

Dental Stem Cells and their Applications in Dental Tissue Engineering

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Abstract: Tooth loss or absence is a common condition that can be caused by various pathological circumstances. The replacement of the missing tooth is important for medical and aesthetic reasons. Recently, scientists focus on tooth tissue engineering, as a potential treatment, beyond the existing prosthetic methods. Tooth engineering is a promising new therapeutic approach that seeks to replace the missing tooth with a bioengineered one or to restore the damaged dental tissue. Its main tool is the stem cells that are seeded on the surface of biomaterials (scaffolds), in order to create a biocomplex. Several populations of mesenchymal stem cells are found in the tooth. These different cell types are categorized according to their location in the tooth and they demonstrate slightly different features. It appears that the dental stem cells isolated from the dental pulp and the periodontal ligament are the most powerful cells for tooth engineering. Additional research needs to be performed in order to address the problem of finding a suitable source of epithelial stem cells, which are important for the regeneration of the enamel. Nevertheless, the results of the existing studies are encouraging and strongly support the belief that tooth engineering can offer hope to people suffering from dental problems or tooth loss.

Keywords: Dental stem cells, mesenchymal stem cells, tissue engineering, biomaterials, bone regeneration, bioengineered tooth.

INTRODUCTION

Stem cells are undifferentiated cells, with the ability to divide and give rise to identical, undifferentiated cells. Under specific condition, they can differentiate to various cell types that comprise the human body. Therefore, the stem cells are capable of developing to mature cells, with distinctive figures and specific functions, such as neural and muscle cells. The stem cells are divided in two groups: 1) the embryonic stem cells and 2) the adult stem cells. The latter are located in human tissues such as bone marrow, skin, adipose tissue and dental pulp [1-4].

Due to their properties, stem cells have the potency to become an important tool of tissue engineering and regenerative medicine. Tissue engineering is merging the principles of engineering and bioscience aiming to develop biological substances for the restoration, conservation and/or improvement of the tissue function [5]. Tissue engineering is aiming to provide the stimulus to the organism to regenerate the tissue from the inside or to develop the tissue externally which could be transplanted as natural tissue. The applications are based on the ability of the cells to proliferate and differentiate as well as on the construction of structures through cell and bioscaffold interactions [6, 7].

The loss or the absence of a tooth is a situation that could occur due to various conditions such as dental caries, periodontitis, traumatic injury or various pathological syndromes. The replacement of the lost tooth is very important not only for aesthetic reasons but also for functional purposes. Recent efforts in the field of biomaterials have led to the creation of osseointegrated implants from biocompatible materials which invade in the jaw bone to replace the missing tooth or to provide support. However, the successful outcome of this application depends on many parameters. Although the success rate of the technique is relatively high it is not always adequate as the implant is of different consistency to the recipient substrate. To overcome this shortcoming novel approaches are suggested, deriving from the field of stem cells and tissue engineering. Recently, tooth tissue engineering is providing the scientific ground for novel therapeutic applications regarding the tooth loss, compensating the existing prosthetic methodology. Dental tissue engineering is focusing on three main parameters: the type of cells that should be used, the scaffolds where the cells should be seeded on and the growth factor/molecular signals that should be applied. The present review is focusing on the properties of dental mesenchymal stem cells and the current studies in the field of dental engineering and the applications of these cells.

DENTAL EPITHELIAL CELLS

Two main cell types are involved in dental tissue formation: the ameloblasts, of epithelial origin, that form enamel and the odontoblasts of mesenchymal origin, that are responsible for dentin production.

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Enamel is formed from ameloblasts that derive from epithelial stem cells. They are the only cells of ectodermal origin that play a role in tooth development. These cells and their ancestor are lost just after the eruption of the tooth and as a result they do not exist in permanent teeth and cannot therefore be stimulated *in vivo*, to produce enamel.

In animal models, epithelial stem cells were isolated from the 3rd molar of newborn or young (still growing) animals. Initially, the epithelium was isolated and the cells were enzymatically separated and propagated, *in vitro*. They were combined with mesenchymal stem cells that were isolated from the same tooth and exposed to biomaterials [8-10].

The above approaches are promising regarding the tooth formation/regeneration. However, the clinical applications are challenging because a tooth germ from children is required. The use of autologous stem cells is most wanted and a good reliable source is needed.

