Review

Towards a New Concept of Regenerative Endodontics Based on Mesenchymal Stem Cell-Derived Secretomes Products

Luis A. Costa, Noemi Eiro [ID]**, Andrea Vaca and Francisco J. Vizoso ***[ID]

Research Unit, Fundación Hospital de Jove, 161, 33920 Gijón, Spain
* Correspondence: investigacion@hospitaldejove.com; Tel.: +34-98-5320050 (ext. 84216); Fax: +34-98-5315710

Abstract: The teeth, made up of hard and soft tissues, represent complex functioning structures of the oral cavity, which are frequently affected by processes that cause structural damage that can lead to their loss. Currently, replacement therapy such as endodontics or implants, restore structural defects but do not perform any biological function, such as restoring blood and nerve supplies. In the search for alternatives to regenerate the dental pulp, two alternative regenerative endodontic procedures (REP) have been proposed: (I) cell-free REP (based in revascularization and homing induction to remaining dental pulp stem cells (DPSC) and even stem cells from apical papilla (SCAP) and (II) cell-based REP (with exogenous cell transplantation). Regarding the last topic, we show several limitations with these procedures and therefore, we propose a novel regenerative approach in order to revitalize the pulp and thus restore homeostatic functions to the dentin-pulp complex. Due to their multifactorial biological effects, the use of mesenchymal stem cells (MSC)-derived secretome from non-dental sources could be considered as inducers of DPSC and SCAP to completely regenerate the dental pulp. In partial pulp damage, appropriate stimulate DPSC by MSC-derived secretome could contribute to formation and also to restore the vasculature and nerves of the dental pulp.

Keywords: dentinogenesis; conditioned medium; MSC; DPSC; dental pulp; cell-free therapy in odontology; regenerative endodontics

1. Introduction

The oral cavity is a gateway to the human body and the general health of an individual, so the teeth are key structural and functional elements of this cavity. They are essential for the initial processing of food, for effective speech, and are also a very important factor for correct facial aesthetics, as well as for interpersonal and psychosocial health and well-being. Due to their morphology and tissues that compose them, teeth represent functioning structures of the oral cavity with unique characteristics in the human body. In them, a series of complex and dynamic interactions take place between their different cell types: odontoblasts, immune cells, fibroblasts, endothelial cells, nerves, and pulpal stem cells. But the tooth can be affected by a series of pathologies, among which are mainly caries, periodontal disease, trauma or leakage due to therapeutic procedures. These processes, in turn, can lead to a chain of events such as loss of enamel and dentin, infection, pulpitis, pulp necrosis, and ultimately tooth loss.

The loss of teeth can lead to physical and mental trauma, considerably reducing the quality of life of the subject. More than 50% of the population aged 70 and over are partially or totally toothless [1]. With an aging population, it is estimated that by 2030 more than 50% of people will be 65 years or older. Therefore, the problem of tooth loss is expected to increase in the population [2]. Already in 2006, and without taking into account the recent substantial increase in the age of the population, the journal Dental Economics reported that >30 million Americans were missing all of their teeth in one or both jaws [3] which gives an idea of the socio-sanitary and economic dimensions of the problem.

The alternatives usually employed that arise in this health and social situation are replacement therapies, such as endodontics for damaged pulp or implants for missing teeth. Of fact, for example, each year, around 22.3 million endodontic procedures are performed in the USA alone [4]. Considering the limitations of these procedures, in this review we analyse the possibility of regenerative endodontics based on mesenchymal stem cell-derived secretomes products. For this propose, we revised all published report in Pubmed during the last 25 years on the followed key words: regenerative endodontic, dental pulp tissue engineering, mesenchymal stem cells from the oral cavity and cell-free regenerative endodontic. In addition, we proposed a new concept of regenerative endodontic therapy based on products derived from the secretome from mesenchymal stem cells.

2. Dental Pulp Tissue Engineering

The therapy methods currently used to repair pulpal damage and tooth loss are based on replacement materials for restoring structural defects.

In necrotic teeth with immature roots, the possibility of restoring their full functionality is an attractive goal, since the regeneration of lost tissues improves the quality of life of patients [5]. The continuous advances in scientific knowledge have as a practical purpose the development of regenerative therapies of the dental pulp by tissue engineering.

Tissue engineering is an interdisciplinary science that combines engineering and biology principles to generate functional substitutes for damaged tissue [6]. In this field of science, three essential components interact: progenitor cells, inducing biomolecules and a scaffold [7,8]. In the search for a new fully functional dental pulp, various approaches have been developed such as the use of biomaterials, living cells, growth factors and, more recently, mesenchymal stem cell (MSC)-derived products. These new technologies have the potential to change the therapeutic scenario in regenerative dentistry. Therefore, new questions arise about what is the most effective way to apply them to obtain the desired results. For this reason, many studies have focused on the development of personalized scaffolds for pulp tissue engineering, optimizing characteristics and performance [9,10].

A scaffold is basically a natural, synthetic or mixed product that serves as a transport, containment and delivery of bioactive elements to obtain a desired effect [11]. Therefore, it follows that every scaffold must mimic the fundamental characteristics of the native extracellular matrix (ECM) in order to promote and regulate regenerative cellular events [10,12]. Currently, natural biomaterials such as collagen or fibrin are widely used in periodontal guided tissue regeneration, but they are difficult to apply in the reduced endodontic space. For this reason, polymer-based synthetic hydrogels have aroused great interest as custom-made scaffolds suitable for insertion into root canals. As a basic condition within the root canal, a hydrogel must be biocompatible, degraded by the host tissues, and its physical properties and mechanical resistance must match those of the tissue to be regenerated [10,13]. The framework or polymeric network of the hydrogels is customizable and can be obtained, for example, by gas foaming techniques, as well as leaching with salts, to obtain macroporous scaffolds. Whereas, nanofibrous scaffolds have been processed by electrospinning, self-assembly, and phase separation [9,14].

Cell cultures on self-assembling peptide hydrogels have been shown to result in high cell viability [15] and even significantly better results are reported with natural scaffolds [9]. Recent studies suggest that bovine decellularized pulp extracellular matrix could be a promising scaffold in dental pulp regeneration [12].

In addition, given the difficulty of obtaining new vascularization throughout the root canal through the small foramen, there are potential supportive scaffolding strategies for the growth of new dental pulp in the root canals. Thus, for example, there is the possibility of transplanting into the human tooth without pulp a cell-based vascularized construct, a prospective biomaterial as an implant for the developing dental pulp, thus facilitating blood supply and improving pulp regeneration [16]. Scaffolds can also be preloaded with antimicrobials to optimize disinfection as a prerequisite for dental pulp regeneration. An in vivo study using poly (vinyl alcohol)-chitosan nanofiber scaffold with ciprofloxacin and

synthetic cationic peptide IDR-1002, had anti-biofilm activity against *Enterococcus faecalis*, *Staphylococcus aureus*, and a multispecies oral biofilm [17].

To date, research on pulp tissue regeneration through tissue engineering has not yet fully restored biological functions, such as promoting blood vessels and nerve supply. Thus, the ideal would be the implementation of regenerative endodontics procedures (REP), fundamentally based on the fact that the oral cavity is a large source of mesenchymal stem cells (MSC).

3. MSC of the Oral Cavity

The first group of stem cells of buccal origin was isolated from human dental pulp and known by the name of dental pulp stem cells (DPSC) [18,19]. Subsequently, other MSC have been isolated from oral tissues, receiving names according to the source of origin: pulp tissue of exfoliated primary teeth (SHED) [20]; periodontal ligament (PDLSC) [21]; apical papilla of developing teeth (SCAP) [22]; dental follicle (DFSC) [23]; gingiva (GFSC) [24] and buccal fat pad (BFPSC) [25]. The MSC of the tissues that make up the oral cavity have the main function of maintaining its integrity and homeostasis, as well as its repair in case of injury [26] (Figure 1).
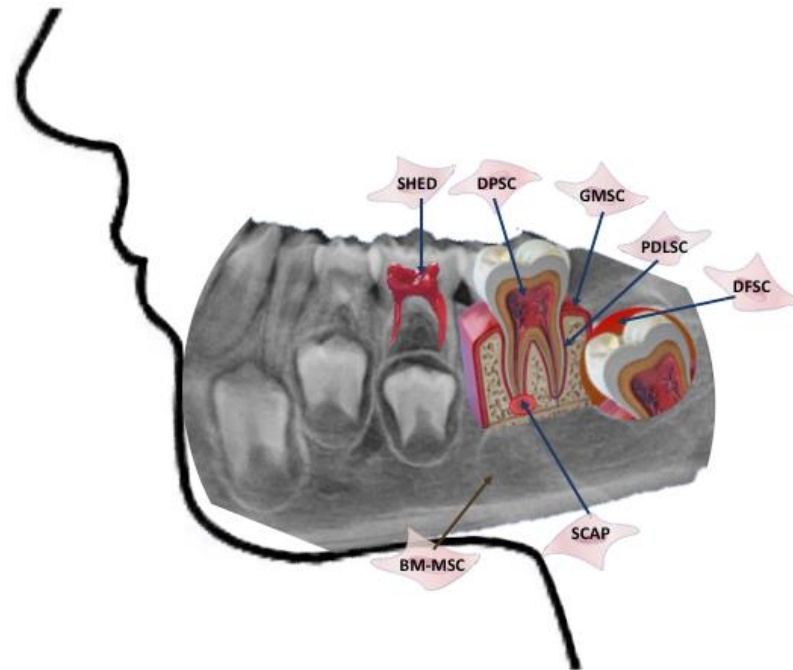

Figure 1. Sources of MSC from oral cavity. SHED: stem cells from human exfoliated deciduous teeth; DPSC: dental pulp stem cells; GMSC: gingiva-derived mesenchymal stem cells; PDLSC: periodontal ligament stem cells; DFSC: dental follicle stem cells; BM-MSC: bone marrow mesenchymal stem cells; SCAP: stem cells from apical papilla.

Postnatal Dental Pulp Stem Cells

Teeth are considered a possible source of MSC, due to their relatively easy access and even with reports of isolation of pulps affected by caries [27], pulpitis [28–30], and third molars [31,32]. In addition, due to the unique ability to regenerate dental tissue, DPSC have been of great interest in various medical applications due to their characteristics. There are reports indicating that DPSC maintain their genomic stability even after multiple passages in vitro [33]. This cell express markers typical of MSC with proliferative and differentiation capabilities across multiple lineages, even when they come from inflamed pulp. Although it was observed that in such circumstances they seem to be dysfunctional, since they present a decrease in immunomodulatory capacity and in their osteogenic or dentinogenic potential [34,35].

DPSC represent a very small group of cells located generally in the central zone of the pulp, whose number decreases as the individual ages [36,37]. Although there is no consensus in the literature about the methods of isolation and expansion of DPSC. The most widely used methods for obtaining DPSC are (i) from pulp of exfoliated primary teeth or extracted permanent teeth in a sterile environment, or (ii) performing a pulpotomy preserving the tooth. The most widely used obtaining method for DPSC is from pulp tissue of extracted third molars [31,38]. Pulpal MSC present a fibroblast-like morphology under the microscope and they express the typical surface molecules that characterize MSC [19,39,40].

In cultures, DPSC showed a high level of colony formation and proliferative capacity, including differentiating into various cell types. Particularly, DPSC have the potential to osteogenic differentiation [41], and to differentiate into dentin-forming odontoblasts [42] as well as neurogenic capability, perhaps due to their neural crest origin [43]. When DPSC were co-cultured with trigeminal neurons, high levels of neurotrophin expression demonstrated the neurogenic potential of these cells [44,45]. Interestingly, it has been showed an enhanced effect of ascorbic acid on differentiation, secretome and stemness of DPSC [46]. On the other hand, similarly to other MSC, DPSC exhibit immunomodulatory activity [19,47] by releasing cytokines and growth factors with paracrine action into their environment. They carry out paracrine action both in immunoregulatory functions and in the regeneration of pulp damaged tissues, which makes them a therapeutic strategy to consider in the context of cell therapies [48–52].

4. Types of Regenerative Endodontics Procedures (REP)

On the basis of the presence of DPSC in dental pulp, there are two alternatives to REP: cell-based REP and cell-free REP (without exogenous cell transplantation).

4.1. Cell-Based REP

Cell-based REP consists of the implantation of autologous or exogenous DPSC inside the root canal, so that they proliferate and differentiate in the different cellular phenotypes of the dental pulp [53]. In dentistry, cell-based REP using exogenous DPSC or SHED have been considered a good alternative, which combined with a custom scaffold, the result is the regeneration of pulp-like tissues [32]. Preclinical studies allow translation from the laboratory to the living being, for a better understanding of the interaction of the host with the scaffolds and their biological content. In vivo studies can be based on ectopic, semi-orthotopic and orthotopic approaches, which have their advantages and disadvantages. Orthotopic regenerative studies with procedures similar to those used in humans but using animal teeth are the most relevant, since they allow evaluating the safety and efficacy of the new approach, and provide information on the changes or adjustments necessary for future clinical protocols. [54,55]

A number of case reports suggest that DPSC can be used for the treatment of intra-bone defects. In these patients with bone defects caused by periodontal disease, collagen sponges and autologous/allogeneic DPSC have been applied through minimally invasive surgeries. The results showed decreased probing depth, new fiber reinsertion, and new bone formation with few adverse effects, indicating that DPSC have regenerative potential for periodontal tissue [56,57]. DPSC have the potential for neurogenic, angiogenic, and odontoblastic differentiation and are therefore considered the main contributor to complete pulp regeneration. In addition, some studies have shown that transplantation of cultured stem cells is effective in the regeneration of the dentin-pulp complex. Studies in vivo, demonstrate that it has been possible to form a tissue similar to dentin by means of DPSC and hydroxyapatite/tricalcium phosphate (HA/TCP) scaffolding, presenting a layer of cells similar to odontoblasts that expressed dentin sialophosphoprotein (DSPP), a specific marker related to with differentiation into odontoblasts [58,59].

The first report on regeneration of the dentin-pulp complex in large animals was made by Iohara et al. [29] who got it by implanting canine pulp-derived stem/progenitor cells into pulpotomized teeth in dogs. The same group reports complete pulpal regeneration

after pulpectomy in dogs by autologous transplantation of a combination of CD105 (+) pulpal stem cells with stromal cell-derived factor-1 [60].

In in vitro and in vivo studies in animal models, showed that SHED combined with a self-assembly peptide scaffold like Puramatrix™ (BD Biosciences, Bedford, MA, USA) or Collagen type I (human recombinant BD™ Fibrogen Collagen Type I; BD Biosciences) presented similar cellularity and vascularization when compared with control human dental pulps [61].

A pilot clinical study in five patients with irreversible post-traumatic pulpitis in permanent incisors, G-CSF-pretreated DPSC were implanted into the empty root canal, observing in 3 patients a vascularized and neural reconstruction of the pulp tissue with new dentin formation at 24 months of follow up [30]. In a randomized clinical trial in teeth with necrotic pulps, they obtained complete regeneration of all tissues of the dental pulp, with connective tissue, blood vessels, odontoblasts arranged in layer and neuronal markers after transplantation of DPSC in situ [62]. Nevertheless, there are relatively few studies reporting the safety use of MSC. Recently was informed the regenerative potential of the pulp tissue and the safety of DPSC transplantation in pulpectomized teeth in dogs [63] and clinical trials [64], without showing toxicity or subsequent adverse events. Other studies indicate the efficacy and safety of MSC from other origins, such as allogenic umbilical cord MSC which have been proved in a preclinical trials [53,65]. However, it is likely that DPSC were not good candidates for periodontal regeneration due to their limited ability to form cementum [66,67].

On the other hand, we also have to mention the possibility of obtaining cells and odontoblastic potential through other origins. Thus, it is of interest the finding that BMP-4 and FGF-8, induces human embryonic stem cell differentiation into odontoblastic lineage [68]. In addition, the induced pluripotent stem cells (iPSC) have capability for indefinite self-renewal and large-scale expansion. In vitro and in vivo studies using iPSC from DPSC with proper stimulation can differentiate into fully functional odontoblasts [69]. In fact, it has been shown that iPSC can be used for test the bioactivity of materials and other regenerative therapies including the treatment of dentin and/or dental pulp damage [69,70].

Limitations of the Cell-Based REP

There are several drawbacks in relation to stem cell-based REP, which make it difficult to transfer this technology to daily clinical practice.

The main drawback comes from the isolation and expansion of the cells, since healthy teeth must be provided by donors or, it is necessary to store them in a cell bank [71]. Furthermore, obtaining enough MSC in 2D or 3D cultures for regenerative treatments is time-consuming, expensive, and usually requires a specialized laboratory [54,72]. To this fact it is necessary to highlight recent reports that implanted MSC do not survive long [73–75]. Evidence demonstrates that MSC transplantation results in a low rate of engraftment at the target site. Inflamed tissues are a hostile environment that reduces the survival of MSC to a half-life of 24 h [76]. As an example, injection of SHED into a bone defect due to periodontitis diffused slightly for 3 days post-grafting and then decreased rapidly [77]. It follows that the injection of a high number of MSC is not related to the beneficial results observed [78].

4.2. Cell-Free REP

There are alternatives for REP by using cell-free strategies, such as based on either blood or MSC derived products.

4.2.1. Cell-Free REP Based on Blood Derived Products, Bioactive Molecules or Bioingenniering Materials

Within cell-free REP strategies there are two therapeutic procedures such as pulpal revascularization and active induction of local cells. The first evidence of attempts to regenerate pulpal tissue dates back to the 1960s by Nygaard-Ostby [79], whose research in

animal models and in humans describes an alternative treatment for the regeneration of dental pulp in permanent teeth. This consists in a certain way similar to what we currently known as revascularization, through a blood clot inside the root canal. Subsequently, alternative scaffolds have been proposed to replace the blood clot, such as autologous platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) and take advantage of its regenerative potential [80] to resolve apical periodontitis and complete root development [81,82]. A REP standard protocol was proposed by the American Association of Endodontics (AAE) in 2016 [83]. This strategy includes a multi-step procedure: control of the infection of the affected tooth, stimulation of bleeding of the periapical tissues by gentle irritation with a pre-curved K-file passing 1–2 mm through the apex, and to perform a blood clot in the root canal, which serves as a suitable scaffold for the growth of new tissue. Subsequently, a biomaterial is placed in contact with the blood clot at the cervical level of the root, usually with mineral trioxide aggregate (MTA), followed by the permanent coronal seal to avoid bacterial reinfection [84,85].

Within the cell-free approach, it has been proposed to regenerate pulp-like tissue by means of molecules that induce the recruitment of the patient's endogenous MSC into the root canal [86,87]. It is believed that this strategy without transplantation of exogenous cells is capable of achieving regeneration of the dentin-pulp complex more effectively than simple revascularization. For this purpose, in preclinical studies several molecules have been used as homing factors, including VEGF, basic fibroblast growth factor (FGF), bone morphogenetic protein-7 (BMP-7), platelet-derived growth factor (PGF), and nerve growth factor (NGF) showing promising results [88]. As a result of these investigations, it can be inferred that cell-free REP show a therapeutic effect on necrotic immature teeth. It has been shown that MSC expressing CD105, CD73 and STRO1 migrate in large numbers into the empty root canal by periapical bleeding [80]. In these studies of necrotic immature permanent teeth treated with the revascularization technique, the MSC found within the canal were expected to come from the adjacent apical papilla, but MSC from other surrounding tissues are probably present. In fact, the newly formed tissues were cementum-like and bone-like, which were confirmed by immunohistochemical staining. Therefore, it is presumed that MSC from the all periapical tissues also entered into the root canal and were co-responsible for the formation of these new mineralized tissues during root maturation [89,90].

There are also several materials that can be used to induce odontoblastic differentiation. Of fact, for now, the gold standard material for vital pulp capping or apexification is MTA, first introduced to the dental market in 1998 [91]. It is also of note that graphene induced osteogenic differentiation of DPSC without the use of chemical inducers for osteogenesis [92]. In addition, in order to promote bone regeneration, growth factors with osteoinductive activity have been used, such as bone morphogenic protein (BMP)-2, embedded in various scaffolds or osteoconductive materials [93,94]. However, there are several issues with the use of some of these factors for tissue regeneration. Thus, for example, recent studies on bone regeneration through the clinical application of BMP-2, have described unexpected side effects [95], such as the induction of a severe inflammatory response, probably due to a higher dose than necessary [96–98].

In some case reports it is suggested that cell-free REP based could eliminate apical periodontitis and even revitalize immature necrotic teeth, but their results still inconsistent. When the dental pulp, apical papilla, or follicle are severely damaged, there are no MSC to differentiate into odontoblasts and promote dentin formation, resulting in the interruption of dental root development. True regeneration of the dentin-pulp complex is rarely seen or detected in the root canal [99]. Hence, there is great interest in developing new strategies to achieve pulp regeneration and return its organized structure to the dentin-pulp complex with original homeostatic functions.

4.2.2. A New Concept of Regenerative Endodontics Based on Secretome-Derived Products from Mesenchymal Stem Cells

Accumulated knowledge reveals that soluble factors with paracrine action secreted by MSC are responsible for the observed beneficial effects. Biological products are present in the secretome of the MSC, which contain a large number of bioactive proteins, such as growth factors and cytokines, as well as extracellular vesicles (EV). EV represent an important component of the MSC secretome that has aroused great interest as a true option for therapeutic applications. EV can be categorized as (i) exosomes (30–120 nm in diameter), that come from intracellular multivesicular bodies (MVB) and are released by the fusion of MVB with the plasma membrane; (ii) microparticles (150–1000 nm in diameter), that originate from blebs that emerge from the plasma membrane and are released into the external environment by proteolytic cleavage of the cytoskeleton; and (iii) apoptotic bodies (500–2000 nm in diameter), originating during the process of programmed cell death. Among these microparticles, exosomes aroused great general interest for their role in cell biology and their potential therapeutic and diagnostic applications. Exosomes are secreted by cells to facilitate intercellular communication. This is carried out through their components, such as lipids, several types of RNA, cytokines, chemokines, integrins (CD81, CD63 and CD9), as well as signal transduction factors, transport proteins (annexins and Rab GTPases), cytoskeleton proteins and metabolic enzymes. After the discovery of their role in the transfer of mRNA, miRNA and active factors, exosomes represent a powerful intercellular communication pathway in organ homeostasis and disease. These mechanisms can be achieved by binding to target cell surface receptors that activate signalling cascades, internalization of cell surface-bound exosomes, and direct fusion with the target cell to release its cargo to the cytoplasmic membrane and cytosol [100].

MSC-derived secretomes, obtained from culture conditions of MSC and named conditioned medium (CM), can be considered as promising candidates for direct medical biotechnology, due to their anti-inflammatory, angiogenic, anti-oxidative stress, anti-apoptotic and regenerative activity [98,100,101] including for REP (Figure 2).

This cell-free strategy would avoid the drawbacks derived from the graft of proliferating stem cells, among which the following stand out: (i) immunological incompatibility, tumorigenicity, formation of emboli, transmission of infections and potentially entry into senescence or apoptosis; (ii) It is possible to better control the biosafety, dose and potency of a secretome, similar to conventional therapeutic agents; (iii) the lyophilized secretome can be stored without the addition of potentially toxic preservative substances; and (iv) using secretome-derived products, such as conditioned medium or exosomes, is more cost-effective and suitable for clinical use, as it would be "ready to use". This is related to the fact that a secretome could be available for treatment when needed, being a product prepared in advance [72].

Previous studies using MSC conditioned medium (MSC-CM) have been shown to be safe with less inflammatory signs and appear to have great osteogenic potential for alveolar bone regeneration [102] and for periodontal tissue regeneration [103]. Several studies also revealed that MSC-CM contains diverse cytokines, such as IGF-1, VEGF, TGF-β1, and HGF, that enhances early bone and periodontal tissue regeneration [104–107]. The growth factor IGF-1 is known to induce osteoblast proliferation and migration [108,109] and stimulate periodontal ligament (PDL) cells through the Pi3 K pathway, enhancing periodontal tissue regeneration [110]. In addition to enhancing endothelial cell survival and differentiation, VEGF is also believed to be involved in the process of osteogenesis [111]. There is another factor that has a direct effect on angiogenesis, such as HGF and TGF-β1 [112]. Among the main functions of TGF- β1 are that of increasing the migration of osteoprogenitor cells, regulating cell differentiation and proliferation, and the production of extracellular matrix [113]. It has been detected that TGF- β1 is expressed during the development of alveolar bone and cement, stimulating the regeneration and repair of the PDL (112) [114].
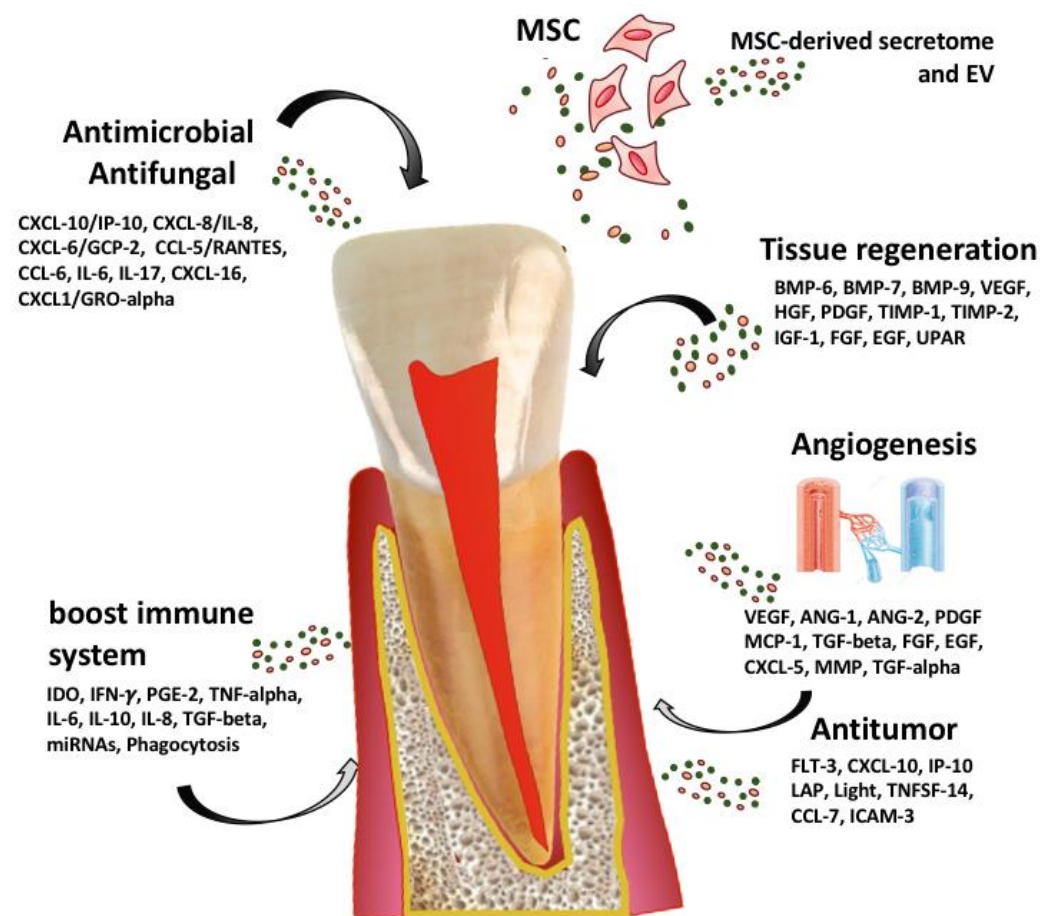
Figure 2. Potential functions and applications of MSC-derived secretome and extracellular vesicles (EV).

All this is especially interesting if we take into account that a previous studies showed that the combination of several growth factors would have an additive effect on cell migration and osteogenic differentiation [115,116].

The anti-inflammatory effect can be especially interesting to control inflammation in the context of bone regeneration. In fact, it was shown that only a few inflammatory cells were detected at the site where MSC-CM was placed in an allogeneic animal study on calvarial bone defects [104,105] or periodontal tissue regeneration [106,107].

Interestingly, there are data indicating the regenerative interest of the DPSC secretome. An early report indicates that direct co-culture of DPSC and endothelial cells enhances the in vitro differentiation toward osteogenic-/odontogenic and angiogenic phenotypes, which suggests that those oral stem cells display paracrine mechanisms [117]. More recent in vivo data indicate that DPSC condition medium (DPSC-CM) could efficiently prevent neurodegeneration, neuroinflammation and enhance hippocampal neurogenesis in a mouse model of hippocampal neurodegeneration [118]. In addition, DPSC secretome had higher expression of the cytokines like GCSF, IFNγ, and TGFβ that promote neural differentiation [119].

Apart from the potential beneficial effects on pulp regeneration of the high concentrations of soluble factors in MSC-CM, a large part of these effects may be due to extracellular vesicles (EV) and, especially, exosomes that have been detected in appreciable quantities in those biological media. Experimental studies reveal that exosomes play an emerging role in a wide range of pathologies [47] and even trigger the increased expression of genes required for odontogenic differentiation and regeneration of dental pulp-like tissue [120]. In relation to oral diseases, there is increasing evidence suggesting that exosomes act as a fundamental regulator of homeostasis and the immune response. Therefore, a new therapeutic approach through the application of exosomes derived from oral MSC could represent a great ad-

vance in the treatment of different oral pathologies, such as osteonecrosis of the jaw (ONJ), periodontal disease and oral cancer [121–123].

In chronic periodontitis, the mechanism of local inflammation and its relationship with systemic conditions is complex, in which exosomes play a key role. In peripheral blood tests of patients with this pathology, an imbalance of T-helper17 (Th17)/T-regulatory (Treg) cells had been detected. PDLSC-derived exosomes from healthy tissue alleviated the inflammatory microenvironment through the Th17/Treg/miR-155-5p/SIRT1 regulatory network. Consequently, in the face of periodontal disease, PDLSC-exosomes make a fundamental contribution to the maintenance of tissue homeostasis [124]. Moreover, during periodontal inflammation PDLSC-exosomes are involved in the regulation of bone remodelling [125]. Anti-inflammatory activity has been reported of PDLSC-derived exosomes during their interaction with macrophages [126].

Regarding SHED-derived exosomes, their effectiveness in stimulating the osteogenic capacity of PDLSCs was observed by activating Wnt/-catenin and BMP signalling pathways [127]. In a mouse model of induced acute lung injury, SHED-exosomes also upregulated the immune response with anti-inflammatory effects [128]. Derived exosomes of the same cellular origin cultured under odontogenic differentiation conditions promoted regeneration of dental pulp-like tissue using both DPSC and bone marrow stem cells in a dental root fragment model, with increased expression of DMP1 and DSPP [120]. GMSC-derived EV have anti-inflammatory potential by downregulating TNF-α through the significant production of interleukin-1 receptor antagonist, during gingival and cutaneous wound healing in mice [129].

Oxidative stress is a condition capable of stimulating inflammation and inducing various oral pathologies, such as periodontal disease. One study indicates that GMSC-derived exosomes reduce entry into cellular senescence of inflamed tissues [130]. In addition, GMSC exosomes promoted wound healing in a diabetic mouse study by stimulating collagen remodelling, angiogenesis, and re-epithelialization [131].

All these data on the biological effects of exosomes may be relevant if we consider the potential advantages of these EV. They are comparatively smaller, their membrane is less complex and contains lower levels of membrane-bound proteins, so they develop less immunogenicity than their parent cells, and their production and storage is also easier than with parent cells.

Recent studies show similar potential beneficial effects of both MSC-CM and EV for pulp regeneration. It has been shows that CM from human DPSC induce osteoblast differentiation in vitro [132], and it was effective in vivo in controlling pulp tissue inflammation and improve dentin bridge formation and its morphological quality [133]. With regard to exosomes derived from human MSC or DPSC, it was proposed their therapeutic effects on the basis of their angiogenic [134,135] and neuroprotective function [120]. The alternative use of CM or Exosomes has advantages and disadvantages. The production of an adequate amount of MSC-derived exosomes in cell cultures is complex, as is their isolation and purufication despite the various technical options for it (centrifugation, ultracentrifugation or size exclusion chromatography) [92]. However, these microvesicles are better defined products with a view to approval by regulatory agencies for their therapeutic use. Even so, future comparative in vivo studies will be necessary in order to select the most powerful and suitable derivative of the MSC secretome for pulp regeneration.

Other possible interest of MSC-CM is in the field of scaffolding. Excitingly, US FDA approved a xenogeneic extracellular matrix (ECM) scaffold for human therapeutic procedures [136], so the biomimetic tooth bud model could be a hopeful treatment for the goal of dental regeneration [137]. Scaffolds preloaded with MSC derivatives be able to stimulate host tissue stem cells to migrate and differentiate thereby contributing to tissue regeneration [138]. Thus, engineering for scaffolds development is constantly evolving in order to provide effective tools for the regeneration of dental tissues. A key factor in the development of preloaded scaffolds is the careful selection of growth factors and cytokines capable of promoting the chemotactic and proliferative activity of host MSC [139,140]. On

the other hand, there are many studies focused on the development of optimal material characteristics, such as tailor-made scaffolds for therapeutic applications in dental pulp engineering [9,10,141–143]. In cases where part of the dental pulp was intact and vital, adequate induction would result in the mobilization of progenitor cells from it onto a scaffolding material to harbor, proliferate, and differentiate [144]. In a recent review, the method of using scaffolds for odontoblast induction appears to be more convenient than without it [145].

It is widely recognized that, when possible, the best option is the conservation of the tooth with its proprioception versus rehabilitation with dental implants. The exquisite tactile sensitivity of natural teeth, such as biting with the incisors or limiting excessive chewing loads, cannot be replaced by osteointegrated implants [146]. In addition, the tooth has the periodontium that exerts a protective and defensive function, such as the junctional epithelium and the perpendicular insertion of Sharpey's fibers from the bone to the root cement. These structures are absent in the implant, since on their surface there is only a longitudinal organization of collagen fibers in the area close to the abutment/implant interface, thus being less resistant to microbial aggression [147].

In this sense, we can consider that, starting from the concept that nothing can overcome nature itself, we propose a new strategy for regeneration of the dental pulp, recovering all anatomical characteristics and functions of the original pulp. This involves new blood supply, innervation and all cell types including dentinogenic ones [148]. This proposed strategy is based on the theory that DPSC are residents close to the perivascular niches in the postnatal dental pulp [27,149] or the SCAP from apical papilla [150] and could be induced by MSC-CM or EV to increase their regenerative potential (Figure 3). In addition, we added a propose on the application of these MSC-derived products into root canals. Of fact, there is the possibility to combine the MSC secretome-derived products with new technologies. Hydrogels are employed due to their biocompatibility, similarity to the extracellular matrix, permeability for oxygen, small molecules and nutrients [151,152], converting them into optimal carriers of MSC secretome to regenerate dental pulp.

On the other hand, furthermore, soluble factors of potential interest in pulpal regeneration have been identified. Thus, it was showed the capacity of DPSC and platelet-rich plasma (PRP) to regenerate dental pulp in canine mature permanent teeth [153]. Recently, hypoxia-inducible factor-1α (HIF-1α) has been found to exert a protective effect on implanted SHED in relation to their survival and angiogenic activity, by regulating reactive oxygen species homeostasis and glycolysis-related gene expression as factors essential in the early post-implantation stages [154,155].
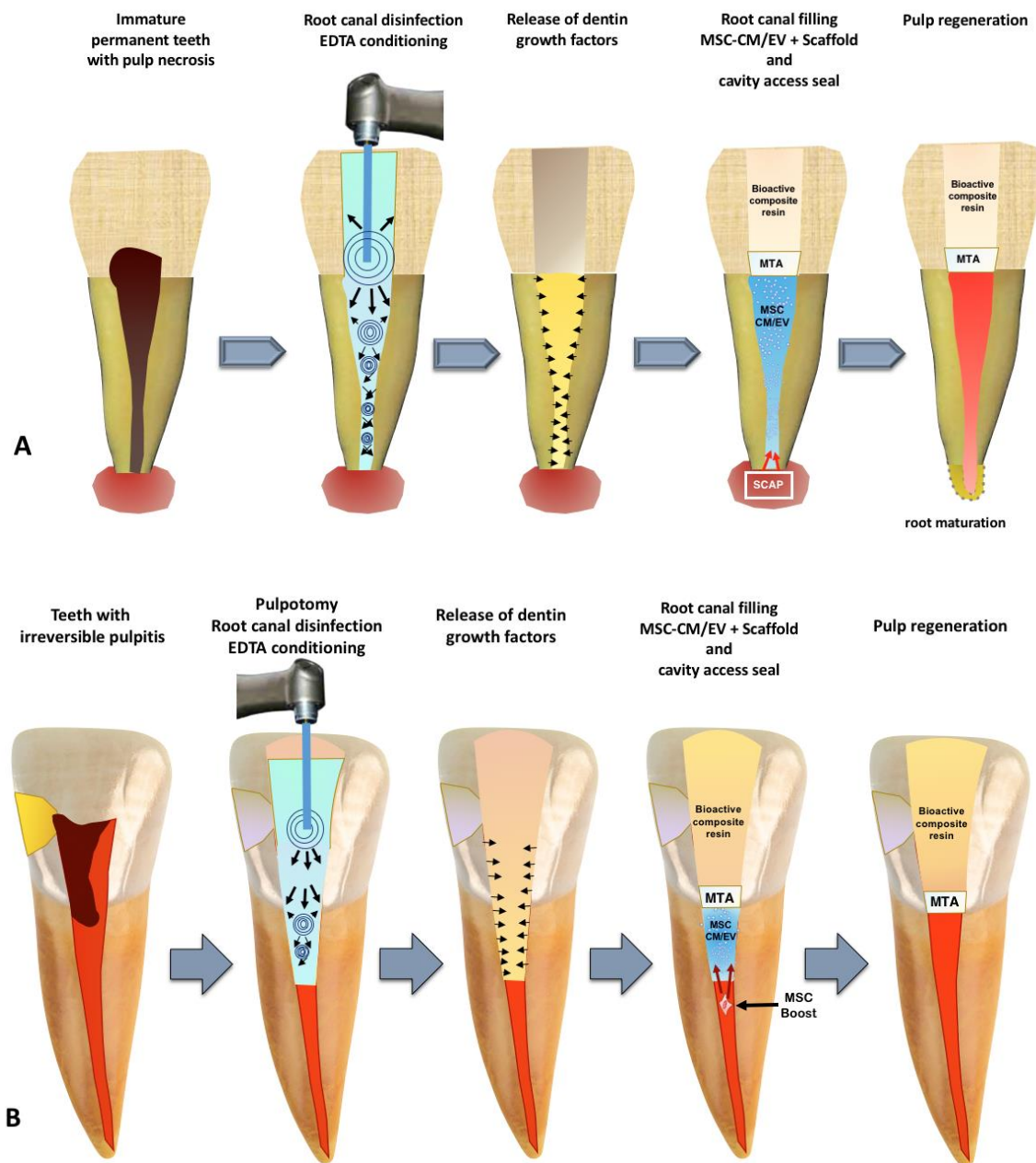
Figure 3. Proposal for a cell-free approach for dental pulp regeneration by using MSC-derived CM/EV. In (**A**) the treatment sequence for immature permanent teeth with pulp necrosis is outlined. In (**B**) the regenerative procedure for cases with partially damaged pulp is summarized. MTA: mineral trioxide aggregate; SCAP: stem cells from apical papilla; EDTA: Ethylenediaminetetraacetic acid.

5. Conclusions and Future Perspectives

The use of MSC-derived secretome, such as CM or EV, instead of cell therapy can promote the stimulation of proliferation and differentiation of MSC from dental pulp towards odontoblasts, blood vessels and nerves. This concept may represent the basis for a new regenerative endodontics strategy. For this purpose, and due to their multifactorial biological effects, MSC-derived secretomes might be considered from non-dental sources. In addition to more often used sources such as adipose tissue, bone marrow or UC, other secretomes sources are having interest. Thus, for example, both human GMSC [156–159], or uterine cervical-MSC (hUCESC) [160–162] secretomes may be good candidates as regenerative inducers in cell-free REP due their potent regenerative and anti-inflammatory biological effects. Besides, hUCESC are easy to isolate and culture, and proliferative faster than other

MSC, such as bone marrow mesenchymal stem cells (BM-MSC) [163] or adipose derived stem cells (AD-MSC) [164]. Nevertheless, further studies should be carried out to explore this hypothesis, as well as to develop methods directed to adequate clinical applications of MSC-derived secretomes, such as their well standardized and mass production and in vitro in bioreactors, as well as the use of functional assays to test the obtained biological products and appropriate experimental models to explore the mechanisms [165].

Author Contributions: L.A.C., N.E. and A.V. analysed the bibliography and prepared the tables and figures. L.A.C. and F.J.V. designed the project and wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Kailembo, A.; Preet, R.; Stewart Williams, J. Common risk factors and edentulism in adults, aged 50 years and over, in China, Ghana, India and South Africa: Results from the WHO Study on global AGEing and adult health (SAGE). *BMC Oral Health* **2016**, *17*, 29. [CrossRef] [PubMed]
2. Schwendicke, F.; Nitschke, I.; Stark, H.; Micheelis, W.; Jordan, R.A. Epidemiological trends, predictive factors, and projection of tooth loss in Germany 1997–2030: Part II. Edentulism in seniors. *Clin. Oral Investig.* **2020**, *24*, 3997–4003. [CrossRef] [PubMed]
3. Journal of Oral Implantology. AAID: American Academy of Implant Dentistry. Available online: https://www.aaid.com/news_and_publications/Journal_of_Implantology.html (accessed on 10 October 2022).
4. Azimi, E.; Song, T.; Yang, C.; Dianat, O. Endodontic Guided Treatment Using Augmented Reality on a Head-Mounted Display System. *Healthc. Technol. Lett.* **2018**, *5*, 201–207. [CrossRef]
5. Schmalz, G.; Widbiller, M.; Galler, K.M. Clinical Perspectives of Pulp Regeneration. *J. Endod.* **2020**, *46*, S161–S174. [CrossRef] [PubMed]
6. Langer, R.; Vacanti, J.P. Tissue engineering. *Science* **1993**, *260*, 920–926. [CrossRef]
7. Oshima, M.; Mizuno, M.; Imamura, A.; Ogawa, M.; Yasukawa, M.; Yamazaki, H.; Morita, R.; Ikeda, E.; Nakao, K.; Takano-Yamamoto, T.; et al. Functional tooth regeneration using a bioengineered tooth unit as a mature organ replacement regenerative therapy. *PLoS ONE* **2011**, *6*, e21531. [CrossRef]
8. Hashemi-Beni, B.; Khoroushi, M.; Foroughi, M.R.; Karbasi, S.; Khademi, A.A. Tissue engineering: Dentin-pulp complex regeneration approaches (A review). *Tissue Cell* **2017**, *49*, 552–564. [CrossRef]
9. Galler, K.M.; Brandl, F.P.; Kirchhof, S.; Widbiller, M.; Eidt, A.; Buchalla, W.; Göpferich, A.; Schmalz, G. Suitability of Different Natural and Synthetic Biomaterials for Dental Pulp Tissue Engineering. *Tissue Eng. Part A* **2018**, *24*, 234–244. [CrossRef]
10. Fukushima, K.A.; Marques, M.M.; Tedesco, T.K.; Carvalho, G.L.; Gonçalves, F.; Caballero-Flores, H.; Morimoto, S.; Moreira, M.S. Screening of hydrogel-based scaffolds for dental pulp regeneration—A systematic review. *Arch. Oral Biol.* **2019**, *98*, 182–194. [CrossRef]
11. Bottino, M.C.; Pankajakshan, D.; Nör, J.E. Advanced Scaffolds for Dental Pulp and Periodontal Regeneration. *Dent. Clin. N. Am.* **2017**, *61*, 689–711. [CrossRef]
12. Bakhtiar, H.; Pezeshki-Modaress, M.; Kiaipour, Z.; Shafiee, M.; Ellini, M.R.; Mazidi, A.; Rajabi, S.; Zamanlui Benisi, S.; Ostad, S.N.; Galler, K.; et al. Pulp ECM-derived macroporous scaffolds for stimulation of dental-pulp regeneration process. *Dent. Mater. Off. Publ. Acad. Dent. Mater.* **2020**, *36*, 76–87. [CrossRef] [PubMed]
13. Piva, E.; Silva, A.F.; Nör, J.E. Functionalized scaffolds to control dental pulp stem cell fate. *J. Endod.* **2014**, *40*, S33–S40. [CrossRef] [PubMed]
14. Wang, L.; Deng, F.; Wang, W.; Li, A.; Lu, C.; Chen, H.; Wu, G.; Nan, K.; Li, L. Construction of Injectable Self-Healing Macroporous Hydrogels via a Template-Free Method for Tissue Engineering and Drug Delivery. *ACS Appl. Mater. Interfaces* **2018**, *10*, 36721–36732. [CrossRef] [PubMed]
15. Cavalcanti, B.N.; Zeitlin, B.D.; Nör, J.E. A hydrogel scaffold that maintains viability and supports differentiation of dental pulp stem cells. *Dent. Mater. Off. Publ. Acad. Dent. Mater.* **2013**, *29*, 97–102. [CrossRef]
16. Katata, C.; Sasaki, J.I.; Li, A.; Abe, G.L.; Nör, J.E.; Hayashi, M.; Imazato, S. Fabrication of Vascularized DPSC Constructs for Efficient Pulp Regeneration. *J. Dent. Res.* **2021**, *100*, 1351–1358. [CrossRef]
17. da Costa Sousa, M.G.; de Almeida, G.C.; Martins Mota, D.C.; da Costa, R.A.; Dias, S.C.; Limberger, S.N.; Ko, F.; Lin, L.T.; Haney, E.F.; Etayash, H.; et al. Antibiofilm and immunomodulatory resorbable nanofibrous filing for dental pulp regenerative procedures. *Bioact. Mater.* **2022**, *16*, 173–186. [CrossRef]
18. Gronthos, S.; Akintoye, S.O.; Wang, C.Y.; Shi, S. Bone marrow stromal stem cells for tissue engineering. *Periodontol. 2000* **2006**, *41*, 188–195. [CrossRef]

19. Gronthos, S.; Mankani, M.; Brahim, J.; Robey, P.G.; Shi, S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 13625. [CrossRef]

20. Miura, M.; Gronthos, S.; Zhao, M.; Lu, B.; Fisher, L.W.; Robey, P.G.; Shi, S. SHED: Stem cells from human exfoliated deciduous teeth. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 5807–5812. [CrossRef]

21. Seo, B.M.; Miura, M.; Gronthos, S.; Bartold, P.M.; Batouli, S.; Brahim, J.; Young, M.; Robey, P.G.; Wang, C.Y.; Shi, S. Investigation of multipotent postnatal stem cells from human periodontal ligament. *Lancet* **2004**, *364*, 149–155. [CrossRef]

22. Sonoyama, W.; Liu, Y.; Yamaza, T.; Tuan, R.S.; Wang, S.; Shi, S.; Huang, G.T. Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: A pilot study. *J. Endod.* **2008**, *34*, 166–171. [CrossRef]

23. Morsczeck, C.; Götz, W.; Schierholz, J.; Zeilhofer, F.; Kühn, U.; Möhl, C.; Sippel, C.; Hoffmann, K.H. Isolation of precursor cells (PCs) from human dental follicle of wisdom teeth. *Matrix Biol. J. Int. Soc. Matrix Biol.* **2005**, *24*, 155–165. [CrossRef] [PubMed]

24. Mitrano, T.I.; Grob, M.S.; Carrión, F.; Nova-Lamperti, E.; Luz, P.A.; Fierro, F.S.; Quintero, A.; Chaparro, A.; Sanz, A. Culture and characterization of mesenchymal stem cells from human gingival tissue. *J. Periodontol.* **2010**, *81*, 917–925. [CrossRef] [PubMed]

25. Farré-Guasch, E.; Martí-Pagès, C.; Hernández-Alfaro, F.; Klein-Nulend, J.; Casals, N. Buccal Fat Pad, an Oral Access Source of Human Adipose Stem Cells with Potential for Osteochondral Tissue Engineering: An In Vitro Study. *Tissue Eng. Part C Methods* **2010**, *16*, 1083–1094. [CrossRef] [PubMed]

26. Gan, L.; Liu, Y.; Cui, D.; Pan, Y.; Zheng, L.; Wan, M. Dental Tissue-Derived Human Mesenchymal Stem Cells and Their Potential in Therapeutic Application. *Stem Cells Int.* **2020**, *2020*, 8864572. [CrossRef]

27. Shi, S.; Gronthos, S. Perivascular Niche of Postnatal Mesenchymal Stem Cells in Human Bone Marrow and Dental Pulp. *J. Bone Miner. Res.* **2003**, *18*, 696–704. [CrossRef]

28. Feng, J.; Mantesso, A.; Bari, C.D.; Nishiyama, A.; Sharpe, P.T. Dual origin of mesenchymal stem cells contributing to organ growth and repair. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 6503–6508. [CrossRef]

29. Iohara, K.; Zheng, L.; Ito, M.; Ishizaka, R.; Nakamura, H.; Into, T.; Matsushita, K.; Nakashima, M. Regeneration of dental pulp after pulpotomy by transplantation of CD31-/CD146- side population cells from a canine tooth. *Regen. Med.* **2009**, *4*, 377–385. [CrossRef]

30. Nakashima, M.; Iohara, K.; Murakami, M.; Nakamura, H.; Sato, Y.; Ariji, Y.; Matsushita, K. Pulp regeneration by transplantation of dental pulp stem cells in pulpitis: A pilot clinical study. *Stem Cell Res. Ther.* **2017**, *8*, 61. [CrossRef]

31. Vendramini, V.O.; Pouraghaei, S.; Barbosa, R.M.; Aloise, A.C.; Muniz, J.R.F.; Sperandio, M.; Moy, P.K.; Pelegrine, A.A.; Moshaverinia, A. Influence of Dental Pulp Harvesting Method on the Viability and Differentiation Capacity of Adult Dental Pulp-Derived Mesenchymal Stem Cells. *Stem Cells Int.* **2021**, *2021*, 1–8. [CrossRef]

32. Huang, G.T.J.; Yamaza, T.; Shea, L.D.; Djouad, F.; Kuhn, N.Z.; Tuan, R.S.; Shi, S. Stem/Progenitor Cell–Mediated De Novo Regeneration of Dental Pulp with Newly Deposited Continuous Layer of Dentin in an In Vivo Model. *Tissue Eng. Part A* **2010**, *16*, 605–615. [CrossRef]

33. Huang, G.T.; Gronthos, S.; Shi, S. Mesenchymal stem cells derived from dental tissues vs. those from other sources: Their biology and role in regenerative medicine. *J. Dent. Res.* **2009**, *88*, 792–806. [CrossRef] [PubMed]

34. Alongi, D.J.; Yamaza, T.; Song, Y.; Fouad, A.F.; Romberg, E.E.; Shi, S.; Tuan, R.S.; Huang, G.T. Stem/progenitor cells from inflamed human dental pulp retain tissue regeneration potential. *Regen. Med.* **2010**, *5*, 617–631. [CrossRef]

35. Yazid, F.B.; Gnanasegaran, N.; Kunasekaran, W.; Govindasamy, V.; Musa, S. Comparison of immunodulatory properties of dental pulp stem cells derived from healthy and inflamed teeth. *Clin. Oral Investig.* **2014**, *18*, 2103–2112. [CrossRef]

36. Iohara, K.; Murakami, M.; Nakata, K.; Nakashima, M. Age-dependent decline in dental pulp regeneration after pulpectomy in dogs. *Exp. Gerontol.* **2014**, *52*, 39–45. [CrossRef]

37. Carvalho, T.S.; Lussi, A. Age-related morphological, histological and functional changes in teeth. *J. Oral Rehabil.* **2017**, *44*, 291–298. [CrossRef] [PubMed]

38. Zhang, W.; Yelick, P.C. Tooth Repair and Regeneration: Potential of Dental Stem Cells. *Trends Mol. Med.* **2021**, *27*, 501–511. [CrossRef] [PubMed]

39. Sakai, K.; Yamamoto, A.; Matsubara, K.; Nakamura, S.; Naruse, M.; Yamagata, M.; Sakamoto, K.; Tauchi, R.; Wakao, N.; Imagama, S.; et al. Human dental pulp-derived stem cells promote locomotor recovery after complete transection of the rat spinal cord by multiple neuro-regenerative mechanisms. *J. Clin. Investig.* **2012**, *122*, 80–90. [CrossRef]

40. Kawashima, N. Characterisation of dental pulp stem cells: A new horizon for tissue regeneration? *Arch. Oral Biol.* **2012**, *57*, 1439–1458. [CrossRef]

41. Bhandi, S.; Alkahtani, A.; Mashyakhy, M.; Abumelha, A.S.; Albar, N.H.M.; Renugalakshmi, A.; Alkahtany, M.F.; Robaian, A.; Almeslet, A.S.; Patil, V.R.; et al. Effect of Ascorbic Acid on Differentiation, Secretome and Stemness of Stem Cells from Human Exfoliated Deciduous Tooth (SHEDs). *J. Pers. Med.* **2021**, *11*, 589. [CrossRef]

42. Smith, A.J.; Tobias, R.S.; Cassidy, N.; Bégue-Kirn, C.; Ruch, J.V.; Lesot, H. Influence of Substrate Nature and Immobilization of Implanted Dentin Matrix Components During Induction of Reparative Dentinogenesis. *Null* **1995**, *32*, 291–296. [CrossRef] [PubMed]

43. Achilleos, A.; Trainor, P.A. Neural crest stem cells: Discovery, properties and potential for therapy. *Cell Res.* **2012**, *22*, 288–304. [CrossRef]

44. Jung, J.; Kim, J.W.; Moon, H.J.; Hong, J.Y.; Hyun, J.K. Characterization of Neurogenic Potential of Dental Pulp Stem Cells Cultured in Xeno/Serum-Free Condition: In Vitro and In Vivo Assessment. *Stem Cells Int.* **2016**, *2016*, 6921097. [CrossRef] [PubMed]

45. Kawase-Koga, Y.; Fujii, Y.; Yamakawa, D.; Sato, M.; Chikazu, D. Identification of neurospheres generated from human dental pulp stem cells in xeno-/serum-free conditions. *Regen. Ther.* **2020**, *14*, 128–135. [CrossRef]

46. Bhandi, S.; Alkahtani, A.; Reda, R.; Mashyakhy, M.; Boreak, N.; Maganur, P.C.; Vishwanathaiah, S.; Mehta, D.; Vyas, N.; Patil, V.; et al. Parathyroid Hormone Secretion and Receptor Expression Determine the Age-Related Degree of Osteogenic Differentiation in Dental Pulp Stem Cells. *J. Pers. Med.* **2021**, *11*, 349. [CrossRef] [PubMed]

47. Dominici, M.; Le Blanc, K.; Mueller, I.; Slaper-Cortenbach, I.; Marini, F.C.; Krause, D.S.; Deans, R.J.; Keating, A.; Prockop, D.J.; Horwitz, E.M. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* **2006**, *8*, 315–317. [CrossRef]

48. Morad, G.; Kheiri, L.; Khojasteh, A. Dental pulp stem cells for in vivo bone regeneration: A systematic review of literature. *Arch. Oral Biol.* **2013**, *58*, 1818–1827. [CrossRef]

49. Yamaza, T.; Sonoda, S.; Tomoda, E.; Tanaka, Y. Properties and Possibilities of Human Dental Pulp-Derived Stem Cells. *Arch. Stem Cell Res.* **2015**, *2*, 1012.

50. Yildirim, S.; Zibandeh, N.; Genc, D.; Ozcan, E.M.; Goker, K.; Akkoc, T. The Comparison of the Immunologic Properties of Stem Cells Isolated from Human Exfoliated Deciduous Teeth, Dental Pulp, and Dental Follicles. *Stem Cells Int.* **2016**, *2016*, 4682875. [CrossRef]

51. Özdemir, A.T.; Özgül Özdemir, R.B.; Kırmaz, C.; Sarıboyacı, A.E.; Ünal Halbutoğlları, Z.S.; Özel, C.; Karaöz, E. The paracrine immunomodulatory interactions between the human dental pulp derived mesenchymal stem cells and CD4 T cell subsets. *Cell Immunol.* **2016**, *310*, 108–115. [CrossRef]

52. Wang, X.; Sha, X.-J.; Li, G.-H.; Yang, F.-S.; Ji, K.; Wen, L.-Y.; Liu, S.-Y.; Chen, L.; Ding, Y.; Xuan, K. Comparative characterization of stem cells from human exfoliated deciduous teeth and dental pulp stem cells. *Arch. Oral Biol.* **2012**, *57*, 1231–1240. [CrossRef] [PubMed]

53. Brizuela, C.; Meza, G.; Urrejola, D.; Quezada, M.A.; Concha, G.; Ramírez, V.; Angelopoulos, I.; Cadiz, M.I.; Tapia-Limonchi, R.; Khoury, M. Cell-Based Regenerative Endodontics for Treatment of Periapical Lesions: A Randomized, Controlled Phase I/II Clinical Trial. *J. Dent. Res.* **2020**, *99*, 523–529. [CrossRef]

54. Rosa, V.; Sriram, G. A critical analysis of research methods and biological experimental models to study pulp regeneration. *Int. Endod. J.* **2022**, *55* (Suppl. S2), 446–455. [CrossRef] [PubMed]

55. Tatullo, M.; Codispoti, B. Strategic Tools in Regenerative and Translational Dentistry. *Int. J. Mol. Sci.* **2019**, *20*, 1879. [CrossRef] [PubMed]

56. Aimetti, M.; Ferrarotti, F.; Gamba, M.N.; Giraudi, M.; Romano, F. Regenerative Treatment of Periodontal Intrabony Defects Using Autologous Dental Pulp Stem Cells: A 1-Year Follow-Up Case Series. *Int. J. Periodontics Restor. Dent.* **2018**, *38*, 51–58. [CrossRef]

57. Hernández-Monjaraz, B.; Santiago-Osorio, E.; Ledesma-Martínez, E.; Alcauter-Zavala, A.; Mendoza-Núñez, V.M. Retrieval of a periodontally compromised tooth by allogeneic grafting of mesenchymal stem cells from dental pulp: A case report. *J. Int. Med. Res.* **2018**, *46*, 2983–2993. [CrossRef]

58. Anitua, E.; Troya, M.; Zalduendo, M. Progress in the use of dental pulp stem cells in regenerative medicine. *Cytotherapy* **2018**, *20*, 479–498. [CrossRef]

59. da Silva, G.S.; Moreira, M.S.; Fukushima, K.A.; Raggio, D.P.; V Mello-Moura, A.C.; Lara, J.S.; Gimenez, T.; Junior, S.A.; Morimoto, S.; Tedesco, T.K. Current evidence of tissue engineering for dentine regeneration in animal models: A systematic review. *Regen. Med.* **2020**, *15*, 1345–1360. [CrossRef]

60. Iohara, K.; Imabayashi, K.; Ishizaka, R.; Watanabe, A.; Nabekura, J.; Ito, M.; Matsushita, K.; Nakamura, H.; Nakashima, M. Complete pulp regeneration after pulpectomy by transplantation of CD105+ stem cells with stromal cell-derived factor-1. *Tissue Eng. Part A* **2011**, *17*, 1911–1920. [CrossRef]

61. Rosa, V.; Zhang, Z.; Grande, R.H.; Nör, J.E. Dental pulp tissue engineering in full-length human root canals. *J. Dent. Res.* **2013**, *92*, 970–975. [CrossRef]

62. Xuan, K.; Li, B.; Guo, H.; Sun, W.; Kou, X.; He, X.; Zhang, Y.; Sun, J.; Liu, A.; Liao, L.; et al. Deciduous autologous tooth stem cells regenerate dental pulp after implantation into injured teeth. *Sci. Transl. Med.* **2018**, *10*, eaaf3227. [CrossRef] [PubMed]

63. Zayed, M.; Iohara, K.; Watanabe, H.; Ishikawa, M.; Tominaga, M.; Nakashima, M. Characterization of stable hypoxia-preconditioned dental pulp stem cells compared with mobilized dental pulp stem cells for application for pulp regenerative therapy. *Stem Cell Res. Ther.* **2021**, *12*, 302. [CrossRef] [PubMed]

64. Yamada, Y.; Nakamura-Yamada, S.; Konoki, R.; Baba, S. Promising advances in clinical trials of dental tissue-derived cell-based regenerative medicine. *Stem Cell Res. Ther.* **2020**, *11*, 175. [CrossRef] [PubMed]

65. Zhang, S.; Zhang, W.; Li, Y.; Ren, L.; Deng, H.; Yin, X.; Gao, X.; Pan, S.; Niu, Y. Human Umbilical Cord Mesenchymal Stem Cell Differentiation Into Odontoblast-Like Cells and Endothelial Cells: A Potential Cell Source for Dental Pulp Tissue Engineering. *Front. Physiol.* **2020**, *11*, 593. [CrossRef] [PubMed]

66. Graziano, A.; d'Aquino, R.; Laino, G.; Papaccio, G. Dental pulp stem cells: A promising tool for bone regeneration. *Stem Cell Rev.* **2008**, *4*, 21–26. [CrossRef] [PubMed]

67. Xu, L.; Tang, L.; Jin, F.; Liu, X.H.; Yu, J.H.; Wu, J.J.; Yang, Z.H.; Wang, Y.X.; Duan, Y.Z.; Jin, Y. The apical region of developing tooth root constitutes a complex and maintains the ability to generate root and periodontium-like tissues. *J. Periodontal. Res.* **2009**, *44*, 275–282. [CrossRef] [PubMed]

68. Kidwai, F.K.; Movahednia, M.M.; Iqbal, K.; Jokhun, D.S.; Cao, T.; Fawzy, A.S. Human embryonic stem cell differentiation into odontoblastic lineage: An in vitro study. *Int. Endod. J.* **2014**, *47*, 346–355. [CrossRef]

69. Xie, H.; Dubey, N.; Shim, W.; Ramachandra, C.J.A.; Min, K.S.; Cao, T.; Rosa, V. Functional Odontoblastic-Like Cells Derived from Human iPSCs. *J. Dent. Res.* **2018**, *97*, 77–83. [CrossRef]

70. Ozeki, N.; Mogi, M.; Kawai, R.; Yamaguchi, H.; Hiyama, T.; Nakata, K.; Nakamura, H. Mouse-induced pluripotent stem cells differentiate into odontoblast-like cells with induction of altered adhesive and migratory phenotype of integrin. *PLoS ONE* **2013**, *8*, e80026. [CrossRef]

71. Ohkoshi, S.; Hirono, H.; Nakahara, T.; Ishikawa, H. Dental pulp cell bank as a possible future source of individual hepatocytes. *World J. Hepatol.* **2018**, *10*, 702–707. [CrossRef]

72. Vizoso, F.J.; Eiro, N.; Cid, S.; Schneider, J.; Perez-Fernandez, R. Mesenchymal Stem Cell Secretome: Toward Cell-Free Therapeutic Strategies in Regenerative Medicine. *Int. J. Mol. Sci.* **2017**, *18*, 1852. [CrossRef] [PubMed]

73. Müller-Ehmsen, J.; Whittaker, P.; Kloner, R.A.; Dow, J.S.; Sakoda, T.; Long, T.I.; Laird, P.W.; Kedes, L. Survival and development of neonatal rat cardiomyocytes transplanted into adult myocardium. *J. Mol. Cell Cardiol.* **2002**, *34*, 107–116. [CrossRef] [PubMed]

74. Toma, C.; Wagner, W.R.; Bowry, S.; Schwartz, A.; Villanueva, F. Fate Of Culture-Expanded Mesenchymal Stem Cells in The Microvasculature. *Circ. Res.* **2009**, *104*, 398–402. [CrossRef] [PubMed]

75. Ide, C.; Nakai, Y.; Nakano, N.; Seo, T.-B.; Yamada, Y.; Endo, K.; Noda, T.; Saito, F.; Suzuki, Y.; Fukushima, M.; et al. Bone marrow stromal cell transplantation for treatment of sub-acute spinal cord injury in the rat. *Brain Res.* **2010**, *1332*, 32–47. [CrossRef]

76. Lee, R.H.; Pulin, A.A.; Seo, M.J.; Kota, D.J.; Ylostalo, J.; Larson, B.L.; Semprun-Prieto, L.; Delafontaine, P.; Prockop, D.J. Intravenous hMSCs Improve Myocardial Infarction in Mice because Cells Embolized in Lung Are Activated to Secrete the Anti-inflammatory Protein TSG-6. *Cell Stem Cell* **2009**, *5*, 54–63. [CrossRef]

77. Gao, X.; Shen, Z.; Guan, M.; Huang, Q.; Chen, L.; Qin, W.; Ge, X.; Chen, H.; Xiao, Y.; Lin, Z. Immunomodulatory Role of Stem Cells from Human Exfoliated Deciduous Teeth on Periodontal Regeneration. *Tissue Eng. Part A* **2018**, *24*, 1341–1353. [CrossRef]

78. Prockop, D.J. The Exciting Prospects of New Therapies with Mesenchymal Stromal Cells. *Cytotherapy* **2017**, *19*, 1–8. [CrossRef] [PubMed]

79. Ostby, B.N. The role of the blood clot in endodontic therapy. An experimental histologic study. *Acta Odontol. Scand.* **1961**, *19*, 324–353. [CrossRef] [PubMed]

80. Lovelace, T.W.; Henry, M.A.; Hargreaves, K.M.; Diogenes, A. Evaluation of the Delivery of Mesenchymal Stem Cells into the Root Canal Space of Necrotic Immature Teeth after Clinical Regenerative Endodontic Procedure. *J. Endod.* **2011**, *37*, 133–138. [CrossRef]

81. Torabinejad, M.; Turman, M. Revitalization of Tooth with Necrotic Pulp and Open Apex by Using Platelet-rich Plasma: A Case Report. *J. Endod.* **2011**, *37*, 265–268. [CrossRef]

82. Shimizu, E.; Ricucci, D.; Albert, J.; Alobaid, A.S.; Gibbs, J.L.; Huang, G.T.J.; Lin, L.M. Clinical, Radiographic, and Histological Observation of a Human Immature Permanent Tooth with Chronic Apical Abscess after Revitalization Treatment. *J. Endod.* **2013**, *39*, 1078–1083. [CrossRef] [PubMed]

83. Sedgley, C.M.; Cherkas, P.; Chogle, S.M.A.; Geisler, T.M.; Hargreaves, K.M.; Paranjpe, A.K.; Yamagishi, V.T.-K. Endodontics: Colleagues for Excellence Newsletter. Available online: https://www.aae.org/specialty/newsletter/regenerative-endodontics/ (accessed on 1 November 2022).

84. Banchs, F.; Trope, M. Revascularization of immature permanent teeth with apical periodontitis: New treatment protocol? *J. Endod.* **2004**, *30*, 196–200. [CrossRef] [PubMed]

85. Murray, P.E.; Garcia-Godoy, F.; Hargreaves, K.M. Regenerative endodontics: A review of current status and a call for action. *J. Endod.* **2007**, *33*, 377–390. [CrossRef] [PubMed]

86. He, L.; Zhong, J.; Gong, Q.; Cheng, B.; Kim, S.G.; Ling, J.; Mao, J.J. Regenerative Endodontics by Cell Homing. *Dent. Clin. N. Am.* **2017**, *61*, 143–159. [CrossRef]

87. Yin, Y.; Li, X.; He, X.T.; Wu, R.X.; Sun, H.H.; Chen, F.M. Leveraging Stem Cell Homing for Therapeutic Regeneration. *J. Dent. Res.* **2017**, *96*, 601–609. [CrossRef]

88. Kim, J.Y.; Xin, X.; Moioli, E.K.; Chung, J.; Lee, C.H.; Chen, M.; Fu, S.Y.; Koch, P.D.; Mao, J.J. Regeneration of Dental-Pulp-like Tissue by Chemotaxis-Induced Cell Homing. *Tissue Eng. Part A* **2010**, *16*, 3023–3031. [CrossRef]

89. Nosrat, A.; Kolahdouzan, A.; Hosseini, F.; Mehrizi, E.A.; Verma, P.; Torabinejad, M. Histologic Outcomes of Uninfected Human Immature Teeth Treated with Regenerative Endodontics: 2 Case Reports. *J. Endod.* **2015**, *41*, 1725–1729. [CrossRef]

90. Becerra, P.; Ricucci, D.; Loghin, S.; Gibbs, J.L.; Lin, L.M. Histologic Study of a Human Immature Permanent Premolar with Chronic Apical Abscess after Revascularization/Revitalization. *J. Endod.* **2014**, *40*, 133–139. [CrossRef]

91. Parirokh, M.; Torabinejad, M. Mineral Trioxide Aggregate: A Comprehensive Literature Review—Part III: Clinical Applications, Drawbacks, and Mechanism of Action. *J. Endod.* **2010**, *36*, 400–413. [CrossRef]

92. Xie, H.; Chua, M.; Islam, I.; Bentini, R.; Cao, T.; Viana-Gomes, J.C.; Castro Neto, A.H.; Rosa, V. CVD-grown monolayer graphene induces osteogenic but not odontoblastic differentiation of dental pulp stem cells. *Dent. Mater. Off. Publ. Acad. Dent. Mater.* **2017**, *33*, e13–e21. [CrossRef]

93. Herford, A.S.; Boyne, P.J. Reconstruction of mandibular continuity defects with bone morphogenetic protein-2 (rhBMP-2). *J. Oral Maxillofac. Surg. Off. J. Am. Assoc. Oral Maxillofac. Surg.* **2008**, *66*, 616–624. [CrossRef]

94. Casagrande, L.; Demarco, F.F.; Zhang, Z.; Araujo, F.B.; Shi, S.; Nör, J.E. Dentin-derived BMP-2 and odontoblast differentiation. *J. Dent. Res.* **2010**, *89*, 603–608. [CrossRef]

95. Woo, E.J. Adverse events reported after the use of recombinant human bone morphogenetic protein 2. *J. Oral Maxillofac. Surg. Off. J. Am. Assoc. Oral Maxillofac. Surg.* **2012**, *70*, 765–767. [CrossRef]

96. Perri, B.; Cooper, M.; Lauryssen, C.; Anand, N. Adverse swelling associated with use of rh-BMP-2 in anterior cervical discectomy and fusion: A case study. *Spine J. Off. J. N. Am. Spine Soc.* **2007**, *7*, 235–239. [CrossRef]

97. Vaidya, R.; