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Challenges for stem cells teeth-based: a literature review

Débora Drummond Hauss Monteiro [1], Diego da Cunha Fontoura [1], Karina Imaculada Rosa Teixeira [1,2], Maria Esperanza Cortés [1,2,*]

[1] Restorative Dentistry Department, Dentistry Faculty, *Universidade Federal de Minas Gerais* (UFMG), Belo Horizonte, Minas Gerais, Brazil

[2] National Institute of Science and Technology in Nano-Biopharmaceutical INCT/Nanobiofar

*corresponding author e-mail address: ***mecortes@ufmg.br; mecortes@yahoo.com***

ABSTRACT

Medicine and dentistry stands to benefit greatly from advances in the field of molecular biology. Since oral tissues are a rich source of stem cells, novel applications have been proposed to better induce differentiation to pulp, bone or neuronal tissues or even can be applied for personalized regenerative treatments. Especial attention has been given to pulp stem cell to differentiate features including into specific types to facilitate repair of the damaged tissues. Evaluations of characteristics and properties of these stem cells may allow diverse applications on tissues repair or regeneration. Within this context of mesenchymal stem cells (MSC) found in the healthy pulp of deciduous and permanent teeth, recent findings and scientific research support the therapeutic use of adult dental stem cells. Although, the MSC from oral tissues has limitation to provide adequate cell number for transplantation therapy. The new knowledge is growing widely and further efforts are essential to advance on this area to maximize the possibilities of treatments and therapeutic efficacy. This review summarizes the current strategies to utilize stem cells for pulp regeneration and tissue repair and regeneration in order to know if stem cell-based therapy could be useful in tissue regeneration or even contribute to the development of a new tissue or organ.

Keywords*: stem cells, dentistry, biomaterials, regenerative procedures.*

1. INTRODUCTION

Tissue engineering is a science based on fundamental principles that focuses on the ability to stimulate or enhance repair of specific tissue [1] the identification of appropriate cells, the development of conductive scaffolds and the understanding of the morphogenic signals [2]. On the most recent concept the regenerative engineering aims cells to regenerate a tissue or organs. Those areas need the integration of multidisciplinary strategies [1]. Whereas regenerative medicine places more emphasis on cell based therapy, particularly stem cells, to repair or replace damaged tissue/organs, regenerative engineering focuses on using biomaterials with or without cells to make bioartificial tissues or organs [3]. 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 seed on the surface of biomaterials (scaffolds) in order to create a biocomplex [4].

Nowadays, it was verified that stem cells from oral cavity are master cells that could generate tissues and organs. They are present in periodontal ligament, alveolar bone and in the pulp of both deciduous and permanent teeth [5]. Dental stem cells display multifactorial potential such as high proliferation rate, multi-differentiation ability, easy accessibility, high viability and easy to be induced to distinct cell lineages [6]. Stem cells have been increasingly used in clinical trials but the manufacturing of clinical grade MSC poses substantial challenge to scientists all over the world. This may in part due to the fact that implementation of stringent good manufacturing practices regulations is indeed impractical in scale-up manufacturing processes that are generally used in stem cell banks [7].

Pioneering research on bone marrow transplantation by E. Donnall Thomas established that injecting bone marrow cells into the bloodstream could repopulate the bone marrow and produce more blood cells [8]. Nevertheless, the study of human stem cell can be traced back since the lattes 50th; only recently the adult MSCs have been identified in several oral and maxillofacial tissues, suggesting that oral tissues are a rich source of stem cells [9]. Teeth are non vital organs that, remarkably, have proven be a surprisingly rich source of multipotent ectomesenchymal stem cell. The majority of live adult tooth tissues derive from the neural crest, and therefore all dental stem cells considered are collectively termed ecto MSC [10].

The rational use of stem cells from the oral cavity has been based on some advantages, such as the fact that it does not only depend on ease of use and accessibility, but also on the efficiency and quality of repair in relation to cost. Furthermore, oral tissues are expected to be not only a source but also a therapeutic target for stem cells, as stem cell and tissue engineering therapies in dentistry continues to attract increasing clinical interest.

The purpose of this review was to highlight the state of the art of the application of stem cells in dentistry, with emphasis on pulp regeneration, since 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.

2. METHOD

The literature was acquired in order to identify the state of art of the application of oral stem cells for repair or substitution of organs, focusing on dentistry and pulp regeneration. An advanced search of the terms “stem cells” and “dentistry”, “stem cells” and

"pulp", "stem cells" and "biomaterial" and "dental cell" on the titles listed in PubMed databank was performed, and the terms "stem cells" and "regenerative therapy" and "dental" on all fields. Additional sources (secondary literature) became available through references in the literature thus found. The articles titles and abstracts were reviewed by the authors for relevance to the study, and they were first judged based on their titles, then on the abstracts, and finally on the entire text. Articles containing no information about dental stem cells were excluded, as were doctoral theses, case reports, and expert opinions. The search period taken into consideration was 2008 to 2014, and the review was restricted to current sources in English language. Thus, the articles selected for the arrangement of the literature review were 54 of a total of 92, since 38 were discarded because they did not fit the selection criteria used.

