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Stem cells in periodontal regeneration

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Abstract

Objectives: The aim of this thesis is to illustrate which stem cells, both dental and non-dental, are available, compare them and clarify their differences, discover the most appropriate for periodontal application and shed some light on the scaffolds and bioactive molecules which can be used, in conjunction with the stem cells, to amplify their clinical outcome.

Material & methods: An electronic search of the Pubmed literature was performed between March 2020 and June 2021 to identify all articles investigating information about the stem cell biology and the different categories of both dental and non-dental stem cells, the different scaffolds and bioactive molecules and their applications so far in animal and human trials.

Results: The database search yielded 59 studies, 50 in animals and 9 in humans.

Conclusion: The abundance of different dental and non-dental stem cells as well as of different scaffolds and bioactive molecules indicates a wide spectrum of possibilities since there are limitless ways to combine them. So far, most promising seems to be the periodontal ligament stem cell (PDLSC) with the bone marrow mesenchymal stem cell (BMMSC) in the second place. However, more studies are needed to prove this result and discover how to maximize its potential by placing it in conjunction with the most fitting scaffold and perhaps with another category of stem cells.

Keywords: Periodontal regeneration, stem cells, PDLSCs, BMMSCs, scaffolds

Introduction

Periodontium consists of the tissues that en masse invest and surround the teeth. The periodontal tissues include the gingiva, the periodontal ligament (fibrous connective tissue), the alveolar bone (force-responsive mineralized tissue), and the cementum (the mineralized layer on the tooth root surface) (Kim et al. 2014). These constructs play a critical role in supporting the tooth structures

under occlusal and masticatory loading conditions (Poiate et al. 2009) and in defending against the invasion of various oral microorganisms (Tribble and Lamont 2010, Pihlstrom et al. 2005).

The structure and composition of the periodontium are affected by both acquired and heritable diseases, the most important among them being the periodontal diseases (Grzesik and Narayanan 2002). Periodontal diseases represent a type of infectious, bacterial-induced chronic inflammation that induces destruction of tooth-adjacent supporting structures (periodontal complex), including the gingiva, the periodontal ligament (PDL), the cementum, and the alveolar bone (Pihlstrom et al. 2005). Based on its ability to induce alveolar bone loss, periodontal disease can be divided into gingivitis and periodontitis (Armitage 1999).

The mild form of periodontal disease restricts itself to the gingiva, thus the term gingivitis, and appears with extremely high prevalence. There is a proven connection between gingivitis and the local bacterial biofilms attached to the surface of the tooth and the gingiva. The gingiva is attached to both the alveolar bone and the enamel surface and constitutes an important part of the oral cavity soft tissue. Its most significant component is the junctional epithelium, because it creates a tight connection between the tooth surface and the gingiva, thus protecting the periodontal ligament, the cementum and the alveolar bone from bacterial invasion and possible infection (Pihlstrom et al. 2005).

When the defense line of the junctional epithelium is breached, oral microorganisms intrude and provoke gingival bleeding, periodontal attachment loss and alveolar bone absorption (Amano 2017). In particular, periodontitis is triggered by the interplay between the local bacterial biofilm and the host immune response against these bacteria (Paster et al. 2001). Not all the bacterial species are equally responsible though. Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, Fusobacterium nucleatum, Peptostreptococcus micros, and Campylobacter rectus are key microorganisms for rapid and generalized destruction (Saini et al. 2009). The bacteria invade the PDL, a precious collagen fiber connection between the cementum and the alveolar bone, full of cells and vessels, and damage it through the release of proteolytic enzymes, cytokines and other bacterial products such as lipopolysaccharides (LPS), which stimulate cytokines to signal precursor cells to differentiate and activate osteoclastic cells as well as the periodontal inflammatory process (Taba et al. 2005, Darveau 2010). Therefore, the host immune response releases also proteolytic enzymes and cytokines. These bioactive molecules create an inflammatory microenvironment which in turn provokes important loss of attachment and apical movement of the junctional epithelium. The height from the top of the junctional epithelium to the alveolar bone crest (known as the biologic width) is fixed, thus the loss of the PDL initiates the resorption of the alveolar bone and leads to tooth loss (Nugala et al. 2012).

The presence and severity of periodontal disease is determined by pocket probing depth (PPD), clinical attachment loss (CAL) and bleeding on probing (BOP) measurements and by evidence of radiographic alveolar bone loss. These data are collectively interpreted contextually for each patient in order to establish a diagnosis of periodontal disease that includes both the extent and severity of disease (Cobb et al. 2009). The current classification distinguishes between chronic and aggressive periodontitis, localized (up to 30% of all tooth surfaces are affected) and generalized (more than 30% of all tooth surfaces are affected) and determines

the severity of the disease according to the loss of attachment into mild (1-2 mm), moderate (3-4 mm) or severe (≥5 mm) (Armitage 1999, Carranja et al. 2006, Wiebe and Putnins 2000).

From an epidemiological point of view, around 35.0% of adults suffer from moderate form of periodontitis, while 15.0% are affected by severe, generalized form of periodontitis at some point at their lives (Albander 2005). Periodontitis is highly prevalent, and in fact severe periodontitis is the sixth-most prevalent health condition worldwide (Kassebaum et al. 2014).

The association and possible cause-effect correlation between periodontitis and multiple systemic diseases such as diabetes, cardiovascular diseases, chronic kidney diseases, and pulmonary infections as well as a higher probability of adverse pregnancy outcome is also reported (Borgnakke et al. 2013, Fisher et al. 2008, Friedewald et al. 2009, Scannapieco et al. 2003, Kinane and Bouchard 2008, Williams et al. 2008), thus highlighting the urgency of treating effectively such a major public health issue and succeeding in regenerating the periodontal tissues (Intini 2010).

