The Diversity of Pulp Stem Cells, Tools for Regenerative Biodentistry

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INTRODUCTION

Regenerative dentistry

Since the publication of Spallanzani, it is known that following surgical amputation, the tail of the urodele amphibians Xenopus tadpole regenerates [1,2]. Restoration of tails, legs and jaws also occurs in the salamander after surgical removal [3]. At the same period, Trembly split hydra heads and obtained multiheaded individuals [4,5]. By bisecting a hydra, two complete animals were produced. During many years, a gap persisted between the biological regenerative capacities and what was requested for surgical therapies. This gap was gradually reduced, and now nearly disappeared. Successive steps highlight the fundaments of regenerative biodentistry.

Implantations of stem cells and surgical approaches in dentistry are derived from epithelio-mesenchymal assembly of embryological tissues. After reactivation of the concepts ahead of clinical applications, new treatments guiding organ repair and regeneration in mammals pave the way for biodentistry improvements. Indeed, applied to the field of human therapies the replacement of lost teeth by tissue-engineered dental recombinants appears as a fascinating goal. The initially aim of dental tissue engineering was to generate a whole tooth, or within some limits took into consideration, to concentrate on the regeneration of a missing part. The aim of dental tissue engineering may been limited to enamel repair, but this is still at an experimental stage. Or a more realistic objective was to heal the dental pulp, and consequently to provide a contribution to dentin mineralization as a substitute to endodontic therapy.

Restorative approaches of root canal treated tooth have relatively limited long-term survival and many failures are associated to diverse complications such as transversal or longitudinal fissures followed by subsequent fractures, and/or chronic periapical inflammation [6]. Substitutive therapies to endodontic treatments have been suggested and innovative strategies have been envisioned, including metallic implants or bridges. However, during the last decades, advances in the understanding of tooth development, as well as stem cell biology permit to consider the biological replacement of lost dental tissues and provide the foundation of novel opportunities in dental tissue engineering.

Developing dental substitutes appear not only as a fascinating goal in restorative dentistry, but raises also the question of technical feasibility. It is clear that many open questions take origin from a series of interrogations and the answers that are given are still confusing. What is exactly our goal? Are we willing to create or recreate a whole tooth? Are we willing to regenerate only a small part of dental tissues? Our aim may be limited to the healing and regeneration of a dental pulp? But will it be possible to use the newly formed pulp as a support for pulp mineralization, playing a role as a potential substitute for endodontic treatment? Each attempt to provide an efficient clinical answer implicates another strategy. Alternatively, it seems sometimes mandatory to evaluate the value of another method, another technic or to test another biomaterial. Consequently, any improvement needs firstly the identification of the question to be answered, and afterward the development of adapted cellular or biological materials [7].

The present approaches imply stem cells, scaffolds, structural and signaling molecules, transcription and growth factors. In this review we concentrate exclusively on the current knowledge related to pulp stem cells and we discuss their potential functions and implications in regenerative dentistry.

Pluripotent embryonic stem cells (ESCs)

A variety of stem cells are found in the dental pulp. Embryonic, post-natal and adult, monopotent or totipotent, immortalized and induced pluripotent cell lines have been identified and they have been used specifically as tools for engineering the dental pulp. During embryogenesis, reciprocal interactions between the oral epithelium and

neural crest-derived ectomesenchyme lead to the initial formation of dental placode and guide through sequential morphogenetic events the building of a tooth [8]. Tooth patterning also involves a spatio-temporal control of signaling pathways that sustain interdependent inductive interactions and signal exchanges between epithelial and mesenchymal cells. Ectomesenchymal cells give rise to odontoblasts, which synthesize the dentin extracellular matrix (ECM), and guide the terminal differentiation of dental pulp cells.