3. LITERATURE REVIEW

3.1. Types of stem cells.

Based on their origin, there are two main types of stem cells: embryonic and postnatal or adult stem cells. Embryonic stem cells are pluripotent cells which mean that they can give rise to all differentiated cell types [11, 12]. In normal condition of tissue repair, adult stem cells are thought to migrate to the area of injury and differentiate into specific types to facilitate repair of the damaged tissues including dental tissues as dental pulp, periapical follicle, periodontal ligament [12]. Human induced pluripotent stem cells, which have similar properties to human embryonic stem cells, can be successfully generated from adult human gingival, periodontal ligament fibroblasts [13].

According to Sreenivas *et al.* (2011) [14] and Sial *et al.* (2013) [15], to be classified as a stem cell, a cell must satisfy three main criteria: i) It is not terminally differentiated: the cell is not committed to differentiate into a single cell type; ii) It can divide without limit; iii) It can renew the stem cell pool: during cell division each daughter cell chooses whether to commit to differentiation into single cell type or remain as a stem cell. Other classifications for stem cells are in relation with their ability to differentiate as totipotent [16, 14]. To date, embryonic stem cells have hardly been examined in dentistry. In contrast to them, adult stem cells pose no ethical conflicts [14, 17]. They, too, have the potential to differentiate into dental structures**.**

3.2. Pulp tissue.

The vital pulp is a heterogeneous soft tissue located in the center of teeth which contains a variety of cell types and extracellular matrix molecules. Both dentin and pulp are derived from neural crest cells. Because of their close relationship, especially during embryonic stages of tooth development it is difficult to discuss these two types of tissues separately. Anatomically, dental pulp is almost fully encapsulated by hard dentin. All of the main blood vessels and lymph drainages of dental pulp pass though tooth apexes, which make the apex the main pathway for tooth nutrition. This limited accessibility and unyielding environment or dental pulp makes it difficult to eliminate inflammation once it has occurred and makes the dental pulp a tissue with limited potential for self-recovery [12].

The cell types that take part in generation of teeth come from different embryonic origins: surface epithelial (ectoderm) and ectomesenchymal (neural crest). Those tissues are precursors of enamel organ and dental papilla, which will generate tooth enamel and dentin-pulp complex, respectively [10]. Dentin and pulp work as a physiological and functional unit, also denominated pulp-dentine complex. Dental pulp consists of heterogeneous cell populations, among which stem/progenitor cells are anticipated to replenish odontoblasts upon infection or trauma in adulthood [18]. The pulp is a richly vascularized and innervated tissue, which is very important for homeostasis and pathology healing.

With the exception of ameloblasts progenitor cells, all stem cells involved in odontogenesis originate in mesenchyme. The various mesenchymal stem cell populations are usually found in prevascular niches of their corresponding tissue. MSC can differentiate into nerve, muscle, vascular, fat, cartilage or bone cells [17]. Dental pulp undifferentiated mesenchymal cells can differentiate into odontoblasts or other cell types such as fibroblasts, to repair the damaged soft pulp tissue [12] but the developmental ability of dental pulp stem cells in vitro is limited. In vivo, more complex tissues can arise [17]. Dental tissues are considered a rich source of mesenchymal stem cell types, including odontoblasts, neural progenitors, osteoblasts, chondrocytes and adipocytes [6] and the main characteristics of them are summarized in Table 1.

Table 1. Main characteristics of MSC from teeth and oral tissues.

	MSC from teeth and oral tissues
Advantages	Multifactorial potential; High viability and plasticity; Richest source of MSC when compare with aspirate of bone marrow; Self-renewal capacity; Easily isolated, expanded and transplanted; Stem cell marker expression; Very promising for tooth regeneration; If donor and receptor of cells is the same the resulting cells from the culture cell are not recognized as foreign by the immune system
Disadvantages	Long time for differentiation; Limited accessibility; There is a need to be patient-specific cells (to obtain the same genetic information); Ethical concerns

Karaöz *et al.* (2011) [19] isolated and characterized putative stem cells derived from human impacted third molar dental pulp and compared their properties with human bone marrow-derived MSC. The authors confirmed the presence of an adult MSC population in human impacted third molars. These cells are not only capable of differentiating into adipogenic, osteogenic, and chondrogenic lineages, but they also have some special characteristics similar to epithelial and neural stem cells,

explained the authors. Moreover, human dental pulp stem cells are also able to differentiate into vascular endothelial cells in vitro.