The goal of periodontal treatment is twofold. The treatment must successfully stop the progression of the disease and prevent further destruction. Subsequently it should regenerate the function and the structure of the damaged periodontal tissues.

In the case of gingivitis, the situation can be readily reversed by supragingival scaling and effective oral hygiene. In the case of periodontitis, however, conventional treatment modalities either non-surgical like scaling and curettage or surgical like open flap debridement result in control of inflammation and formation of a long junctional epithelium and hence only in periodontal repair without any predictable regeneration (Narayanan and Bartold 1996).

Several procedures have been tried to achieve periodontal regeneration, posing, however, important limitations regarding the predictability of the outcome and the complexity of the underlying surgical procedure. The most suited site for a regenerative surgical technique is a deep intrabony defect (Ramsier et al. 2012). Regarding the different materials and procedures involved:

The use of bone grafts is linked with autogenous bone grafts-donor site morbidity and complications as well as limited graft availability (Hjørting Hansen E. 2002, Burchardt H 1983, Cordaro L et al. 2002). On the other hand, xenografts and alloplasts present only osteoconductive properties and an inclination to fibrous encapsulation (Garraway R et al. 1998). The use of enamel matrix derivative (Emdogain) or platelet rich plasma (PRP) is correlated with limited predictability and high degree of variability in results (Needleman IG et al. 2006, Esposito M et al. 2009). Finally, the guided tissue regeneration (GTR) presents successful outcomes in cases of narrow 2/3 walled defects, circumferential defects and class 2 molar furcations (Wang HL and Cooke J 2005). It is completely ineffective in class 3 molar furcations (Sanz M and Giovannoli JL 2000). Since the use of a membrane is obligatory in GTR, there is either the possibility of a resorbable or of a non-resorbable membrane, each presenting its own disadvantages. The resorbable membranes can collapse, therefore they are placed along with bone grafts. The non-resorbable are prone to infection and need a surgical re-entry procedure to be removed (Nguyen TT et al., 2013).

A novel approach is introduced, through the application of stem cells, towards a predictable and successful outcome. It requires the recruitment of progenitor cells that can differentiate into periodontal ligament cells,

mineral forming cementoblasts and bone forming osteoblasts. These cells would, at least theoretically, form a functional epithelial seal, insert new connective tissue fibers into the root, reform the acellular cementum on the tooth and restore the alveolar bone height (Bartold PM and Narayanan AS, 1998, Giannoble WV et al., 2001) Two important parameters in this pursuit are the scaffolds as wells as additionally administered bioactive molecules, which in collaboration with the stem cells, can provoke the desirable regenerative processes so that a true regeneration is established.

Material and methods

An electronic search of the literature was performed between March 2020 and June 2021 to identify all articles investigating the above addressed questions. Information about the stem cell biology and the different categories of both dental and non-dental stem cells, as well as about the different scaffolds and bioactive molecules, was retrieved.

The first part of the thesis will use a selection of retrieved articles dealing with more general aspects such as possible donor areas, cell features, cell identification and isolation approaches, capacity of the isolated cells to differentiate as well as potential scaffolds and their properties and bioactive molecules. The search was conducted using MEDLINE (National Library of Medicine)-PubMed without restrictions concerning the date of publication. The following keywords were used (connecting different keywords with AND, OR): dental regeneration, periodontal regeneration, bone regeneration, tooth regeneration, tooth repair, tooth development/aging, regenerative dentistry, cell-based therapy, cell sheet engineering, cell aggregates/cell sheets, cell culture techniques, tissue engineering, tooth engineering, 3D printing technology, stem cells, tooth stem cells, dental stem cells, dental mesenchymal stem cells, periodontal stem cells, mesenchymal stem cells, immature stem cell, periodontal ligament stem cells, dental pulp stem cell, induced pluripotent stem cells, skeletal stem cells, adult stem cells, stem cell transplantation, stem cell research, periodontitis, periodontal diseases, periodontal treatment, periodontal ligament, cementum, dental pulp, bioengineered tooth, periodontal tissue complex, multiple tissue formation, scaffold conditioned medium, bioactive molecules and tissue banks. This was followed by a manual search, and references were used to identify relevant articles.

In the second part of this review, the search focused on evaluation of the outcome of a cell-based treatment in comparison to conventional regenerative approaches. For selection of animal studies, the filters “animal” and “trials” were used, whereas for identification of studies performed in human patients the filters “human”, “trials” and “clinical trials” were applied. 50 records were obtained for the selection of animal studies while 9 studies were retrieved for the analysis of human clinical trials.

The articles identified from the electronic and manual search were screened to eliminate those that failed to meet the respective inclusion and exclusion criteria as listed below.

Inclusion criteria for analysis of animal studies:

- Animal studies analyzing the impact of stem cell administration on periodontal regeneration.

Exclusion criteria for analysis of animal studies:

- Studies in a language other than English or without an English abstract.

Inclusion criteria for analysis of human studies:

- Randomized controlled clinical trials, prospective and retrospective clinical studies, Case reports or case studies that evaluated the efficacy of stem cell application

Exclusion criteria for analysis of human studies:

- Studies in a language other than English or without an English abstract.

After this screening 50 studies remained for full text analysis for the animal study selection and 8 for the human clinical trial selection.

Both in animal and human studies the following outcome parameters were extracted: formation/regeneration of periodontal tissues such as bone, cementum and periodontal ligament, formation of Sharpey's fibers, formation of cementum/periodontal ligamentlike complex, periodontal ligament/bone-like complex and root/periodontal ligamentlike complex, formation of new vessels and formation of bone implant contact.