DENTAL MESENCHYMAL STEM CELLS

The mesenchymal stem cells are non hematopoietic, multipotent cells that can proliferate and differentiate into a range of cell types comprising various tissues. They were characterized for the first time, 48 years ago, by Dr Friedenstein and his scientific group, when they identified a specific cell population in the bone marrow [11]. These cells demonstrated specific properties such as:

- 1) fibroblast-like morphology,
- 2) ability to adhere on plastic tissue-culture surfaces, and
- 3) osteogenic potency.

They express explicit protein markers (proteins of the cell membrane existing in these cells) such as CD90, CD73, CD105, CD44, and they are negative for CD34, CD31, CD45 [12]. They are multipotent and they can differentiate to osteoblasts, neuroblasts, cartilage, endothelial, muscle and adipose cells [13]. Mesenchymal stem cells are also found in: adipose tissue, skeletal muscle, joint fluid, amniotic fluid, cord blood, umbilical cord and teeth. Due to their presence in the teeth, their properties and the relatively easy isolation technique, the dental mesenchymal stem cells are currently studied extensively, becoming a potential, novel, important tool of tissue engineering and regenerative medicine.

Various mesenchymal stem cell populations exist in the tooth. According to their position in the tooth they can be grouped as:

- Dental Pulp Stem Cells, DPSCs [14]
- Stem cells from Human Exfoliated Deciduous teeth, SHEDs [15]
- Periodontal Ligament Stem Cells, PDLSCs [16]
- Dental Follicle Stem Cells, DFSCs [17]
- Stem Cells from the dental Apical Papilla, SCAPs [18].

Although the above groups are similar they also demonstrate specific characteristics relevant to each population that will be analyzed in the following paragraphs.

DENTAL PULP STEM CELLS (DPSCS)

The regenerative properties of the dentin/dental pulp complex, has lead the scientists to the hypothesis that dental pulp could contain stem cells responsible for the restoration/repair of the dentin (replacing and reparative tertiary dentin). In 2000, Gronthos *et al.*, reported for the first time, the presence of stem cells in the dental pulp of adults [14]. This study reported the isolation of DPSCs from adult 3rd molars (19-29 years old) and demonstrated that they have a typical fibroblast shape, expression pattern of protein markers similar to mesenchymal stem cells of the bone marrow (BM MSC) and higher proliferation rate *in vitro*, compare to the latter. This particular feature could be attributed to the fact that 3rd molars are the last permanent teeth regarding growth, formation and eruption. Thus, they exhibit a more primitive state compared to the mesenchymal stem cells of the bone marrow.

DPSCs were then transplanted along with HA/TCP powder into immunocompromised mice. After 6 weeks, they observed a dentin-pulp-like structure, similar to the one observed in human teeth. Collagenous matrix was deposited perpendicular to the odontoblast-like layer and the odontoblast-like cells extended cytoplasmic processes into the dentinal matrix, which interfaced with a pulp-like interstitial tissue infiltrated with blood vessels.

The same group, in 2002, demonstrated that DPSCs can proliferate giving rise to identical cells and differentiate to various cell types such as neuro- and adipose cells (stem cell properties). Moreover, they can generate dentin with attached pulp, *in vivo* [19].

According to a recent study, it was observed that DPSCs can generate bone, following subcutaneous transplantation in mice, using ceramic HA/TCP, as carrier [20]. Additionally, DPSCs stored and defrosted after 2 years remain capable of differentiating to pro-osteoblasts; the presence of specific surface antigens remained unchanged and the cells produced dental tissue [21].

The ability of these cells to differentiate into odontoblasts, osteoblasts, muscle, adipose, neurons and chondrocytes, has been verified *in vitro*, while their capability to develop hepatocyte morphological features and functions has been recently demonstrated [4, 22].

A study in 2006, reports that when DPSCs were placed in three different 3D scaffolds (a spongy collagen, a porous ceramic, and a fibrous titanium mesh) and transplanted into mice for 6 or 12 weeks, they formed a tissue that was more like a connective tissue rather than dental-pulp-like tissue [23]. All these studies demonstrate that DPSCs can be used in dental tissue engineering. The major issue is to provide the scaffold, which in endodontics could be the sterile root canal inside of which the new pulp could be recreated. The stem cells could be found in the residuals parts of the live pulp if the pulp or other regions of the tooth like the dental apical papilla or the bone of periapical region has not undergone necrosis.

Researchers all over the world are still trying to find a competent scaffold to place DPSCs as well as the ideal microenvironment that will promote their differentiation. Re-

search on the microenvironment and its role on self-renewal, differentiation and proliferation of the cells and tissue are of crucial importance for the future of regenerative medicine.