In 2004, murine tooth structures were successfully bioengineered after implantation of dissociated rat tooth bud cells-seeded scaffolds in ectopic site and/or after combination of non-dental mesenchymal cells with embryonic oral epithelium and transplantation into mice adult jaw [9,10]. Recombining the tissues lining the oral stomodeum and the mesenchyme seems to mimic developmental events and leads after implantation to the formation of tooth structures with a similar morphology as a natural tooth, when combined with a suitable scaffold. In vivo, interactions between the epithelium and connective tissues are instrumental for the building of a tooth. Recombining the tissues lining the oral stomodeum and the mesenchyme specifically located within the first branchial arch (and/or the naso-frontal bud), stem cells-based murine teeth were successfully bioengineered [9,10]. Some authors put emphasis on the importance of the epithelial cell layer lining the stomodeum, whereas others were focusing mostly on the effectiveness of the condensing connective tissue as a key factor of success [11-15]. Regeneration of an organ or a tooth involves cells expressing a complex cascade of genes, structural molecules, transcription and growth factors, associated with specific scaffolds.

In the dental pulp, stem cells are scarce. These unique cells endowed the dual capacities to ensure self-renewing and generate the differentiated cells types required to carry out specialized functions. They are implicated in the repair and regeneration of deficient tissues and organs. Pluripotent embryonic stem cells (ESCs), derived from the inner cell mass of mouse blastocysts, differentiate into dental epithelial-like cells and/or odontoblast-like cells [16-18]. Furthermore, ESCs recombined with embryonic oral epithelium express odontogenic genes [10].

Initially, embryonic stem cells acquire the final cell phenotype and become secretory cells, a process followed by the mineralization of the tissue. The major difficulties focus on how to obtain functional embryonic stem cells. It is quite unrealistic to use stem cells located within the embryonic blastula, even amplified by cell culture. More realistic, hematopoietic progenitor cells are present in umbilical cord blood. Placental blood (PB) previously considered as waste product constitutes nowadays an alternative source of hematopoietic stem cells for bone marrow reconstitution.

The origin of post-natal and adult stem cells (ASCs)

Initially, embryonic stem cells (ECSs) are totipotent cells, clonogenic, and capable of self-renewal. At later stages, adult stem cells have been shown to undergo asymmetric cell division, one daughter remaining in the stem cell compartment, whereas the other daughter cell undergoing further cell division gives rise to differentiated cells [19,20]. They may differentiate into each of the more than 200 cell types of the adult body. Stem cells are also present in adult organism, maintaining tissue homeostasis and ensuring tissue repair after a lesion. Postnatal or adult stem cells (ASCs) are no more totipotent [21]. They divide and differentiate into diverse specialized cell types. They have been used extensively in the field of repair and regeneration. Stem cells display multilineage differentiation potential. When they are activated, they are maintaining the capacity to self-renewal and "stemness".

After isolation, expansion, and characterization of the multipotent human mesenchymal stem cells, 5 to 7 days after initial plating, committed progenitor cells displaying a restricted potential were evaluated by Pittenger et al. [22] as being about 0.001 to 0.01% of the grand total of cells [22]. According to Sloan and Waddington [23], the subset of undifferentiated cells can represent in the dental pulp as little as 1% of the total cell population. Side population (SP) cells in human deciduous dental pulp were evaluated as 2% of the total cells [24]. According to Kenmotsu et al. [25] approximately 0.40% of the pulp cells may be stem cells or side population when they are found in young rats, whereas only 0.11% is found in old rats.

Pluripotent adult stem cells display a restricted lineage potential. Interestingly, mesenchymal stem cells (MSCs) derived from bone marrow display an odontogenic potential [9,17]. Co-culture of these non-dental stem cells with oral epithelial cells derived from embryos renders the MSCs able to express odontogenic genes such as Pax9, Msx1, Lhx7, DMP1, and DSPP. The transplantation of such cell mixture in murine renal capsule resulted in the formation of tooth-like structures [9]. The MSCs may thus represent an autologous source of cells for tooth regenerative research.