Results

Stem cells biology

Stem cells are distinguished by other cell types based on three main and unique characteristics (Fortier 2005). Firstly, they are capable of self-division and self-renewal. This ability to go through numerous cycles of cell division without losing the undifferentiated state remains for quite a long time. Secondly, since they are undifferentiated and as long as they remain so, they do not have any tissue specific structures that would allow specialized functions to be performed. The third and final property is the potency, the ability to differentiate after exposure to various signals into specialized cell types. These signals can be either external such as chemicals secreted by neighbouring cells, physical contact with adjacent cells and molecules in the microenvironment or internal such as the expression of cell genes. Depending on their differentiating potential, stem cells can further be classified as (Smith 2006):

- Totipotent stem cells (also known as omnipotent): Stem cells can differentiate into embryonic and extra-embryonic cell types.
- Pluripotent stem cells: Stem cells which are the descendants of totipotent cells and can differentiate into most cells from any germ layer.
- Multi potent stem cells: stem cells which can differentiate into cells that is restricted to a particular germ cell origin.
- Oligopotent stem cells: Stem cells that can form two or more lineages within a tissue.
- Unipotent stem cells: can produce only one cell type, their own but have the property of self-renewal which distinguishes them from non-stem cells.

Theoretically speaking, stem cells should be either totipotent or pluripotent, although multipotent or unipotent progenitor cells are also referred to as stem cells.

Stem cells are of two types, embryonic stem cells and adult stem cells. The embryonic stem cells can differentiate into virtually any cell type as well as propagate indefinitely in an undifferentiated state, thus having pluripotent and totipotent differentiating capacity (Evans & Kaufman 1981, Keller 1995). Large numbers of them can be easily grown in culture and they are immortal but at the same time it is difficult to control their proliferation and they can provoke transplant rejection. The immortality is based on the high expression of telomerase enzyme which is responsible for maintaining chromosomal stability.

The adult stem cells only differentiate into different cell types of tissue of origin and replace cells lost through wear or tear, injury or disease (Ivanovski et al 2006). They are relatively rare in mature tissues and a method of expanding them in cell culture has yet to be implemented successfully. The main advantage is that

they come from the same patient they aim to treat, thus guarantying a safe transplant without risk of rejection. Unfortunately, they undergo senescence and considered to have a finite life span since telomerase enzyme is absent in most mesenchymal stem cells.

Stem cells categories and features

The stem cells used for periodontal regeneration are dental and non- dental mesenchymal stem cells (MSCs). Stem cells (SCs) of dental origin are the following ones:

1. Dental pulp stem cells (DPSCs)
2. Mesenchymal stem cells from human exfoliated deciduous teeth (SHEDs)
3. Stem cells from the dental apical papilla (SCAPs)
4. Periodontal ligament stem cells (PDLSCs)
5. Stem cells from the dental follicle (DFSCs)

Stem cells of non-dental origin are the following ones:

1. Bone marrow mesenchymal stem cells (BMSCs)
2. Adipose tissue derived stem cells (ATSCs)
3. Embryonic stem cells (ESCs)
4. Induced pluripotent stem cells (iPSCs)

A detailed description of every feature of both dental and non-dental stem cells is reported by several authors (Huang et al. 2009, Inanc & Elcin 2011, Ullah et al. 2015).

In the following the main features will be summarized.

DPSCs and SHED express multipotential differentiation (osteo/dentinogenic, adipogenic, chondrogenic, myogenic, neurogenic) and can form de novo dentin–pulp like tissue, bone- like tissue, blood vessels and neuronal tissue. They can be put into practice in tooth and periodontal regeneration to create dentin/pulp, roots and periodontal tissue.

SCAPs express multipotential differentiation (osteo/dentinogenic, adipogenic, neurogenic) and can form de novo dentin–pulp- like tissue and blood vessels, thus are used in tooth and periodontal regeneration to create dentin/pulp and roots. PDLSCs have also multipotential differentiation (osteo/cementogenic, adipogenic, chondrogenic, neurogenic) and can form de novo cementum- like tissue and PDL- like tissue with wide application in tooth and periodontal tissue regeneration.

DFSCs have cementogenic, odontogenic, adipogenic and chondrogenic differentiation. They can form PDL- like tissue and cementum- like tissue and can therefore be used in regeneration of roots and periodontal tissues.

BMSCs have long been regards to have capability for osteogenic, odontogenic, adipogenic, chondrogenic differentiation but are nowadays also discussed to express myogenic and in particular cardiomyogenic potential and even the ability to form neural cells. However, the mechanisms underlying this trans differentiation remain unclear and it is still questionable if functional neuronal cell can be obtained. Because of this broad spectrum of regenerated tissues, they can regenerate both the whole tooth and the periodontal tissues.

ATSCs demonstrate osteogenic, adipogenic, chondrogenic, myogenic and also for these cells neurogenic differentiation is described. They create bone- like tissue, cartilage, blood vessels and adipose tissue and are capable of periodontal tissue regeneration.

ESCs and iPSCs can differentiate into almost all cell lineages including endodermal, mesodermal, and ectodermal. They can form everything de novo (bone-like tissue, cartilage, myocardium, blood vessels, adipose tissue, neuronal tissue) and regenerate the whole tooth as well as the periodontal tissues.

Stem cells markers, identification and isolation

Stem cell markers help to identify, characterize and isolate stem cells.

Embryonic stem cells markers

Oct-4, Nanog, SSEA-3, SSEA-4, TRA-1–60 and TRA-1–81, and CD31 are expressed by ESCs and immature dental pulp stem cells. Transcription factors Oct-4, Nanog, and Sox2 form the core regulatory network that ensures the suppression of genes leading to differentiation and maintenance of pluripotency. Cell surface antigens most commonly used to identify ESCs are the glycolipids SSEA3 and SSEA4 and the keratan sulfate antigens Tra-1–60 and Tra-1–81 (Oswal et al. 2011)

Adult stem cells markers

Stro-1, trypsin-resistant cell-surface antigen, is one of the early surface markers of MSCs. It is a commonly used stem cell marker for all dental MSCs. Its expression diminishes gradually during cultivation of the stem cells. It is also present in bone marrow stem cells.

