentiation ability; and the demonstrated interactivity with biomaterials makes them ideal for tissue reconstruction. SBP-DPSCs are a multipotent stem cell subpopulation of DPSCs which are able to differentiate into osteoblasts, synthesizing 3D woven bone tissue chips in vitro and that are capable to synergically differentiate into osteoblasts and endotheliocytes. Several studied have been performed on DPSCs and they mainly found that these cells are multipotent stromal cells that can be safety cryopreserved, used with several scaffolds, that can extensively proliferate, have a long lifespan and build *in vivo* an adult bone with Havers channels and an appropriate vascularization. A definitive proof of their ability to produce dentin has not been yet done. Interestingly, they seem to possess immunoprivileges as they can be grafted into allogenic tissues and seem to exert anti-inflammatory abilities, like many other mesenchymal stem cells. The easy management of dental pulp stem cells make them feasible for use in clinical trials on human patients.

Keywords Stem cells · Dental pulp · Embryonic origin · Development · Differentiation · Cryopreservation · Bone tissue engineering · Tissue repair · Cell therapy

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Introduction

Human tissues are extremely different in term of regenerative properties; actually, epithelia can repair defects occurred after an injury and regenerate themselves all life long; the connective tissues, like bone or cartilage can regenerate only small defects and in particular conditions; neural tissues or myocardium, for the high cellular specialization reached by their citotype, do not show this ability.

In this framework, stem cells are a promising tool for tissue repair [1], thanks to their extensive proliferation and differentiation plasticity, characteristics that make them able, theoretically, to regenerate the structure of injured tissues. One of the main problems of the therapeutic use of

stem cells remains the identification of accessible sites within the human body where collecting an adequate amount of stem cells. Although their number is higher before the birth, also within the adult human body there are several “loci” or “niches” inhabited by a significant number of stem cells [2].

Anyway the surgical access to the collection site remain a limiting point, due to the morbidity of the site itself. Stem cells are virtually in every tissue of the human body, but collecting stem cells for example from CNS, would bring such residual deficit after collection procedures that possible advantages from the use of stem collected would be made not useful. On the other hand the selected site must contain an high percentage of stem cells compared to the volume of the collected tissue: the adipose tissue for example, is not rich in stem cells; but this problem can be counteracted increasing the volume of tissue source. Unfortunately, in most of human tissues, the available mass is very limited, to avoid an excessive weakening of the organ or the apparatus of the body. Actually, homologous stem cells transplantations can cause pathogen transmission and need immunosuppression as long as any other tissue or organ transplantation procedure, so the golden standard today is the use of an autologous stem cell source.

The second major problem is the nature of the cells collected, linked to their functional properties: cells must be able of extensive proliferation in order to repair macroscopic defects and to represent a therapeutic alternative, but this proliferation ability has to follow a pre-determined and repeatable scheme [3].

The last point that needs to be considered, for therapeutic use of stem cells, is the interaction with the biomaterials [4]. Building “de novo” tissues trying to transfer a 2D technology, like an in vitro cell culture, usually performed in the first steps of identification and differentiation procedures, in a 3D organism, like the human body; brings lots of difficulties and often fails. The whole dynamic process of the organogenesis is far to be understood, so the only way to develop a 3D structure today is to use a scaffold to seed the cells, mimicking the volume and shape of the part to be replaced. Actually, to reach this goal we need stem cells able to interact with biomaterials, because these are able to interact with stem cells, driving them to right architecture and helping them to establish a cell to cell and cell to matrix contacts.

Dental pulp stem cells have efficiently demonstrated to answer to all these questions due to easy surgical access, the very low morbidity of the anatomical site after the collection of the pulp, the high efficiency of the extraction procedure of the stem cells from the pulp tissue, their differentiation ability, demonstrated interactivity with biomaterials for tissue engineering applications. The aim of this review is to underline the state of the art on dental pulp stem cells research and focus on their clinical potential application for craniofacial tissue engineering [5].

Origin of the Dental Pulp

During the sixth week of embryogenesis, after the migration of neural crest cells into head and neck mesenchyme, the ectoderm covering the stomodeum begins to proliferate, giving rise to the dental laminae. From the dental lamina, following ecto-mesodermic interactions [6, 7], ovoidal structures start to separate and then develop into tooth germs, where neural crest cells differentiate into the dental organ, dental papilla and dental follicle, forming the main part of the dental and periodontal structures [8]. Therefore, dental pulp is made of both ectodermic and mesenchymal components, containing neural crest cells that display plasticity and multipotential capability [9]. After crown mineralization, dental pulp tooth germ remains entrapped within a hard structure that preserves it from environmental differentiation stimuli. [10]. Also after the crown eruption the dental pulp, the soft connective tissue remains entrapped within the pulp chamber, a sort of “sealed niche” and may explain that it is possible to found, within it, a rather large number of stem cells, although in an adult tissue. In the case of the third molar, the develop begins at the sixth year of life: it means that until this time, embryonic tissues from dental lamina remain quiescent and undifferentiated within the jaws, the only organogenesis event that completely occurs after the birth.