MESENCHYMAL STEM CELLS FROM DENTAL PULP OF EXFOLIATED DECIDUOUS TEETH (SHED)

In 2003, Miura M *et al.*, presented the existence of multipotent mesenchymal-type stem cells in the dental pulp of the deciduous teeth [15]. For their isolation incisors from 7-8 years old children were used and they demonstrated that SHEDs have the ability to differentiate *in vitro* to neuro-like cells, odontoblasts, osteoblasts, and adipocytes. Based on transplantation experiments in mice, it was shown that they have the capacity to produce dentin, as well as bone forming ability. In contrast to the mesenchymal cells from the adult dental pulp (DPSCs), they were not able to form dentin-pulp complex.

Deciduous teeth are different compared to the permanent teeth regarding developmental processes, tissue structure and function. Thus it is plausible that SHED have a higher proliferation rate, better osteoinductive ability *in vivo* and inability to form a dental-pulp complex [15].

Based on these observations, several studies suggest the use of SHEDs in dental tissue engineering [24, 25]. Cordeiro *et al.*, also suggested that stem cells from deciduous teeth could also be the ideal source of stem cells for repairing damaged teeth or for the induction of bone formation [26]. This research group seeded SHEDs on a biodegradable scaffold composed of poly-L-lactic acid (PLLA) prepared inside human tooth slices and transplanted these scaffolds/slices subcutaneously in mice. SHEDs differentiated into odontoblasts and when endothelial cells were co-transplanted the scaffold was vascularised. Thus, SHEDs could be an important source of stem cells for repairing damaged dental tissues and for the induction of bone regeneration. This means that in the future therapeutic approaches the restoration of damaged dentin and pulp could be successful with the use of autologous stem cells of the deciduous dentition which could have been previously extracted and preserved. However, the emerging question is whether we could successfully use heterologous stem cells for such therapeutic approaches.

MESENCHYMAL STEM CELLS FROM THE PERIODONTAL LIGAMENT (PDLSCS)

Periodontal ligament is found between the tooth and the alveolar bone and it consists of fibers that keep the tooth attached to the jaw. It can be isolated from the root of extracted teeth and it contains stem cells that self-renew and differentiate to form other tissues, like cementum and the alveolar bone [16, 27]. PDLSCs can be differentiated *in vitro* into adipocytes, osteoblasts and chondrocytes [28].

Upon transplantation in mice, these cells formed structures similar to bone, cementum, cartilage and periodontal ligament, whereas in a study in pigs, PDLSCs were used to cure periodontal lesions [29]. In another study, when PDLSCs combined with stem cells from the dental apical papilla, (SCAPs), isolated from the 3rd molar, were seeded in a scaffold and transplanted in the alveolar bone of young

pigs, the formation of root and periodontal complex were observed and were able to support an artificial ceramic crown. This restored the normal function of the tooth [18].

A more recent study of Orciani *et al.*, showed that human PDLSCs were differentiated into osteoblasts which exhibited a high production of Ca²⁺ and nitric oxide. Based on these observations they suggest that the local transplantation of proliferating PDLSCs, together with nitric oxide could be a new promising method for the treatment of periodontal lesions [30]. Moreover, PDLSCs that had been previously cultured with APTG-CM (apical tooth germ cells) produced structures of cementum / periodontal ligament, upon transplantation in mice [31]. It seems that the periodontal ligament is another alternative source of stem cells, which could be used in cellular therapies to restore lesions.

MESENCHYMAL STEM CELLS FROM DENTAL FOLLICLE (DFSCS)

The dental follicle surrounds the developing tooth and plays an important role in the formation of cementum, periodontal ligament and alveolar bone. DFSCs were isolated from the follicle of 3rd molars and it was documented that they can remain in cell cultures until passage 15 [17]. DFSCs were differentiated into osteoblasts, adipocytes and nerve-like cells *in vitro* [32-34], whilst they were able to form cementum *in vivo* [35]. Another study revealed that DFSCs transplantation into mice resulted in the formation of a new periodontal ligament, 4 weeks post-transplantation [36]. These cells could be a new tool for the study of the development of the periodontal ligament and for the development of regenerative therapies and reconstructive treatments.

MESENCHYMAL STEM CELLS FROM APICAL PAPILLA (SCAPS)

SCAPs are the stem cells isolated from the upper dental papilla, which is the precursor tissue of the dental pulp. An important source of SCAPs is the 3rd molars and teeth with open apices.