Diversity of adult dental SC

Since 2000, several adult stem cells have been identified in tooth. Many type of dental stem cells have been recognized in this context. Dental pulp stem cells (DPSCs) are derived from the pulp of the human permanent third molar [19]. Other cells have been isolated from exfoliated deciduous teeth (SHED) [20]. Apical papilla stem cells (SCAPs) cells are derived from the apical part of the papilla of growing tooth roots [26]. In vitro, SCAP have been shown to exhibit dentinogenic and adipogenic properties. In addition, they also express neuronal markers. We have reported that in the open apical papilla, some cells have the capacity to multiply, as shown by the Proliferating Cell Nuclear Antigen immunostaining (PCNA labeling). They migrate from the central part of the pulp to the lateral sub-odontoblastic boundaries. Then, they slide from the root toward the coronal part of the pulp where they underwent terminal differentiation [27].

At the chair side, adult stem cells are easily accessible from extracted wisdom molars or from their surrounding tissues. All these heterogeneous cell populations have the ability to differentiate in vitro into odontoblasts, osteoblasts and adipocytes, and into neuron-like cells. They form dentin-like structure in vivo after transplantation with hydroxyapatite/tricalcium phosphate in immunocompromised mice [28,29]. A few stem cells are also found in the periodontal ligament (PDLCs) and dental follicle (DFSCs) [30-32].

Table 1: shows the origin of adult postnatal stem cells:

Stem cells permanently present in the adult tooth:

-Dental pulp stem cells isolated from the pulp of permanent teeth.

-Exfoliated deciduous teeth stem cells and immature dental stem cells from deciduous teeth.

-Apical papilla stem cells.

Stem cells present in periodontal tissues:

-Periodontal ligament stem cells.

-Dental follicle stem cells, differentiating in odontoblast-like cells or endothelial cells.

SM taking origin from other tissues:

-Induced pluripotent stem cells.

-Hematopoietic stem cells.

-Neuronal stem cells.

Hence, instead direct pulp capping by implanting in the coronal pulp calcium hydroxide in a pulp exposure, or inserting a few pulp stem cells, apical progenitor cells may contribute to pulp regeneration. Apparently stem cells are not easily accessible, they are still difficult to detect and isolate. These cells are rare, and in addition, they may de-differentiate after a limited number of passages.

Induced pluripotent cell lines

In this context, the strategy involving Induced Pluripotent Cell Lines (IPs) was developed, expecting to obtain large clusters of stem cells and cell collections allowing easier implantation within the dental pulp. By over-expressing some transcription factors, including Oct3/4, Sox2, Nanog, Klf4, c-Myc, and Lin28, it was shown that adult somatic cells may be reprogramed, even if they were previously differentiated. The iPS can de-differentiate, and further re-differentiate according to the site where the cells are implanted. It appears that any adult differentiated cell has the potential to step back to an embryonic stage. Many dental cells such as DPSCs, exfoliated deciduous teeth stem cells, SCAPs, periodontal ligament and gingival fibroblasts have been used to generate iPS. These cells provide a promising unlimited source of autologous cells for regenerative dentistry. However, it comes out that after preliminary differentiation, iPS does not keep a stable terminal phenotype [7].

Immortalized cell lines

Another strategy was used to establish immortalized and stable cell lines. A few were obtained either after spontaneous transformation, but more were got from virally transformed cloned 3T6 cell lines [33]. Immortalized bovine dental papilla cells, or viral transformation of MO6-G3 odontoblast cells were also used successfully [34-36]. Cells immortalized by telomerase as well as cells modified by human telomerase transcriptase (hHTERT) gene were exploited in the course of such investigations [37,38]. These cell lines were employed to characterize dental pulp progenitors and elucidate the molecular and cellular mechanisms governing their differentiation. In our laboratory, we have obtained clonal cell lines from tooth germs of day 18 mouse embryos transgenic for an adenovirus-SV40 recombinant plasmid (pK4). Transformed odontoblast cell lines were created with high proliferative capacity, allowing ultimately studying the regeneration and dentin repair [39].