The pulp is divided into four layers, from the outer to the inner part: (1) the external layer made up of odontoblast producing dentin; (2) The second layer, called “cell free zone”, poor in cells and rich in extracellular matrix; (3) the third layer, called “cell rich zone” , containing progenitor cells that display plasticity and pluripotential capabilities [11]; 4) the inner layer, that comprise the vascular area and nervous plexus.

Identification of the Dental Pulp Stem Cells

Firstly Gronthos and colleagues, in 2000, isolated stem cells from human dental pulp, calling them DPSCs (dental pulp stem cells). These cells exhibited differentiation potential into odontoblastic, adipogenic and neural citotype; the same group isolated a similar citotype in deciduous teeth that have been called SHEDs (stem cells from human exfoliated deciduous). When compared with bone marrow stromal cells, the DPSCs showed superimposable ability in terms of calcified tissues formation, although in different lineages: after loading on HA-TCP scaffold DPSCs underwent odontoblastic differentiation instead of osteo-

blastic differentiation like BMSCs [12]. The same authors reported the immaturity of SHEDs when compared with DPSCs [13]. The in vivo transplantation experiments of these cells into immunodeficient mice [14, 15] confirmed the mineralized tissue commitment of these cells [15, 16], compatible with their collection site [17] and with supposed physiologic activity exhibited in vivo by this cells in tertiary dentin formation after crown injury; when the dental pulp stem cells have been placed on the surface of human dentin “in vivo” [12] or when exposed to tooth germ conditioned medium [18], they differentiate in odontoblastic-like cells [19]; confirming the needing of esogen factors for odontogenic differentiation [20].

Actually during the odontogenesis the same cells forming the odontoblasts, coming from the Hertwig lamina, are involved in the development of several but different hard-tissues, including crown and root dentin, cementum and alveolar bone [8]; their physiologic activity does not end with the eruption because for DPSCs it has been hypothesized a role in resorption of deciduous teeth too [21].

After Gronthos studies [22] other researchers confirmed how these stem cells, under specific stimuli [23], differentiated into several cell types, including neurons, adipocytes and chondrocytes [24, 25] although the main commitment remains to form bone [26–28].

The clinical application of these cells was better addressed in 2005 the group of Laino and Papaccio, who isolated a selected subpopulation of DPSC called SBP-DPSCs, that, already in vitro were capable of woven bone tissue formation. Previously, stem cells from permanent teeth have been shown to produce in vitro only sporadic but densely calcified nodules [22], exhibiting the formation of ectopic mineralized tissue only when grafted in vivo. The approach of Laino and colleagues was different because these authors selected the cells before performing the experiments, using an antibody formula to specifically select pulp cells, such as STRO-1, CD34, and c-Kit. 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 [29]; this antibody identifies a cell surface antigen expressed by the osteogenic fraction of stromal precursors in human bone marrow . In the dental pulp it has been suggested that STRO-1 recognizes also a stromal cell precursor of pericyte cells within dental pulp and identifies cells with both odontogenic and multilineage potential [20, 30].

The SBP-DPSCs represents roughly 10% of dental pulp cells, extensively proliferate under standard culture conditions, have a long life-span, and maintain their multipotential capabilities for generations and are pluripotent cells, as assessed by their differentiation into smooth muscle cells, adipocytes, neurons and osteoblasts, under particular conditions [31–33]. Head and neck hard tissues of the body have, other than a mesodermal origin, a neural crest source: the expression of c-kit has been shown in neural crest-derived cells, such as melanocyte precursors other than in the area of developing teeth; all the citotypes obtained from DPSCs are compatible with this embryonic origin due to the well-known role of neural crest cells in the human organogenesis.

Experiments performed with SBP-DPSCs, in presence of 20% of FBS, confirmed that bone was the main commitment of dental pulp stem cells, as substantiated by the RUNX-2 expression, a transcription factor essential for inducing osteoblast differentiation, and involved, with other Runx-related genes, in dental pulp mineralization processes [34]. In this setting, SBP-DPSCs produce bone but not dentin, as shown by in vitro mRNA transcripts, by their high expression of alkaline phosphatase [31, 32] and by in vivo histomorphometry [33].

When transplanted in vivo, this tissue was then remodeled in a lamellar bone. [31, 32] through co-differentiation of SBP-DPSC into osteoblasts and endotheliocytes [35]. During the in vitro ossification process, the SBP-DPSCs cells give rise to both osteoblasts and endotheliocytes, and to bone containing vessels, leading to the formation of an adult bone tissue after in vivo transplantation. The presence of these vessels and their complete integration with host, other than being the first demonstration of a complete tissue growth from stem cells, is of great importance for its use in therapy. Therefore, this is a model of synergic differentiation, whose key aspect is the expression of flk-1, pivotal for the coupling osteogenesis and vasculogenesis. Angiogenesis is itself one of the default program of differentiation for dental pulp stem cells [36, 37], due to the importance that angiogenetic factors [38] play in the native tissue [39] and due to the observation that the vascular event seem to be supported by non-stem population such as fibroblasts [38]. The latter also represents an interesting aspect for development of a complete and efficient three-dimensional tissue reconstruction therapy.

Dental Pulp Stem Cells and Bone Regeneration

The need for bone is increasing in western countries in the past few decades as result of the large increase of age of the average population. In the elderly, fractures tend to occur more frequently because of bone weakening due to a decrease in calcium content in ECM matrix and slowing of bone remodelling mechanisms. In addition, the periostium and the bone marrow stromal fraction is rich of bone progenitors, so that the bone, since early 90s, has been one of the approachable goal of clinical tissue engineering.

Dental pulp stem cells showed differentiation profiles similar to those showed during bone differentiation [40] and this event make them very interesting as a model to study the osteogenesis [41] and the relationship with scaffolds [42]. Laino and colleagues demonstrated that SBP-DPSCs, when undergo their differentiation to pre-osteoblasts, deposit an extracellular matrix which becomes a calcified woven bone tissue called LAB (living autologous bone). The LAB can be produced already in vitro on 3D pre-carved scaffold [43]. 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 of cells surrounded by a mineralized matrix. Due 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 bone marrow stem cells (BMSCs), whose efficiency is limited by the fact that they differentiate into osteoblasts and produce small calcified nodule, but not chips of bone tissue. In this way in pre-clinical phase it is possible to assess the osteoconductivity of a biomaterial. Moreover, a complete differentiation with subsequent tissue formation, including an adequate blood supply is of paramount importance for tissue repair and transplantation.

In addition, it has been shown [32] that no differences were found when comparing stem cells and differentiated cells obtained from young (up to 29 years) and old (30–45 years) subjects, regarding their expansion rate and number of calcification centers and LAB nodules obtained per well. This make the LAB a promising tool not only to study the bone regeneration but as therapeutic tool. Therefore, these cells appear to be good candidates for bone-tissue reconstruction protocols and bone regeneration models, thanks to the high BMP-2 and VEGF secretion and cellular morphology [44]. Transplantation results obtained with SBP-DPSCs are of extreme interest for therapeutic use. In fact, both woven chips and stem cells challenged with a scaffold, after transplantation, become adult bone, with a complete vascularization, of human origin, containing host blood. These results demonstrate that chips obtained in vitro, as well as cells, become adult bone tissue when transplanted into immunosuppressed rats. In addition, the dimensions of the obtained bone are the same of the grafted chips or of the scaffolds and that bone differentiation and maturation is almost complete, as an evident and functionally efficient vascularization takes place. In particular, complete Haver's channels containing blood vessels and surrounded by bone arranged in a lamellar configuration have been obtained.

On the other hand, this study of the osteoblast genesis led to the challenge of managing the bone differentiation stimulating quiescent progenitors resident in the tissue itself. The advantage of this approach is the use of autologous pre-committed cells, decreasing the dangers of ex-vivo stimulation.

Therefore, SBP-DPSCs may be a good standard to study the ossification process along substrates suitable for clinical application in bone reconstruction [31–33] as assessed in experiments performed challenging the stem cells and scaffolds, both in vitro [43] and in vivo [42, 44].

Cryopreservation of DPSCs

Cryopreservation of cells and tissue, mainly of the reproductive system, has been significantly improved lately, but up to now only hematopoietic stem cells have been cryopreserved and then successfully utilized for transplantation. Moreover, dental pulp represents an easily accessible source of stem cells that can be cryo-stored for long periods [33] and used to establish a cryobank for adult tissue regeneration [45]. Dental pulp stem cells retain their potential after cryopreservation, as observed by Zhang [46]. Therefore, the group of Laino and Papaccio undertook to observe the ability of SBP-DPSCs, and of their derived osteoblasts, to be cryopreserved and recovered after a 2-year period. This study provides evidence that dental pulp stem cells and their differentiated osteoblasts can be easily cryopreserved and recovered. This renders them a potentially useful and reliable source of cells for delayed therapies, designed for tissue repair upon patients' needs.

After long-term cryopreservation, 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 [31, 32]. Furthermore, proliferation was comparable to that of fresh cells, with no apoptotic cell death. In addition, cells were found to retain their multipotency, all which is of interest when assessing the suitability of stem cells for use after cryopreservation. Moreover, osteoblasts produced a large-scale woven bone, which we observed in at least 100 25 cm^2 flasks. Samples of this bone, when transplanted into immunosuppressed rats, were remodelled into lamellar bone, further demonstrating their vitality.

A study was performed on cryopreserved tissue samples of minced periodontal ligament [47]. Cryopreservation of whole dental pulp does lead to safe recovery. Different cryopreservation techniques are probably required for whole pulp.

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.

Perspectives

The extreme feasibility to manage the dental pulp stem cells make them ready to use in clinical trial on human patients: autologous stem cells collected from dental pulp of extracted wisdom teeth or from pulpectomy of teeth left in situ are a chance that should be improved in clinical trial. In this field, the observation made by Pierdomenico et al. [48] of a supposed immunosuppressive activity of the dental pulp stem cells, if confirmed, it would be of extremely importance. Although in the future dental pulp could play a fundamental role in all human tissues regeneration [49], the main commitment is bone and bone regeneration is the goal where these cells can find an immediate applications [50].

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