Noncell-based methods can be considered in tissue engineering, nonetheless, it is widely accepted that most of the developing and established strategies rely on the use of cells of different origins. To date, five different human dental stem/progenitor cell populations have been isolated and characterized: dental pulp stem cells (DPSCs), stem cells from deciduous teeth (SHEDs), stem cells from apical papilla (SCAPs), periodontal ligament stem cells (PDLSCs), and dental follicle precursors cells (DFPCs). These post natal populations have MSC like characteristics, including the capacity for self-renewal and multilineage differentiation potential [20].

Stem/progenitor cells in dental pulp are derived from dental papilla during tooth development [18]. Depending on specific signals from their environment, dental pulp stem cells can either regenerate new stem cells or undergo the differentiation process [17].

Although adult stem cells commonly behave in a very similar manner both in vivo and in vitro, they represent some individual characteristics; especially for the tissue they were isolated from [19]. Dental pulp stem cells are isolated by opening the pulp chamber and root canal of an extracted or exfoliated tooth to remove cells out of the extracellular matrix. The isolated cells are then stored under ultra-low temperature to induce the arrest of cellular activities [5]. After dental stem cells isolation and sequential cultivation of cell population, certain properties could be induced with the use of an appropriate environmental condition.

Pulp regeneration by cell transplantation dictates a point-of-care therapy. Unless the clinician is trained to isolate and culture cells, one must rely on commercial stem cell products. Point-of-care refers to one doctor treating one patient using the patient's own cells, from the point of cell harvesting to transplantation [18].

To assess cell function and tissue morphogenesis, dental stem cells were isolated, cultivated and injected in a model of dental root matrix in a recent study of Nakashima and Iohara (2011) [21]. The teeth were extracted, and the apical portion of the root was cut out and transplanted again into the alveolar bone after whole pulp removal and enlargement of the apical foramen, followed by biological filling ex vivo. Autologous cell injection was performed with collagen types I and III (1:1) scaffold in the lower part of the root canals. These cells were stimulated with different angiogenic, neural, enamel or odontoblast substances in the medium of culture, and cultivated until the third to fourth passages. Seven days after transplantation of the different stimulated cells, the lower part of the root canal was filled with loose connective tissue containing capillaries and spindle-shaped cells. Pulp-like tissue with vasculature and nerves was formed 14 days after transplantation. The odontoblast-like cells attached to the dentinal wall in the root canal and produced dentin-like tissue, extending their processes into dentin tubules at 35 days, expressing enamelysin, an odontoblast marker. When unfractionated total pulp cells were implanted instead of the fractionated pulp stem cells, less tissue was observed on day 14, and matrix formation and mineralization were seen in the regenerated tissue on day 35. Their results are demonstrating complete pulp regeneration by cell therapy by harnessing pulp stem/progenitor cells with high angiogenic/neurogenic potential with stromal-cell-derived factor-1 in endodontic treatment.

3.3. Pulp regeneration and hard tissue formation.

Diverse substances and biomaterials have been used aiming pulp regeneration. Hypothetically, mesenchymal stem/progenitor cells in pulp replenish all pulp cells including odontoblasts in post-natal life. Kim et al. (2013) [18] consider that it is, therefore, intuitive to transplant mesenchymal stem/progenitor cells from dental pulp directly into endodontically treated root canals in order to regenerate the dental pulp–dentin complex.

To regenerate dentin or pulp tissue, dental pulp stem cells, stem cells of human exfoliated deciduous teeth and stem cells of apical papilla can be used [17]. Dental stem cells have been successfully tested in tissue engineering research, where full generation of dentin pulp complexes and even whole teeth out of isolated cells (complete organ restoration) has proven to be possible [22]. The regeneration of dentin-pulp complex with stem cells might be clinically achievable. However, this may not be applicable in clinical scenarios [23].

Huang et al. (2010) [24] tested the possibility of regenerating vascularized human dental pulp in emptied root canal space and producing new dentin on existing dentinal walls using a stem/progenitor cell-mediated approach with a human root fragment and an immunocompromised mouse model. Stem/progenitor cells from apical papilla and dental pulp stem cells were isolated, characterized, seeded onto synthetic scaffolds consisting of poly-D, L-lactide/glycolide, inserted into tooth fragments, and transplanted into the subcutaneous space of the mice. The results showed the formation of a continuous layer of dentin-like tissue on the existing canal dentinal walls. Expression analysis of several key genes indicate that the regenerated pulp tissue closely resemble natural pulp tissue.

