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REVIEW

Somatic stem cells for regenerative dentistry

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Abstract Complex human tissues harbour stem cells and/or precursor cells, which are responsible for tissue development or repair. Recently, dental tissues such as periodontal ligament (PDL), dental papilla or dental follicle have been identified as easily accessible sources of undifferentiated cells. The dental stem cell biology might provide meaningful insights into the development of dental tissues and cellular differentiation processes. Dental stem cells could also be feasible tools for dental tissue engineering. Constructing complex structures like a periodontium, which provides the functional connection between a tooth or an implant and the surrounding jaw, could effectively improve modern dentistry. Dental precursor cells are attractive for novel approaches to treat diseases like periodontitis, dental caries or to improve dental pulp healing and the regeneration of craniofacial bone and teeth. These cells are easily accessible and, in contrast to bone-marrow-derived mesenchymal stem cells, are more closely related to dental tissues. This review gives a short overview of stem cells of dental origin.

Keywords Stem cells · Tissue engineering · Differentiation · Stem cell marker · Implantology

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Introduction

Tooth maladies are widespread in industrial countries. For example, approximately two thirds of German citizens suffer from periodontitis, which is a frequent cause of tooth loss. Moreover, there is an association between presence and severity of periodontitis and coronary artery disease [4]. For many years, new opportunities arising from stem cell research and advances in tissue engineering have been discussed for periodontal treatments and dental implantology. Several interesting studies about guided tissue regeneration for periodontal disease have been published recently [1, 31]. However, the use of dental stem cells to create a physiological anchorage of dental implants to the surrounding alveolar bone would mark a revolutionary achievement in the dental field.

There are two major categories of stem cells, which are discussed for dentistry: (1) embryonic stem cells; (2) somatic or adult stem cells. Isolation and use of human embryonic stem cells is ethically controversial, but first evaluations are on the way for actual treatments [28]. Somatic stem cells have a limitation in their potentials of differentiation. However, we think that somatic or adult stem cells are a better option for dentistry, as these cells are easily accessible, and their use does not bring up ethical concerns.

This review will give an overview on recent knowledge about somatic stem cells for their prospective use in regenerative dentistry. Here, we portray studies with non-hematopoietic mesenchymal stem cells for potential dental treatments. Later, we analyse dental epithelial stem cells and various ectomesenchymal dental cell types, which are dental pulp stem cells (DPSCs), periodontal ligament (PDL) stem cells, dental follicle precursor cells (DFPC) and stem cells from the apical papilla. Finally, these ectomesenchymal cell lines are classified.

Non-hematopoietic mesenchymal stem cells

Today, there is an abundance of adult stem cells available for possible cell-based therapies. Since a long time, bone marrow-derived mesenchymal stem cells have been studied and discussed for research and therapeutics. These cells are in close neighbourhood to hematopoietic stem cells, which have successfully been used to treat certain types of leukaemia for many years. Non-hematopoietic bone-marrow-derived mesenchymal stem cells are also known as "bone marrow stromal cells (BMSCs)", as described decades ago [3]. BMSCs can be isolated from single cell suspensions from bone marrow aspirates, as they adhere to cell culture plates and display the characteristic of clonogenicity, defined as the ability of a single cell to produce a colony when cultured at extremely low densities [29]. These stem cells are capable of migrating to organs such as liver and brain after transplantation into immunocompromised mice [13, 21]. Mesenchymal stem cells are capable of differentiating into osteoblasts, chondrocytes or retinal cells, which means a transdifferentiation into cells of at least two different germ layers [20, 29, 41, 42]. However, it is still controversial whether transplanted stem cells acquire tissue-specific markers by transdifferentiation or by cell fusion [30]. Nonetheless, current studies support the hypothesis that the process of transdifferentiation is possible [2]. In recent times, dental cell therapies have been discussed by combining non-dental mesenchymal stem cells and dental stem cells [18, 27, 28, 35]. A new study demonstrated the positive effect of enamel matrix proteins on porcine BMSC differentiation into cementoblasts [36]. Moreover, a recent study demonstrated that the use of MSCs in combination with platelet-rich plasma resulted in a reduction of probing depths by 4 mm and a clinical attachment gain of 4 mm, while bleeding and tooth mobility disappeared [43].