Among the cell lines that were obtained, A4 cells appear to be multipotent [29]. In the presence of glycerophosphate (GP), ascorbic acid (AA) and dexamethasone (Dex), three-dimensional cultures of A4 cells differentiate into osteoblasts. In contrast, after the addition of TGF1 and dexamethasone to the culture medium, the 2D cultured cells expressed a chondrogenic phenotype. In addition, using some other culture conditions (IBMX, indomethacin, insulin and dexamethasone), the A4 cell lines were implicated in adipogenesis, whereas GP, AA and Dex induce an osteo/odontoblast phenotype. Two other cell lines, C5 and H8, were monopotent and displayed a restricted odontoblastic program. We demonstrate the coexistence of multipotential and restricted-lineage progenitors in the dental pulp [29].

After a surgical exposure, following in vivo implantation of cells in the dental pulp of rodent incisors, osteodentin was massively formed in the lumen of the pulp. In the molar, the mesial horn of the dental pulp was filled by osteodentin [40]. This evidence that cell implantation may be a tool stimulating the synthesis and secretion of ECM molecules, followed by pulp mineralization.

Embryonic and adult stem cells, totipotent and monopotent immortalized cells, implanted within the dental pulp release extracellular matrix molecules, transcription and growth factors, influencing the control and efficacy of inflammatory molecules. Implicated in the synthesis and secretion of ECM, the SIBLINGs are actual components of the dental pulp that play a role in pulp mineralization. Conversely, MEPE, which is another member of the SIBLING family, has a more ambiguous role. Acting as mineralization promotor, the C-terminal (ASARM) and the central domain are also performing mineralization inhibition [41,42].

The nature of the pulp cells implicated in such processes remains open. After a given number of asymmetric cell divisions, fibroblasts (or pulpoblasts) divide and differentiate

toward a phenotype characterized as odontoblast and/or osteoblast. Following the formation of clusters, pulp mineralization takes place. Interactions between the feeding pulp cells and structural fibroblasts contribute to the release of a series of differentiation factors, which may influence the number of stem cells, also named side population cells [25]. They behave as autonomous cells, which are primarily responsible for engineering a pulp inside a root canal, which further underwent mineralization.

In addition to the series of stem cells previously cited, it appears that the recruitment and differentiation of pulp stem cells are under the control of two neuromediators regulated by platelets [43]. The A4 cell line behaves as a multipotent mesoblastic stem cell, whereas the H8 cell line corresponds to monopotent progenitors with a restricted potential of differentiation towards the odontogenic fate [29,38]. Both cell clones produce neurotrophins and synthesize, catabolize, store and transport serotonin (5-HT) and dopamine (DA). Injury-activated platelets are the source of systemic 5-HT and DA necessary for dental repair since natural dentin reparation is impaired in two rat models with monoamine storage-deficient blood platelets. Natural reparative dentin formations after pulp injury is impaired in Fawn-hooded and reserpine-treated rats whose platelets are deficient in bioamine storage. Dual 5-HT/DA intrinsic properties of odontogenic stem cells including functional 5-HT and DA auto receptors and the release of 5-HT/DA by activated blood platelets in response to pulp injury are a prerequisite and necessary for stem cells recruitment [43]. These results shed lights on the emergence and differentiation of the stem cells that are located in the dental pulp, but there is still a need to find the keys for the clinical implications of these findings.

Stem cells constitute the major factors concerned by pulp healing and renewal. We are far from the complex regeneration of a tooth, still problematic. We focus here on the first step for tissue engineering, restricted to the restoration of dentin or a dental pulp, followed afterward by the root canal mineralization. This phase may provide a good substitute for endodontic treatment. Altogether, many questions arise in this emerging field of tissue engineering that are not yet answered, but pave the way for the future.

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