Stro-4 binds to heat shock protein 90 beta of multipotent MSCs and is also suited to identifying stem cells.

Markers for differentiated cells can be used to evaluate the differentiation potential of the stem cells, e.g. the osteoblast marker osteocalcin is frequently used as differentiation marker for dental pulp stem cells (DPSCs).

Neural marker nest in on dental stem cells indicates that dental mesenchymal stem cells originate from the progenitor cells of the neural crest, which can also differentiate into neural tissues.

Stro-1, perivascular cell marker CD146, alpha smooth muscle actin, pericyte associated antigen and scleraxis are markers for PDLSCs (Oswal et al. 2011).

Adult stem cells identification and isolation.

For stem cell identification of DPSCs several techniques exist (Murray et al. 2007):

- Fluorescent antibody cell sorting (FACS): Stem cells can be identified from mixed cell populations by staining the cells with specific antibody markers and using a flow cytometer.
- Immunohistochemical staining.
- Physiological and histological criteria, including morphology, proliferation, chemotaxis, specific differentiation potential.

Cell isolation is primarily performed via attachment methods to form colony forming units that are further propagated while more sophisticated methods include immunomagnetic selection or fluorescent antibody cell sorting also for isolation of cells.

Regarding the isolation and culturing procedure, although many different techniques are described for every stem cell available, for the time being, it is challenging, time-consuming, time-sensitive and very costly. Thus, the application of the autologous multipotent stem cells remains a challenge in terms of tissue culturing and ex vivo expansion.

Bioactive molecules

The bioactive molecules that can contribute to the stem cell based periodontal regeneration belong to several categories such as growth factors, pharmaceuticals, matrix proteins or plant extracts.

The growth factors include the recombinantly produced platelet derived growth factor (PDGF) or patient derived growth factor containing mixtures such as the platelet rich plasma (PRP) and the platelet rich fibrin (PRF). PDGF accelerates the regeneration of the periodontal apparatus in periodontal defects in rats and creates an interposed periodontal ligament between the newly formed cementum and the alveolar bone (Chang et al. 2013, Plonka et al. 2016, Kaigler et al. 2011). PRP and PRF present a strong angiogenic capacity, provide a natural scaffold and release slowly the bioactive factors in humans with periodontitis (Castro et al. 2017, Aydemir et al. 2016, Baba et al. 2016).

Enamel matrix derivate (EMD), contains primarily matrix proteins also known as amelogenins and facilitates the adhesion and proliferation of osteoblasts and PDLSCs (Miron et al. 2016a, Miron et al. 2016b, Miron et al. 2017, Zhang et al. 2017) and promotes the periodontal regeneration of Class III furcation defects in monkeys and in humans with periodontitis (Shirakata et al. 2017, Gupta et al. 2014, Aimetti et al. 2016).

Frequently used pharmaceuticals in dental applications comprise aspirin and melatonin. The aspirin controls the inflammation in periodontal defects in rats, while melatonin has an anti-inflammatory and anti-oxidative effect in experimental periodontitis in rats (Du et al. 2018, Kara et al. 2013).

Plant extracts that were reported in dental context include osthole, resveratrol and quercitrin. Osthole improves the capacity of osteogenic differentiation of PDLSCs and promotes the periodontal regeneration in periodontal defects in rats (Sun et al. 2017, Gao et al. 2013).

Resveratrol prevents bone loss and promotes osteogenesis in periodontitis model in rats (Bhattarai et al. 2016, Tamaki et al. 2014). Quercitrin is anti-inflammatory and affects the human gingival fibroblasts (Gomez-Florit et al. 2015, Gomez-Florit et al. 2014).

Animal studies

The database search yielded for full text analysis 50 studies for the animal study selection. The results are illustrated in the form of stating the specific species used, the combination of cell source and scaffold/carrier, the defect type treated and the final outcome of the therapeutic intervention.

Bone marrow mesenchymal stem cells

17 animal studies refer to the usage of bone marrow derived mesenchymal stem cells (BMSCs). BMSCs in conjunction with a tricalcium phosphate/hydroxyapatite (TCP-HA) carrier in canines increased the bone regeneration in alveolar saddle defect (De Kok et al. 2003). The same principle (BMSCs as cell source and HA/TCP carrier in canine species) was applied again, in this case in extraction sockets, where the BMSCs remained and contributed to bone regeneration (De Kok et al. 2005).

BMSCs with collagen gel as a carrier were used to regenerate experimental Class III furcation defects in canines (beagle dogs) and led indeed to more periodontal regeneration (Kawaguchi et al. 2004): Formation of new bone, cementum, and periodontal ligament (PDL) was observed without any complete regeneration of defects. Significantly higher percentages of new cementum length and bone area were observed in defects treated with cells and carrier compared to carrier alone.

BMSCs labeled with green fluorescent protein (GFP) were also combined with atelocollagen in treating experimental Class III periodontal defects in canines (beagle dogs) and enhanced the periodontal tissue regeneration (Hasegawa et al. 2006): Formation of new bone, cementum, and PDL was observed in the test group without achieving complete regeneration of defects. Differentiation of labeled BMSCs into cementoblasts, osteoblasts, osteocytes, and fibroblasts were observed.

An interesting idea was the application of cryopreserved cells in combination with a carrier. Autologous cryopreserved BMSCs were placed with a collagen sponge carrier in surgically created fenestration defects in beagle dogs. There was no major difference between the regeneration of periodontal tissue defects through cryopreserved and no cryopreserved BMSCs. In both cases, amounts of formation of new bone, cementum, and PDL fibers were significantly higher compared to the carrier alone group (Li et al. 2009).

BMSCs can also be used in conjunction with different combinations of platelet rich plasma (PRP) carriers. BMSCs with a PRP/Fibrin carrier increased the bone regeneration and bone implant contact (BIC) in circumferential peri implant defects in canines (Ito et al. 2006). Enhanced bone formation was observed after application of BMSC source and a PRP/ fluorohydroxyapatite (FHA) carrier in alveolar defect of 3.5 mm diameter and 8 mm depth in minipigs (Pieri et al. 2009) while increased bone formation and BIC were observed in swine after sinus augmentation with BMSCs and PRP/ FHA as a carrier (Pieri et al. 2008). Especially for sinus augmentation, increased new bone formation is also possible through the usage of BMSCs and inorganic material as a carrier in rabbits (Sun et al. 2008).

An alternative way of causing periodontitis and delivering the stem cells was applied in the following experiment: Periodontal defects were caused by periodontitis by binding wire around teeth and inoculating P. gingivalis in Sprague Dawley rats while allogeneic

BMSCs were delivered by local injection into defects using 0.9% NaCl solution.

Inhibition of proinflammation factors and enhanced periodontal tissue regeneration were observed in the animals afterwards (Du et al. 2014).

Allogeneic BMSCs labeled with GFP were placed with a gelatin beads carrier in surgically created periodontal window defects on the buccal surface of the mandibular 1st molar in Sprague Dawley rats. As a result, significantly more, new bone formation and greater number of functionally orientated PDL fibers were found in the test defects. GFP-labeled cells were integrated in newly formed periodontal tissue (Yang et al. 2010). Again, allogeneic BMSCs labeled with GFP were put into practice this time however, they were delivered by intravenous (IV) injection in irradiated wild-type mice. The periodontal defects were surgically created in contact to the mesial root of maxillary 1st molars and subsequently filled with ceramic bovine bone. New Periodontium formation was observed and the labeled BMSCs contributed to periodontal regeneration and differentiated into fibroblasts and osteoblasts (Zhou et al. 2011). Allogeneic BMSCs labeled with enhanced-GFP were delivered by intra-bone marrow transplantation four weeks before the creation of the defects. The defects were surgically created as periodontal window defects on the buccal surface of the mandibular 1st and 2nd molars in irradiated Sprague Dawley rats. The GFP-labeled cells were detected in the defects one week after surgery and in the newly formed bone, PDL, and cementum 4 weeks after creation of the defects (Yu et al. 2013).

Autologous BMSCs labeled with bromodeoxyuridine (BrdU) in an alginate hydrogel carrier treated surgically created class III furcation defects in beagle dogs. New periodontal tissue formation with new bone, PDL and cementum was observed while the labeled cells were distributed into PDL, cementum, alveolar bone, and blood vessels. These cells expressed markers of osteoblasts and fibroblasts (Wei et al. 2010).

Autologous BMSCs engineered to overexpress the BMP-2 gene were compared to unmodified BMSCs with Pluronic F-127 gel as a carrier. In that case, in the surgically created transgingival periodontal defects over the palate of maxillary incisors in New Zealand white rabbits, more new bone formation was found in the defects treated with engineered-BMSCs compared to the defects treated with the unmodified BMSCs (Chen et al. 2008).

Autologous BMSCs transfected with human basic fibroblast growth factor (bFGF) in a calcium alginate gel

carrier treated surgically created class III furcation defects in beagle dogs. More new bone formation was observed in sites that were treated with bFGF-modified BMSCs compared to unmodified BMSCs (Tan et al. 2009).

Autologous BMSCs modified to overexpress osteoprotegerin (OPG) in a Poly Lacticco-Glycolic Acid (PLGA) carrier treated surgically created periodontal window defects on buccal surface of mandibular premolars in beagle dogs. The amounts of formation of new bone, cementum, and connective tissue were significantly higher in defects treated with modified or non-modified BMSCs compared to the control defects (Zhou and Mei 2012).

As a result, we can deduct from these data, that BMSCs can, regardless of the carrier or the species involved, contribute to the regeneration of periodontal tissues. Different methodologies of preserving the stem cells and combinations with different carriers, further enhance the clinical outcome.

Periodontal ligament cells/Periodontal ligament stem cells

20 animal studies refer to the usage of periodontal ligament cells (PDLCs) and periodontal ligament stem cells (PDSCs).

PDL cells with a demineralized root as a carrier to bear them were re-implanted in alveolar bone in canines and created a new periodontal ligament (Boyko et al. 1981). PDL cells were also placed in artificial periodontal defects in canines and as a result, a new connective tissue attachment was observed (Van Dijk et al. 1991).

In combination with a collagen sponge as a scaffold, PDL cells induced cementum regeneration in periodontal fenestration defects in canines (Nakahara et al. 2004). In combination with a hyaluronic acid carrier, a PDL cell sheet formed new cementum in dehiscence defects in canines (Akizuki et al. 2005).

The cell sheet engineering proved to be quite useful, avoiding the limitations of other techniques. Multilayered PDL-derived cell sheets were placed in three-wall periodontal defects in canines and regenerated new bone and cementum, in particular cell formation was observed on the defect walls with periodontal ligament and polyglycolic acid stem cells (Iwata et al. 2009). The goal was to examine stem cells derived from multiple layers of periodontal ligament for periodontal regeneration. PDL cell sheets of human origin cultured with osteogenic differentiation medium regenerated periodontal fenestration defects in immunocompromised rats (Flores et al. 2008).

PDL cells have also been combined with Teflon membranes. In minipigs, experimentally induced furcation and interdental defects were filled with new alveolar bone and cementum (Lang et al. 1998).