It is therefore interesting that Hu et al. [12] demonstrated the differentiation of bone-marrow-derived c-Kit^{+}-enriched cells into ameloblast-like cells. Here, Lesot and colleagues cultured murine bone marrow cells with dental epithelial cells and with dental mesenchyme in a re-association experiment. After 20 days, bone-marrow-derived cells acquired a polarized morphology and expressed the ameloblast specific markers amelogenin and ameloblastin. This study demonstrated, for the first time, that bone marrow-derived cells can be reprogrammed to give rise to ameloblast-like cells, offering novel possibilities for regenerative dentistry [12].

These studies are promising, but the use of dental stem cells seems more feasible as they are closely related to mature dental tissues.

Dental epithelial stem cells

Tooth development is based on interactions between the ectodermal and the ectomesenchymal (neural crest) germ layers. The development of a tooth begins in the mandibular arch of the embryo. The oral ectoderm is in close vicinity with the underlying ectomesenchymal tissue and builds the surface of the mandibular arch. It is accepted that dental development is initiated in the oral ectoderm. Previous studies demonstrated that embryonic dental epithelial tissues were capable of inducing the development of a tooth germ in combination with non-dental ectomesenchymal tissues [40]. In contrast, oral ectomesoderm in combination with non-dental epithelial tissues did not induce tooth development. Later steps of the dental development are driven by a complex interaction of both the ectodermal and the ectomesenchymal tissue. BMP-4 and FGF-8, two important growth factors produced by the oral ectoderm, are crucial for the initiation of tooth development [23].

Although it is evident that stem cells or progenitor cells are involved in dental development, the identification of dental stem cells was easier said than done. Opposing to rodents, who show a constant regeneration of their incisors, human teeth do not form new enamel after tooth eruption. The murine incisor therefore is an ideal model to study tooth development and dental diseases. Moreover, the development of the crown and of the ameloblast layer can be analysed in detail. Irma Thesleff's group identified these dental ectodermal stem cells in tissue explants of adult mouse incisors for the first time [11]. BrdU-labelled stem cells enabled a detailed examination of cell migration and ameloblast development in dental explants. The fibroblast growth factor (FGF), in particular, FGF-10 and the activated Notch-pathway are essential to maintain dental stem cells in an undifferentiated state and for the directed differentiation of stem cells into ameloblasts or into cells of the stratum intermedium [9, 10]. In humans, these dental epithelial stem cells are lost after tooth eruption; therefore, they are not available for studies on dental development.

Dental pulp stem cells

In contrast to dental epithelial stem cells, undifferentiated cells of the oral ectomesenchyme are not entirely lost after tooth eruption in human. Already in the middle of the nineties, it became possible to isolate precursor cells from the dental pulp [39]. Later, dental ectomesenchymal stem cells were isolated from the dental pulp (DPSCs) of extracted wisdom teeth [5]. These DPSCs display similar features as bone-marrow-derived mesenchymal stem cells. For example, both cell types adhere to plastic and are colony-forming cells. In contrast to bone-marrow-derived cells, DPSCs were found to differentiate into odontoblast-like cells. These cells also shared characteristics of osteoblast-like cells. A DNA microarray study could distinguish DPSCs from bone-marrow-derived mesenchymal stem cells [34], where DPSCs differentially express cell-cycle-associated genes. These results are in accordance to findings of a high proliferation rate in DFPCs compared to mesenchymal stem cells [5]. In an additional study, DPSCs were also found to differentiate into adipocytes or neural-like cells [6]. Interestingly, dental stem cells are located in the perivascular niche and express the stem cell marker Stro-1 [33]. Recently, a special stem cell population was isolated from the dental pulp [17]. These cells can be induced to undergo uniform differentiation into smooth and skeletal muscle cells, neurons, cartilage and bone cells under chemically defined culture conditions. Stem cells derived from the dental pulp are often mentioned in recent discussions about regenerative endodontics [27, 35]. Furthermore, ectomesenchymal stem cells of human exfoliated deciduous teeth (SHEDs) were isolated from the dental pulp of exfoliated incisors [22]. These cells could be cultivated either as fibroblast-like, adherent cells, or like neural stem cells as neurospheres. SHEDs are capable of differentiation into odontoblast, adipocytes and neural cells [22]. They induced bone formation and produced dentin under in vivo conditions; and they were able to survive and migrate in murine brain after transplantation into immunocompromised animals [22].

Periodontal ligament stem cells

A further class of dental ectomesenchymal stem cells are PDL stem cells, which were isolated from the root surface of extracted teeth [32]. These cells could be isolated as plastic-adherent, colony-forming cells, but display a low potential for osteogenic differentiation under in vitro conditions [32]. PDL stem cells differentiate into cells or tissues very similar to the periodontium [32]. Moreover, PDL stem cells transplanted into immunocompromised mice and rats demonstrated the capacity for tissue regeneration and periodontal repair. Recently, PDL stem cells were also isolated from sheep and pigs [7, 37]. It has been shown that a functional periodontium could successfully be established using PDL stem cells [37]. Interestingly, Kawanabe et al. [15] identified low numbers of cells (0.25%) of a ABCG2-dependent side population in human periodontal ligament, which corresponds to highly proliferating stem cells. Further studies are necessary to delineate PDL stem cells; however, the presence of ABCG2-mediated PDL side population cells could provide new insights useful for regenerative therapy in dentistry.

Dental follicle precursor cells

The PDL-stem cells are capable of differentiation toward periodontal cells; however, the dental follicle, which plays a crucial role in tooth development, contains precursors of the periodontium as well. The dental follicle is separated from developing dentin by epithelial cell layers (Hertwig's epithelial root sheath) during early steps of periodontal development. According to recent observations, disintegration of the Hertwig's epithelial root sheath into epithelial fragments enable contacts between ectomesenchymal cells from the dental follicle with the dentin surface, where they differentiate into mature cells of the periodontium [19, 38]. For this reason, this tissue should contain progenitor cells, capable of forming osteoblasts of the alveolar bone, PDL-fibroblasts or cementoblasts. In a first experiment, progenitor cells were isolated from bovine dental follicles. Interestingly, these cells formed spheroid like cell clusters under in vitro conditions. Surprisingly, differentiation of dental follicle cells toward mineralizing cells was exclusively possible under in vivo conditions [8]. The human dental follicle is a tissue of the tooth germ, which can easily be isolated after wisdom tooth extraction. Likewise, bovine dental follicles, cells of the human dental sac develop into the mature periodontium consisting of alveolar bone, the PDL and cementum [24]. The dental follicle contains ectomesenchymal cells which are derived from the neural crest. DFPCs, like BMSCs, are plastic adherent and colony forming cells, and they can differentiate into osteoblast like cells under in vitro conditions (Fig. 1). Similar to PDL stem cells, DFPCs can also differentiate, form robust connective tissues and produce clusters of mineralized tissue [24–26]. These connective tissues form biological diaphragms [24]. Here, typical markers for PDL and cementum were expressed, such as collagen type XII (Col_12) and the cementum attachment protein (CAP) [25]. Recently, Salles and colleagues published a study about DFPCs, demonstrating that they express the mesenchymal stem cell marker Stro-1 and confirming that human DFPCs have multipotential mesenchymal precursor cell properties [16]. DFPCs

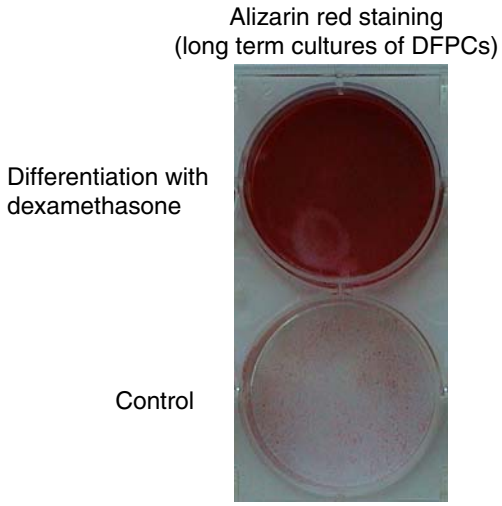

Fig. 1 Differentiation of DFPCs into calcified clusters (alizarin red colouring): 6-week long-term cultures of DFPCs treated with dexamethasone form strong mineralized clusters (*top*) in contrast to untreated DFPC long-term culture used as control (*bottom*)

differentiated toward multiple mesenchymal-derived cell types, such as cementoblasts, chondrocytes and adipocytes.

Stem cells from the apical papilla

A new class of dental stem cells was isolated from the dental papilla of wisdom teeth or incisors of 4 month old mini-pigs (SCAP, stem cells from apical papilla; [14, 37]). The dental papilla is an embryonic-like tissue that becomes also the dental pulp during maturation and formation of the crown. Therefore, SCAPs can only be isolated at a certain stage of tooth development. However, SCAPs have a greater capacity for dentin regeneration than DPSCs because the dental papilla contains a higher number of adult stem cells compared to the mature dental pulp [37]. In addition, SCAPs' are likely to be less differentiated than DPSCs, as they originate from an embryonic-like tissue. Interestingly, only a combination of SCAPs and PDL stem cells induced the formation of a dental connective tissue, namely, the attachment of an artificial tooth crown in the alveolar bone [37]. This experiment is exciting, but many questions are open. For example, we do not know which mechanisms of cell differentiation and which stem cells were important for the synthesis of a de novo peridontium [37]. Further studies can hopefully enlighten these questions.

Classification of dental stem cells

The main differentiation potential of dental stem cells lies within the formation of dentin or periodontium-associated tissues, whether these cells are derived from pulp, PDL or dental follicle. It is obvious that dental ectomesenchymal stem cells can be classified in two different groups with respect to their major differentiation potential. The first group is associated with the dental pulp, consisting of DPSCs, SHEDs and SCAPs; the second group contains PDL stem cells and dental follicle progenitor cells and is related to the periodontium. However, it is difficult to characterize these stem cells by surface protein markers (Fig. 2). Some surface proteins, like Stro-1 and CD73, are ubiquitously expressed by all dental stem or precursor cells. Nonetheless, deeper knowledge of stem cell markers and

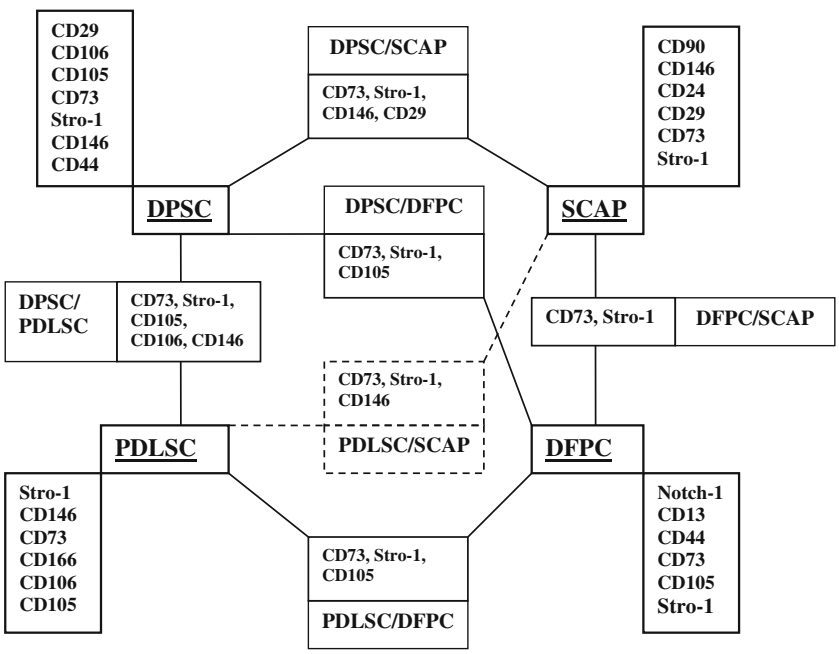

Fig. 2 Selected cell surface markers of DPSCs, PDL stem cells (PDLSCs), DFPCs and SCAPs

their relation to cell differentiation and plasticity will be helpful for stem cell based therapies.

Today, the practical use of dental stem cells might still be problematic, as the availability of dental stem cells is restricted to specific points in time. Whereas bone marrow-derived mesenchymal stem cells are accessible for treatments at nearly all times, dental stem cells can be isolated only under specific circumstances. DFPCs or SCAPs are available only from patients during wisdom tooth eruption, usually between 15 and 28 years of age. Unfortunately, during adolescence, the necessity of sophisticated treatments is a rather rare event. Special storage facilities for stem cells could provide a solution to the problem and make these cells available whenever there is a need for dental treatment. Stem cells from dental pulp or PDL could be harvested from exfoliated deciduous teeth, from extracted wisdom teeth or from teeth extracted during orthodontic treatment. However, the quality of these cells regarding their proliferation rates or differentiation potential may be lower than for dental papilla or dental follicle-derived cells [37].

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