The state of the inflammatory pulp condition was examined as source to obtain stem cells donors. Wang *et al.* (2010) [13] explored whether cells retrieved from clinically compromised dental pulp have stem cell–like properties. They isolated cells from healthy teeth (control group) and from teeth with clinically diagnosed irreversible pulpitis (diseased group). Cell proliferation, stem cell marker expression, and cell odonto-osteogenic differentiation competence were compared. The results showed that cells from diseased group demonstrated decreased colony formation capacity and a slightly decreased cell proliferation rate, but they had similar stem cell marker expression and exhibited a similar percentage of positive ex vivo osteogenic induction and dentin sialophosphoprotein expression. The authors suggest that the existence of functional putative stem cells in clinically compromised dental pulp with irreversible pulpitis is possible.

Human dental pulp stem cells in combination with preameloblast-conditioned medium could be valuable not only for odontoblast differentiation but also for repair and regeneration of the dentine-pulp complex. Lee *et al.* (2011) [25] investigated the effects of preameloblast-conditioned medium on the odontogenic differentiation of human dental pulp stem cells *in vitro* and *in vivo*. When preameloblast conditioned medium-treated human dental pulp stem cells were transplanted into immunocompromised mice, they generated pulp-like structures lined with human odontoblast-like cells showing typical odontoblast processes. However, during

recombinant human bone morphogenenetic protein 2-treated human dental pulp stem cells transplantation, some of the cells were entrapped in mineralized matrix possessing osteocyte characteristics. These findings promote dentin formation *in vivo* and in *vitro*.

Considering the transplanted cells into dental structures to induce regeneration of tissues, there are some experiences to demonstrate that the transplantations of bone marrow-derived stem cells and adipose-derived stem cells can induce pulp regeneration in the root canal after pulpectomy in dogs [26]. When was compared the pulp regeneration potential of stem pluripotent cell subfractions among three different origins: dental pulp, bone marrow and adipose tissue, there were no difference in terms of morphology, growth characteristics and angiogenic/neurogenic potential *in vitro*. The migration activity, however, was higher in pulp and adipose cells compared to that in bone marrow cells. It is noteworthy that there is enhanced matrix formation and root canal obliteration after adipose cell transplantation.

New cell therapies and tissue bioengineering applied to root and tooth formation have progressed considerably. Bioengineering-seeding dental stem cells and appropriate growth factors to drive differentiation onto a matrix, or synthetic scaffold could be an alternative for regenerating periapical tissues in vivo or in vitro in the near future [27].

Others believe that the potential of autologous mesenchymal bone marrow stem cells could promote hard-tissue formation after direct pulp capping procedures. In agreement, Obeid *et al.* (2013) [28] showed that direct pulp capping procedures performed in posterior teeth, and then treated with mineral trioxide aggregate (MTA), hydroxyapatite/tricalcium phosphate, or bone marrow stem cells were used as direct pulp capping agents. Both MTA and bone marrow stem cells had a comparable tendency to produce a hard-tissue barrier that was significantly higher than hydroxyapatite/tricalcium phosphate.

In accord with the origin of the main hard dental tissue formation there are two main cell types 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 [4]. 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 ancestors are lost just after eruption of tooth and as a result they do not exist in permanent teeth and cannot therefore be stimulated in vivo to produce enamel.

A promising approach to tooth regeneration in animal experiments may be to obtain epithelial stem cells from third molars of newborn or juvenile animals [29, 30, 31, 32]. All authors concluded that it has not yet been possible to find a source of human adult ectodermal stem cell to regenerate enamel. Ulmer *et al.* (2010) [17] conducted a systematic literature review including 126 studies with *in vitro* and *in vivo* animal experiments in order to evaluate stem cell biology research in the field of dentistry and identify which methods now being developed have the potential to be used in humans in the future. It has not yet been possible to find a source of human adult ectodermal stem cells to regenerate enamel post-eruptively.

The enamel growth seems to be the biggest challenge, since the successful tissue engineering of complex tooth structures will require the characterization of specific progenitor cell populations that mediate tooth development. The *in vitro* model of dental epithelial differentiation is expected to facilitate a better understanding of this process. The use of feeder layers may facilitate the process of tissue engineering of enamel by enabling expansion of undifferentiated dental epithelial cells in culture [33]. Stem cells have the ability to form dentine or bone, and these tissue formation activities might be useful for osseointegration or tertiary dentine repair with good and similar characteristics to the original ones.

The ultimate goal in dentistry is to have a method to biologically replace lost teeth; in essence, a cell-based implant rather than a metal one. The minimum requirement for a biological replacement is to form the essential components required for a functional tooth, including roots, periodontal ligament, and nerve and blood supplies. Paradoxically, the visible part of the tooth, the crown, is less important because, although essential for function, synthetic tooth crowns function well, and can be perfectly matched for size, shape and colour. The challenge, therefore, for biological tooth replacement is ultimately one of forming a biological root [22]. Human adult teeth and periodontium retain populations of neural crest stem cells that show characteristics of pluripotency Fig 2.

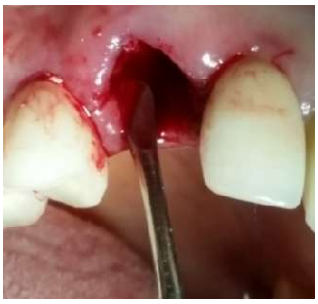

Figure 2. Picture of human periodontum as an exemple of source of stem cells.

3.4. Influence of biomaterials on stem cell-based therapy.

The achievement of good responses in tissue engineering demands the orchestration of three fundamental elements: cells, scaffold and cell signaling [23]. Regeneration of tissues occurs naturally due to the existence of stem cells with the capacity to self-regenerate and differentiate. However, regenerative capacity decreases with age, and in many cases, regeneration is not sufficient to repair the damage produced by degenerative, ischemic, inflammatory or tumor-based diseases [11].

Diverse molecules play a critical role during the development guiding process that determine the fate of stem cells and regulate the generation of all tissues and organs in the developing embryo and in the physiological process of tissue regeneration. The growth and morphogenic factors are proteins that bind to specific membrane receptors and trigger a series of signaling pathways that coordinate all cellular functions [6].

The same growth factors that guide embryogenesis and physiological tissue regeneration can also be used therapeutically to guide stem cell differentiation toward specific cell fates. Horst *et al.* (2012) [3] add that endothelial cells and their paracrine factors such as vascular endothelial growth factor were shown to play important roles in mediating angiogenesis to nurture engineered tissues or organs and facilitate host integration. Other

growth factors and bioactive molecules can be used to guide the formation of engineered tooth tissues/organs in the manner recapitulating development. The ideal matrix for dental pulp regeneration should have good bonding properties, allowing stem cells to proliferate and differentiate, and ensure a good neurovascular supply to the new pulp tissue [27].

Wigler *et al.* (2013) [34] suggest that the new approach to dental treatment can at times be challenging and the outcome of revascularization procedures still remains somewhat unpredictable; they represent an improvement over older treatment protocols that have left the roots short and the walls of the root canal thin and prone to fracture. The bioactive materials was able to stimuli the tissular neoformation with important angiogenesis and cell proliferation The authors advise that like all dental procedures, careful case selection and full disclosure to the patient regarding the goals and limitations of the treatment are essential to make this form of main stream treatment an acceptable alternative in the clinical management of infected immature teeth.

In dentistry many studies have been considered mainly for pulp tissue recovery. Moreover, despite the many therapeutically procedures have been quite successful; the biggest challenge to be achieved is still growing the dentine-pulp complex completely. The molecular mechanisms that controlled the mobilization and homing which required invasion through extracellular matrix (ECM) barriers are almost unknown.

The MSCs (including those of dental origin) express receptors to numerous inflammatory mediators, which can be produced by inflammatory cells, by injured pulp/periapical cells or released from dentine and/or materials during treatment. Cytokines and growth factors can increase or decrease recruitment, proliferation and/or differentiation of MSCs [35]. Local pathology such as the presence of microorganisms in root canals and/or periapical lesions may also play a significant role in the regeneration process with the involvement of neurogenic inflammatory reactions and immune responses [18].

The use of patient-specific cells is clinically beneficial because the cells carry the same genetic information as the host and thereby will efficiently engraft without immune rejection upon transplantation. When allogeneic cells are used, donor-associated variation such as age, gender, and method of isolation may substantially influence the characteristics of harvested cells and will likely cause variable treatment outcomes.

The use of biological signaling molecules for cell homing approach makes pulp regeneration more approachable for practitioners because the delivery of growth factors is not nearly as complicated and costly as cell transplantation. Immune rejection, potential contamination during cell manipulation, and unintended differentiating patterns of transplanted cells leading to tumorigenesis are minimized. Dental pulp–dentin regeneration using cell transplantation has encountered significant hurdles and, to date, has not illustrated a clinically viable pathway. Cell homing is a clinically translatable approach for dental pulp–dentin regeneration and circumvents some of the key challenges associated with cell transplantation. Whereas new knowledge about dental pulp–dentin regeneration will result from both cell transplantation and cell homing studies, cell homing presents clear advantages in terms of clinical applications [18].

Takahashi *et al.* (2012) [36] characterized the expression status of cadherins in dental pulp-derived mesenchymal progenitor/stem cells from deciduous and permanent teeth, and in order to elucidate how cadherins (which are differentially expressed in deciduous and permanent teeth) affect the multipotency of the dental pulp-derived progenitor/stem cells, the ability of the dental pulp cells to differentiate into adipocytes and osteoblasts was evaluated. They observed that R-cadherin is vigorously expressed in dental pulp cells derived from permanent teeth but not in dental pulp cells derived from deciduous teeth. The ability of pulp cells of deciduous teeth to differentiate into adipocytes and osteoblasts was found to be much higher than that of cells obtained from permanent teeth. Figure 2.

For some authors [37, 38], in general, scaffold materials should reflect the microenvironment of target tissues/organs to facilitate cell growth and ultimately integration to the host. A beneficial clinical feature for dental pulp regeneration would be if the scaffold is injectable, as are some of the natural scaffold materials and hydrogels. In these cases, the gelation time would need to be taken in to consideration when seeding cells in a scaffold for implantation into a host.

An essential clinical feature for scaffold selection is biocompatibility. Naturally derived scaffold materials have the advantage that they are generally well tolerated, do not lead to immunogenic response. However, a major drawback is the lack of control over the pore size and heterogeneity of the scaffold. The degradation process of the scaffold is important, and should closely follow the rate of tissue regeneration. When using synthetic polymers, the release of acidic degradation products must be taken into consideration, as well as the resulting drop in pH in the surrounding microenvironment and how that affects the immune response [3, 39].

Bioactive glasses are silico-phosphate chains that have been used in the treatment of periodontal bone defects. These materials have the ability to bind chemically with the bone. Same bioactive glasses may haveosteo-inductive properties and have been tested in animal trials [40].

On tissue-engineering applications, biomaterials often serve as scaffold for a specific cell type. An ideal scaffold should provide chemical stability or degradability and physical properties matching the surrounding tissue to provide cytocompatibility, support adhesion, proliferation, stability, and mechanical strength.

The adaptation of biomaterials for tissue-engineering applications is an iterative process [41, 42, 43]. Usually a biomaterial is tested in combination with only one specific cell type. Emerging understanding about interactions between stem cells, scaffolds, and morphogenic factors has accelerated translational research in the field of dental pulp tissue engineering. Further studies described the use of biomaterial arrays consisting of polymers or extracellular-matrix molecules for dentistry regenerative procedures (Table 2).

Neuss *et al*. (2008) [41] tested the use of various biomaterials with stem cells: synthetic biodegradable and non-degradable polymers were investigated as well as degradable biopolymers. The usage of biomaterial arrays to identify suitable combinations of cells and biomaterials for cellular therapy requires simultaneous assessment of cell morphology, vitality, cytotoxicity, apoptosis, and proliferation. The authors concluded

that combination of dental pulp stem cells and polymers for tissue-engineering applications, supported cell adhesion and proliferation while apoptosis and necrosis are inhibited.

Eslaminejad *et al*. (2013) [44] studied the scaffold of tricalcium phosphate in contact with dental pulp stem cells in 3D culture system. The 3D culture system improves odontogenic differentiation of the stem cells. The differentiation level of the cells in 3D culture is significantly lower than that of odontoblasts present in pulp tissue. TCP biomaterial possesses an odontogenic-inducing property.

Palumbo *et al*. (2013) [45] made a comparison of the behavior of committed (human osteoblast cells – hOB – from bone biopsies) *versus* multipotent (human dental pulp stem cells – hDPSC – from extracted teeth) cells, cultured on shot-peened titanium surfaces, since the kind of cell model considered has been shown to be relevant in techniques widely used in studies on composition/morphology of biomaterial surfaces. The titanium surface morphology, with different roughness, and the behavior of cells were analyzed by confocal microscope (CM), scanning electron microscope (SEM) and X-ray microanalysis. They concluded that multipotent cells could be suitable to obtain *in vitro* osteocyte-like network for regenerative medicine.

Among biomaterials, some types of pluronics have been reported to increase bone formation of stem cells. The effect of these pluronics on odontogenic differentiation has not been addressed yet. Taşlı *et al*. (2013) [46] studied the effect of pluronics F68, F127, and P85 on odontogenic differentiation of stem cells derived from third molar tooth germs of young adults. They shown that ability of stimulate stem cell did not general for the every pluronics. F68 was able to induce odontogenic differentiation but F127 was nontoxic to stem cells but did not have any effect on differentiation. P85 was found to reduce cell viability during differentiation.

Cavalcanti *et al*., (2013) [47] suggested that self-assembling peptide hydrogels might be useful injectable scaffolds for stem cell-based regenerative endodontics. Moshaverinia *et al*. (2013) [48] related the association of dental-derived mesenchymal stem cells (MSCs) and alginate hydrogel as a promising candidate for cartilage regeneration. Their results highlight the vital role played by the microenvironment, as well as value of presenting inductive signals for viability and differentiation of MSCs.