It has been found that SCAPs can be differentiated into osteoblasts, odontoblasts and adipocytes, *in vitro* whereas *in vivo* they can be differentiated into osteoblasts and odontoblasts [37, 38]. It has been demonstrated that human SCAPs possess a higher differentiation rate and are more efficient, compared to DPSCs, for the tooth formation. Furthermore, transplantation of SCAPs and PDLSCs along with HA/TGT (hydroxyapatite/tricalcium phosphate) as carrier on mice resulted in the formation of dentin and cementum/Sharpey's fibers, respectively. Based on these results, it is suggested that the combination of dental mesenchymal stem cells could regenerate the root/periodontal ligament complex [18]. Thus, SCAPs are a category of cells that take part in the regenerative endodontic therapy (revascularization).

EPITHELIAL AND MESENCHYMAL STEM CELLS IN DENTAL TISSUE ENGINEERING

Given that the tooth is formed from two different tissues, its construction requires the cooperation of dental mesenchymal and epithelial cells. Several trials have been at-

tempted for the reconstruction of teeth *in vitro* and *in vivo* using the above combination of stem cells, with encouraging results [39,40].

Dental mesenchymal and epithelial stem cells originating from rats, pigs and mice were cultured in the lab and seeded on the surface of different biomaterials, prior to transplantation in mice. All the present published reports indicated the formation of dentin and enamel concluding that the combination of dental stem cells could lead to their reorganization, the formation of separate independent layers and their differentiation in odontoblasts and ameloblasts [9, 10, 41, 42].

These bioengineered teeth were formed in ectopic site and were characterized by the lack of important elements such as the complete root and the periodontal tissues that allow the correct implantation on the alveolar bone. However, in 2007 Nakao *et al.*, published a study for tooth formation on mouse mandible. In particular, dental epithelial and mesenchymal stem cells were seeded on a drop of collagen gel and implanted in a mouse tooth cavity [43]. The bioengineered tooth germ, lead to the formation of a structurally correct tooth with odontoblasts, ameloblasts, dental pulp, blood vessels, crown, periodontal ligament, root and alveolar bone. It was concluded that the implantation into the mandible allowed the development, the maturation and the emergence of the tooth [43]. These results are an important indication that the dental stem cells could be used for the replacement of a missing tooth, in human.

Another study of 2009, demonstrated the successful functional tooth replacement in mouse by the transplantation of a bioengineered tooth on the alveolar bone, in the area of the missing tooth [44]. Dental epithelial and mesenchymal molar tooth germ-derived stem cells were cultured and combined with a biomaterial, prior to transplantation into the alveolar bone of the mouse in the area of the missing teeth. In particular, the upper first molar was extracted and the transplantation took place three weeks later, to allow the physical rehabilitation of the dental cavity and oral cavity epithelium. Finally, it was demonstrated that the bioengineered tooth that emerged in the oral area had the correct construction and the hardness of the tissues necessary for chewing (measured by the Knoop hardness test). Furthermore, it responded to experimental orthodontic treatment and to noxious stimuli, such as mechanical stress and pain challenge with various thermal and electrical stimuli. Its size was smaller than normal since the configuration of parameters forming the tooth, such as setting the width of the crown, is not possible with the current techniques.

FIRST AUTOLOGOUS DENTAL TISSUES ENGINEERING CLINICAL TRIAL IN HUMAN

In 2009, d'Aquino *et al.* presented the results of their study regarding the restoration of bone loss in the lower jaw, in human, with the implantation of a bio-complex of adult dental pulp mesenchymal stem cells (DPSCs) and a collagen sponge [45]. Initially 17 patients participated in this clinical trial that underwent panoramic radiograph to establish the presence of impacted third molars of the mandible. It was estimated that upon extraction of the 3rd molar of the mandible bilaterally, the patients would present a bone defect, of at least 1.5 cm in height, distal to the 2nd molar secondary to

impaction of the third molar on the cortical alveolar lamina. This clinical condition permits slow bone repair after extraction of the third molar, and may eventually lead to loss of the adjacent second molar. An important parameter for the enrolment of the patients in this study was to have two similar lower molars, so that we could use one as a test (T) site and the other one as a control (C) site for the implantation of the biocomplex. Furthermore, the patients should have untouched 3rd molars, the extraction of which was necessary for the isolation of DPSCs.

Upon the extraction of the maxillary 3rd molars DPSCs were isolated and cultured for 21 days. Flow cytometry was performed to detect the protein markers usually found on the cellular surface of DPSCs and were finally placed on collagen sponge. The day of the application, the patients underwent extraction of the mandibular 3rd molars. In the control site (C), a collagen sponge without DPSCs was placed whereas in the other site, the Test site (T), the biocomplex of collagen sponge filled with DPSCs was positioned. The patients were examined one week post-surgery and they were monitored monthly for the first trimester.