Periodontal ligament stem cells with a HA/TCP carrier regenerated periodontal tissues after inducing inflammation in swine through surgically created periodontal defects in the mesial of 1st molars, thus exploring the potential of using autologous periodontal ligament stem cells to treat periodontal defects and proving their effectiveness in autologous transplantation. Formation of new bone, cementum, and PDL was reported. Significant improvements in clinical parameters such as the height of the regenerated alveolar bone were observed in the stem cells with the HA/TCP carrier group compared to HA/TCP alone and no treatment groups (Liu et al. 2008). The same combination of PDLSCs and HA/TCP carrier increased the new bone formation in peri implant saddle like defects in canines (same effect with BMSCs) (Kim et al. 2009).

An animal trial, trying to determine the spatial distribution of the stem cells in the periodontal ligament, found out that the PDLSCs found on the alveolar bone in immunocompromised mice had a greater potential for multiline age differentiation than those found on the root surface, both in osteogenic and adipogenic differentiation (Wang et al. 2011).

The ideal cell type for clinical application could be the PDLSCs, since they provided new cementum, bone, and PDL fibers, especially incremental lines of new cement, with Sharpey fibers being inserted and cellular cementum at the apex of the root in canines (beagle dogs). The research team compared the effect of autologous PDLSCs to dental follicle stem cells (DFSCs) and to dental pulp stem cells (DPSCs) in surgically created circumferential apical involvement defects without any carrier. PDLSCs had the best regenerative potential while no periodontal regeneration was achieved in the DPSCs group (Park et al. 2011).

To evaluate histomorphometrically the possible use of PDLSCs in the treatment of class III furcation defects, they were placed in canines and succeeded in promoting, in association with guided tissue regeneration, significant periodontal regeneration (Suaid et al. 2012).

In pursuit of developing a feasible allogeneic cell-based method for the treatment of periodontitis, autologous PDLSC sheets, allogeneic PDLSC sheets and autologous heterogenic PDL cells (PDLCs) with a HA/TCP carrier were compared for their clinical efficiency in an experimental model of periodontitis in swine. Significantly improved clinical and histological outcomes and repair of bone defects were observed for allogeneic and autologous PDLSCs sheets compared to PDLCs and control groups. No evidence of immunological rejections of allogeneic PDLSCs was found (Ding et al. 2010).

In the case of large bone defects, the bone regeneration potential of biomimetic intrafibrillarly mineralized collagen (IMC) loaded with autologous periodontal ligament stem cells proved to be promising, since it achieved a significantly higher extent of forming new bone in swine, with the normal architecture of natural bones and blood vessels (Zhang et al. 2017).

An attempt to recreate with PDLSCs a favorable to regeneration microenvironment and to enhance the reconstruction of the physiologic architecture of a dental cementum/PDL-like complex, succeeded in immunocompromised mice. The mixed type PDLSCs pellets supported cementum/periodontal ligament (PDL)-like tissue regeneration with neovascularization (Xie and Liu 2012).

Interestingly enough, Vitamin C, through the induction of telomerase activity in periodontal ligament stem cells, leads to the upregulation of extracellular matrix type I collagen, fibronectin, and integrin β1, stem cell markers Oct4, Sox2, and Nanog as well as osteogenic markers RUNX2, ALP, OCN. Thus, under Vitamin C treatment, PDLSCs can form cell sheet structures because of increased cell matrix production. Autologous vitamin C induced PDLSCs sheets without any carrier regenerated the defects up to normal levels and compared to autologous dissociated PDLSCs with gelfoam as a carrier, led to a significantly higher percentage of newly formed alveolar bone in swine. Formation of Sharpey's fibers was seen in all groups, although the spatial distribution was better in the vitamin C induced PDLSCs group, since they were irregular in the dissociated PDLSCs group (Wei et al. 2012).

Allogeneic PDLSCs with gelfoam sponge carrier in surgically created zero-wall dehiscence defects in Merino sheep led to no significant differences in mean area of new bone formation, length of new cementum

formation, and Sharpey's fiber thickness compared to the Gelfoam alone group. All regenerative parameters were significantly improved in both groups compared to untreated defects (Mrozik et al. 2013).

An animal trial managed to evaluate whether the cell sheet or the pellet is a better way to distribute stem cells. The outcomes of the monolayered PDLSCs cell sheets (MCSs), the monolayer PDLSCs pellets (MCPs) and the multilayered PDLSCs pellets (MUCPs) in surgically created periodontal defects in the mesial region of the maxillary 1st molars in Sprague Dawley rats without the involvement of any carrier (to avoid its co-founder effect) was the perpendicular insertion of fibers into defects in MUCPs and MCPs groups as well as the higher formation of mineralized tissue in MUCPs group rather than in the MCPs group. Therefore, MUCPs functioned better than the MCPs and MCPs functioned better than the MCSs (Guo et al. 2014).

Xenogeneic (human) PDLSCs with a HA/TCP carrier integrated into the periodontal tissue in two out of six samples in surgically created periodontal defects on buccal surface of the mandibular molars in immunodeficient rats (Seo et al. 2004). Xenogeneic (human) PDLSCs with an amniotic membrane enhanced the regeneration of periodontium and induced a higher percentage of bone fill in surgically created class II furcation defects in immunodeficient rats compared to defects treated with the amnion alone (Iwasaki et al. 2014).

PDLCs have proven to be perhaps the most promising stem cell category in achieving periodontal regeneration. The relatively easy gathering of stem cells from the donor site and the wide range of treatment modalities mean that research is going to focus on this specific stem cell, managing hopefully to expand its capabilities.

Dental pulp stem cells

2 animal studies refer to the applications of dental pulp stem cells (DPSCs). Cultured human DPSCs have the capacity to form bone and produced calcified tissue that was histologically proved to be bone when transplanted into immunocompromised mice (Otaki et al. 2007). Another trial investigated the possible positive effect of the hepatocyte growth factor (HGF) in conjunction with DPSCs. The combination significantly improved the periodontal bone regeneration in swine (Cao et al. 2015).

Mesenchymal stem cells from human exfoliated deciduous teeth

2 animal studies refer to the application of stem cells from human exfoliated deciduous teeth (SHED). The first aimed to reveal the characteristics and the potential of development of the SHED stem cells in vivo. They were able to differentiate into odontoblasts and induce osteoblasts to form bone in vivo in immunocompromised mice (Miura et al. 2003). The second investigated the ability of allogeneic SHEDs to regenerate lost periodontium in a swine periodontitis model. The effective repair of the loss of hard and soft tissue caused by periodontitis was observed (Fu et al. 2014).

Stem cells from dental apical papilla

There is only one animal trial, that evaluated the potential application of the stem cells from the dental apical papilla (SCAPs) for cementum and periodontal ligament regeneration and bio root engineering in vivo. Tissue regenerative capacity was observed, that produced a typical cementum/PDL-like complex in immunocompromised mice (Han et al. 2010).

Adipose tissue derived stem cells

3 animal studies investigate the application of adipose tissue-derived stem cells (ATSCs) in the periodontal regeneration. Allogeneic ATSCs, labeled with GFP, together with a PRP carrier formed new bone,

cementum, and PDL fibers in surgically created fenestration defects in wistar rats. GFP-positive cells were observed on the surface of the regenerated alveolar bone and PDL structures (Tobita et al. 2008). The same main researcher (Tobita et al. 2013) carried out another study, this time with autologous ATSCs and a PRP carrier, put together in surgically created class III furcation defects in canines (Beagle dogs). Newly formed PDL fibers were observed in defects treated with ATSCs and PRP, but not in defects treated with PRP alone. No significant differences in the amount new bone and cementum formation were found between the groups. The third study used allogeneic ATSCs in surgically created fenestration defects in F344 and Sprague Dawley rats with a poly lactic-co-glycolic acid(PLGA) carrier. Higher bone formation, greater amount of newly formed cementum and width of newly formed PDL were observed in ATSCs and PLGA group compared to PLGA alone group (Akita et al. 2014).

Induced pluripotent stem cells

3 animals studies go through the application of induced pluripotent stem cells (iPSCs). Systemic and local injections of allogeneic iPS cells and allogeneic iPS cells engineered to overexpress tumor necrosis factor-stimulated Gene 6 (TSG-6) were used to treat an experimental periodontitis, that was established by ligature and inoculation of P. gingivalis around the maxillary 1st molars in Sprague Dawley rats. Inflammation mediators, osteoclasts and bone loss were decreased in the animals treated with modified and unmodified iPS cells compared to no treatment group. The amounts of reduction in proinflammatory mediators were significantly higher in TSG-6 iPS cells (Yang et al. 2014).

Allogeneic iPS cells and xenogeneic iPS cells were also combined with apatite coated silk fibroin scaffolds and enamel matrix derivatives (EMD). After treatment of surgically created fenestration defects in immunodeficient mice, significantly greater amounts of new bone and cementum formation were seen when defects were treated with the combination of cells, scaffold and EMD versus treating the defects with scaffold alone or scaffold and EMD (Duan et al. 2011). Xenogeneic iPS cells labeled with BrdU and clotted with 5 μL fibrinogen and 5 μL thrombin were placed in surgically created fenestration defects in immunodeficient rats. Significantly higher area of mineralized tissue formation was observed in the test group and the labeled cells were integrated into the newly formed tissues (Hynes et al. 2013).

Combinations of different stem cells

2 animal studies investigate combinations of different stem cells. Stem cells from the dental follicle (DFSCs) could enhance the function of PDLSCs by providing a beneficial microenvironment. PDLSCs co-cultured with DFSCs produced a typically arranged tissue with Sharpey's-like perpendicular fibers. In immunocompromised mice, a root/periodontal ligament-like complex and a periodontal ligament/bone-like complex were observed (Liu et al. 2014). Collagen sponges, embedded with cementum-derived cells (CDCs) and PDLSCs, were placed in experimentally created periodontal intrabony defects in canines (beagle dogs). The cellular therapy promoted the periodontal regeneration and resulted in a higher amount of new cementum and a larger quantity of new connective tissue (Nuñez et al. 2012).

Human studies

8 human studies have investigated the application of different stem cells in the oral cavity.

DPSCs demonstrated that as a bio complex with a collagen sponge can be used to repair bone defects in

humans. Autografts produced rapid bone regeneration, which was of optimum quality and quantity compared to standard techniques for guided regeneration (D'Aquino et al. 2009). DPSCs also managed to regenerate the infrabony defect on the mandibular right second premolar in humans and it was filled with bonelike tissue, as confirmed through the reentry procedure (Aimetti et al. 2014).

Application of PDLSCs in humans illustrated the utility of autologous progenitor cell transplantation in tissue repair. A human study with 3 patients investigated the autologous periodontal ligament progenitor cells (PDLPs) and the PDLSCs (from extracted 3rd molars). 16 teeth with at least one deep intrabony defect were treated. PDLPs were analogous to PDLSCs in terms of high proliferation, expression of mesenchymal surface molecules, multipotent differentiation, and in vivo tissue regain. Clinical examination indicated that transplantation of PDLPs may provide therapeutic benefit for the periodontal defects. All treated patients showed no adverse effects during the follow up. The results supported a potential efficacy and safety of utilizing autologous PDL cells in the treatment of human periodontitis (Feng et al. 2010). The safety of autologous transplantation of PDLSCs and its effectiveness as adjuvant to graft materials in the repair of bone defects caused by periodontitis was examined in another study in humans (Chen et al. 2016). The use of stem cells did not produce adverse effects and was effective at repairing bone defects.

BMSCs have also been tried successfully in humans, combined with a HA/TCP carrier in sinus augmentation, achieving 41.34% new bone formation but without any control group to compare to (Shayesteh et al. 2008). Combined with allograft, again in sinus augmentation in humans, high percentage of vital bone content, after a relatively short healing phase, was achieved. Again, unfortunately, there was no control group to compare to (McAllister et al. 2009).

Autologous BMSCs taken from the iliac crest with a PRP carrier were placed in angular interproximal defects, where the highest probing pocket depth (PPD) reduction and clinical attachment level gain were 4mm after 1 year (Yamada et al. 2006). No statistical analysis was possible, since it was a case report with one patient. The same main researcher, this time with 17 patients, treated angular interproximal defects with autologous BMSCs (from iliac crest) with a PRP carrier. The treatment resulted in mean PPD reduction of 5.12± 2.45 mm, clinical attachment level gain of 4.29 ± 1.32 mm, and radiographic bone height gain of 3.12 ± 1.23. No side effects were observed (Yamada et al. 2013).

Discussion

The use of pluripotent stem cells appears far from feasible at the moment. The embryonic stem cells propagate uncontrollably and raise ethical issues regarding their usage. The induced pluripotent stem cells seem promising but even so, the results of their limited in numbers animal trials pale in comparison to the results of the multipotent stem cells. However, the research on these two categories remains important since the theoretical capabilities of ESCs and iPSCs largely exceeds those of regular adult stem cells. Regarding the multipotent stem cells, there is the possibility of placing autologous or allogeneic stem cells. A major challenge of non-autologous stem cell therapy includes graft-versus-host disease. Therefore, autologous transplantation remains the safest approach to the issue. PDLSCs appear to be the most effective stem cell to achieve periodontal regeneration although BMSCS present also good clinical outcomes. Both of them are backed up by a relatively great number of animal studies

and promising early results in human applications. Because of that, it stands to reason that future clinical trials, in animals or even better in humans, will focus on these two categories and probably exclude the rest of the multipotent categories (DPSCs, SHED, SCAPs, ATSCs). The concurrent use of scaffolds/carriers/bioactive molecules provides always a stimulus for much better regeneration, regardless of the stem cell placed in the defect or the species (animal or human) involved. The question is, whether specific scaffolds/carriers/bioactive molecules function better with specific cells or species or in defects of distinct architecture. This question has yet to be answered adequately. Despite that, cell based periodontal regeneration is a fact and provides a safe, reproducible and satisfactory, regarding the amount of regeneration achieved, solution in cases of both small and large defects, thus surpassing the clinical outcomes of GTR and GBR and perhaps replacing them both in the near future.

Conclusions

PDLSCs and BMSCs are the best representatives of the stem cell family. Their results must be further illustrated by prospective clinical trials in humans, progressively in bigger number of subjects, in order to put into practice every possible combination with different scaffolds, bioactive molecules and different stem cells categories since successful combinations provide not cumulatively but exponentially better outcomes. A paradigm (Kramer et al. 2004) suggesting that is the co-culturing of BMSCs with PDLSCs. It resulted in BMSCs which developed periodontal ligament-like characteristics, due to contact-mediated factor both in vitro and in vivo, further suggesting these cells may have the potential to form other periodontal tissues. Also, the co-culturing of BMSCs with PDLSCs led to a significant increase in mesenchymal cell expression of osteocalcin and osteopontin and a decrease in bone sialoprotein characteristic of periodontal ligaments in vivo. Additionally, pluripotent stem cells must not be marginalized. A better knowledge of the underlying biochemical pathways could lead to steadier control of their proliferation rate and predictable regeneration of periodontal tissues. Another issue to be dealt with is the discovery of simpler and costeffective isolation and culturing procedures, thus providing a cell based periodontal regeneration, accessible to every patient.

List of abbreviations

Abbreviation	Full name of abbreviation
ATSC	Adipose tissue derived stem cell
BIC	Bone implant contact
BMSC	Bone marrow mesenchymal stem cell
BOP	Bleeding on probing
BrdU	Bromodeoxyuridine
CAL	Clinical attachment loss
CDC	Cementum-derived cell
DFSC	Stem cell from the dental follicle
DPSC	Dental pulp stem cell
EMD	Enamel matrix derivative
ESC	Embryonic stem cell
FHA	Fluorohydroxyapatite
GBR	Guided bone regeneration
GFP	Green fluorescent protein
GTR	Guided tissue regeneration
HA	Hydroxyapatite
HGF	Hepatocyte growth factor
IMC	Intrafibrillarly mineralized collagen
iPSC	Induced pluripotent stem cell
LPS	Lipopolysaccharides
MCP	Monolayer PDLSCs pellet
MCS	Monolayered PDLSCs cell sheet

MSC	Mesenchymal stem cell
MUCP	Multilayered PDLSCs pellet
PDGF	Platelet derived growth factor
PDL	Periodontal ligament
PDLC	Periodontal ligament cell
PDLP	Periodontal ligament progenitor cell
PDLSC	Periodontal ligament stem cell
PLGA	Poly Lactic-co-Glycolic Acid
PPD	Pocket probing depth
PRF	Platelet rich fibrin
PRP	Platelet rich plasma
SC	Stem cell
SCAP	Stem cell from dental apical papilla
SHED	Stem cell from human exfoliated deciduous teeth
TCP	Tricalcium phosphate
TSG-6	Tumor Necrosis Factor-stimulated Gene-6

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