In a most recent study the same authors studying dental-derived mesenchymal stem cells now associated to alginate microspheres for periodontal therapy concluded that periodontal ligament and gingival tissues can be considered as suitable stem cell sources for tendon engineering. PDLSCs and GMSCs encapsulated in TGF-β3-loaded RGD-modified alginate microspheres are promising candidates for tendon regeneration [49].

Niu *et al*. (2014) [50] investigated the effects of intrafibrillar-silicified collagen scaffolds (ISCS) on the osteogenic differentiation of human dental pulp stem cells (hDPSCs) *in vitro* and *in vivo*. ISCS significantly promoted the proliferation, osteogenic differentiation and mineralization of hDPSCs. Their study demonstrates combining the use of hDPSCs and ISCS to promote bone-like tissue formation is a promising approach for clinical bone repair and regeneration. A critical challenge of the clinical scenario described previously is the need for quick vascularization of the engineered tissue to enable the maintenance of the viability of transplanted stem cells. It appears that necrotic immature teeth with open apices are the prime candidates for dental pulp tissue engineering at this stage of development of the technique. Even in these cases, we believe that the success rate of such therapy would benefit from the delivery of a proangiogenic stimulus [51].

Table 2. Summary of the studies associating stem cells and biomaterial for regenerative techniques.

Authors	Cell type	Scaffold associated
Neuss *et al*., 2008 [41]	Systematic screening assays of dental pulp stem cell	Degradable biopolymers Degradable synthetic polymer Non degradable polymers
Eslaminejad *et al*., 2013 [44]	Dental pulp stem cell	Tricalcium phosphate
Moshaverinia *et al*., 2013 [48]	Dental derived mesenchymal cell	Alginate hydrogel
Palumbo *et al*., 2013 [45]	Human osteoblasts cells compared with human dental pulp cell	Titanium surfaces with alumina particles.
Cavalcanti *et al*., 2013 [47]	Dental pulp stem cell	Self assembling peptide hydrogel
Tasli *et al*., 2013 [46]	Human tooth germ stem cells	Pluronics F68, F127, and P85
Moshaverinia *et al*., 2014 [49]	Dental derived mesenchymal cell	Alginate microspheres
Niu *et al*., 2014 [50]	Human dental pulp stem cell	Intra-fibrilar-silicified collagen scaffolds

This article focused on the use of stem cells for dental applications. Stem cell-based therapy is an exciting field of science that poses a great challenge to scientists all over the world and dentistry may be one of the main specialties to research and clinically use the benefits of this developing science. One of the main advantages of dentistry is due to the ability of dental tissues to provide a rich source of mesenchymal stem cell types [6] and differently from other donor sources in human body, they are easily accessible and pose minimal ethical conflicts when compared with those obtained from embryonic cells.

Dental stem cells have the advantage of easy access by dentists and, until now, five different human dental stem cell populations have been isolated and characterized: dental pulp stem cells, stem cells from deciduous teeth, stem cells from apical papilla, periodontal ligament stem cells and dental follicle precursor stem cells [20].

Scaffolds of biological materials such as collagen, proteoglycans, alginate-based substrates and chitosan have been used for tissue engineering. Natural polymers typically promote good cell adhesion and growth, and are biodegradable, which is very important to allow host cells to produce extracellular matrix and substitute the scaffold. But to fabricate scaffolds from biological materials with homogeneous and reproducible structures is not easy, since they usually have poor mechanical properties and it limits their use. Collagen-based scaffolds can be used alone or with additives to enhance biological and mechanical properties, for example those used for bone regeneration [52].

Little is known about the association of dental stem cells and collagen scaffolds, which would be a great improvement for this expanding field. Deciduous teeth are usually discarded in dental office, but stem cells from deciduous teeth have a great potential in research.

Advances in pulp regeneration have been made and diverse substances and biomaterials had been tested mainly for neo-vascularization. The possibility of regenerate a vascularized human dental pulp in emptied root canal space and produce new dentin on existing dentin walls was tested by Huang *et al.* (2010) [24]. The authors used a stem cell-mediated approach with a root fragment and an immunocompromised mice model. The results showed formation of a continuous dentin-like tissue on the existing dentin walls and that the regenerated tissues closely resemble natural pulp tissue.

Nakashima and Iohara (2011) [21] also demonstrated complete pulp regeneration with a continuous layer of dentin-like tissue by stem cell therapy, and Lee *et al.* (2011) [25] showed that human dental pulp stem cells in combination with preameloblast-conditioned medium could be valuable on repair and regeneration of dentin-pulp complex.