Three months after the surgery the radiographs of the two sites were significantly different. The T site presented a higher rate of mineralization and the cortical level was much higher when compared to the control site (C). The T sites were completely regenerated. No infections or inflammations were observed and the functionality (eg mastication) was normal. Furthermore, a sample from T and C sites was collected for histological and immunofluorescence analyses. In the T sites a well organized and vascularized bone with a lamellar architecture surrounding the Haversian channels was observed, whereas bone from C sites was immature with incomplete and large Haversian channels and signs of bone reabsorption. Immunofluorescent analysis demonstrated the expression of osteonectin, osteocalcin, bone alkaline phosphatase in both sites but with a different allocation, whereas bone morphogenic protein 2 and vascular endothelial growth factor had a higher expression in the T site confirming different levels of bone maturation. In general, this first report of autologous bone regeneration in humans with the use of DPSCs in collagen sponge demonstrates optimal repair of bone defects of the mandible and the restoration of periodontal tissue, below the 2nd molar.

The bones of the maxilla and mandible usually undergo reabsorption following degenerative diseases like periodontal diseases, necrosis of the mandible etc. The authors suggest that DPSCs could be an easy and natural alternative for the restoration/regeneration of damaged tissues and they call them the new "tool" for bone engineering. Of course more clinical trials are necessary before this technique of using autologous stem cells in dental treatments is applied. However, the technique of using stem cell originating from the periapical bone tissue, the dental papillae or the remaining living cells in the pulp cavity to revascularize a sterile pulpal cavity is already applied in endodontics.

DISCUSSION

Tooth loss can cause many problems, like difficulty in chewing and there it can be a problem of aesthetics. The current practises for the replacement of the missing tooth, like

implants and classic prosthetic procedures, are based on external materials which may lead to the final loss of teeth. Dental tissue engineering is a new promising therapeutic approach that aims to replace the missing tooth with a bioengineered tooth or to restore the damaged dental tissue. The main principle is the use of dental stem cells, seeded on the surface of biomaterials that provide the proper stimuli to create a biocomplex.

Numerous studies have shown the formation of dental tissues and/or tooth in animal models. A recent clinical trial in humans demonstrated the potential of adult dental mesenchymal stem cells attached on collagen to regenerate bone of the mandible. These results are promising and prove that dental tissue engineering can offer a new opportunity to the individuals that suffer from dental diseases or have lost teeth due to pathological syndromes, traumatic injuries or congenital absence. However, more research is necessary since the application of these techniques could be time-consuming, difficult and expensive and thus not easily accessible to the general public.

The most direct source of dental stem cells seems to be the adult dental pulp and the periodontal ligament. Even though deciduous teeth are the most easily accessible source of dental stem cells very few patients have saved SHEDs, since their isolation and storage is a very recent service. For DFSCs and SCAPs isolation the presence of the third molar is necessary. DPSCs can be isolated both from the third molar and the teeth predestined to have an endodontic treatment and still have a live pulp (50), whereas PDLSCs are isolated from the roots of extracted teeth. Even though DPSCs and PDLSCs exhibit a lower proliferation potential compared to SHEDs, they are an easy and direct source of dental stem cells for the purposed of dental tissue engineering but also for the restoration of other tissues and organs.

The major obstacle that needs to be overcome is the finding of available dental epithelial stem cells, since their combination with the dental mesenchymal stem cells seems to be the technique with the best results when trying to create a biotooth. Even though the isolation of dental epithelial stem cells for newborn or young animal is feasible, their use in humans is impossible and potentially hazardous, given that it can cause immunoreactions and rejection. Dental epithelial stem cells could be isolated from the tooth germ of childrens' third molar and be used or saved for future use. Since this particular practice refers the child to surgery it is not ethical and thus not an easily applicable technique. Furthermore, in the case of adults, it still remains a problem, since dental epithelial stem cells are already lost after the eruption of the teeth. According to current options, the solution of the problem of enamel regeneration could be the use of an artificial crown, which will be supported for a teeth originating for the mesenchymal stem cells.

Many *in vitro* and *in vivo* studies demonstrate encouraging and promising results for the potential future use of dental tissue engineering techniques. However, more research is needed to better understand odontogenesis and resolve the existing obstacles. The future establishment of these techniques will change the therapeutic approach of many of the dental syndromes and diseases.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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