The hard tissue formation has also been tested**.** Obeid *et al.* (2013) [28] demonstrated that autologous mesenchymal bone marrow stem cells also have the potential to promote hard tissue formation after direct pulp capping procedures. In their study, the results showed that the bone marrow stem cells had significantly higher tendency to produce a hard-tissue barrier than Mineral Trioxide Aggregate (MTA). However, according to Ulmer *et al.* (2010) [17] it has not been possible yet to find a source of human adult ectodermal stem cell to regenerate enamel.

3.5. Pulp stem cells for recovery of a specific condition.

The ability of a stem cell isolated from one tissue to "convert" to cells found in a different tissue was denominated plasticity [14]. These stem cells, similar to other neural crest stem cell types in human body, are highly accessible and offer substantial additional advantages that make them good alternatives for manipulation and clinical use: they present a high multilineage differentiation potential, high proliferative capacity, they are not oncogenic, ant its achievement does not raise ethical concerns.

Another great application ground for dental neural crest stem cells is nervous system repair. Both dental and non dental neural crest stem cells express immature neural/glial cell markers and are particularly amenable to neural/glial differentiation. Remarkable positive results of neural regeneration and functional improvement have been obtained in experimental models of brain, spinal cord and nerve injury [53].

Inoue *et al.* (2013) [54] agree that regenerative therapy using stem cells is a promising approach for the treatment of stroke. In their study, adult male Sprague-Dawley rats were subjected to permanent middle cerebral artery occlusion. Stem cells from human exfoliated deciduous tooth were then administered intranasal and the motor function and infarct volume were evaluated. The transplantation of stem cells ameliorated the ischemic brain injury.

The therapeutic potential of mesenchymal stromal cells depends on their ability to survive and proliferate under adverse in vivo scenarios in a particular disease. In most of the sites of injury, especially in diabetic wounds, there can be hypoxia, hyperglycemia, and ischemia, leading to a lack of nutrients. In a comparative study between human dental pulp stem cells from exfoliated deciduous teeth and permanent teeth it was investigate the influence of hypoxia, high glucose, and low serum concentrations on the growth kinetics and proliferative potential. In this study were isolated two types of specialized stem cells from human dental pulp tissues, which were supposedly of neural crest origin, and cultured them in KO-DMEM medium supplemented with 10% fetal bovine serum. Both exfoliated deciduous pulp stem cells and permanent pulp stem cells were characterized for standard CD surface markers, and their ability to differentiate into adipogenic and osteogenic lineages was tested. Exfoliated deciduous pulp stem cells and permanent pulp stem cells were exposed to either hypoxia or high glucose or low serum conditions, and their growth kinetics and differentiation potentials were compared with those of normal culture conditions. The exfoliated deciduous pulp stem cells retained their phenotypic expression and differentiation potential under hypoxia, high-glucose, and low-serum conditions and exhibited a higher proliferation in terms of cell yield and a reduced doubling time compared to permanent pulp stem cells. Exfoliated deciduous pulp stem cells were superior to permanent pulp stem cells as evidenced by their enhanced proliferation under adverse culture conditions [55].

The great advantage of regenerative therapy with dental stem cells from teeth is that they are easily accessible for research and clinical use and going forward, it is important to base the best research in this area, in future be able to bring the general practitioner to the technique that should be applied for dental regeneration, favoring their understanding about the care and recommendations that should be followed. We are facing a unique opportunity to advance the science and future research in this area should be encouraged until the regenerative therapy becomes a common technique used in dental clinics and further efforts to educate patients and clinicians about the use of stem cells should be stimulated. The dentists and health´s scientist must be encourage and warn over the potential of the use of extracted teeth that can become a source of stem cells , and make them aware of the regenerative potential that is often wasted. Moreover, widespread among professionals and patients could be critical for regenerative stem cell therapy so that more people know of the many forms of treatment and applications that exist.

4. CONCLUSIONS

Until now the use of dental stem cells has limited clinical applications in dentistry. This is because the structural complexity of the teeth. To this end a growing number on research about of the molecular signaling and growth factors are available for specific cells and important advances for pulp regeneration have been made especially to know the correlation between neurotrofic factors and angiogenesis. Moreover, the role of integrated stimuli factor for each kind of cells like fibroblast, endothelial and Schwann cells has never understood completely. On the other hand, teeth cells can be easily obtained in dental office and have some important properties like high proliferation rate and capacity to differentiate into various other cells in dentistry and medicine. Thus, dental stem cells are becoming a great promise in science and the clinical perspectives of their use will become a reality in near future. There are, however, many challenges and hurdles involved to achieve complete success regarding the efficacy of the

process. Finally, the education of patients, physicians and dental practitioners and further studies in areas like growth factors and signaling molecules implicated in tooth development are essential to improve dental stem cells dental stem cells clinical use.

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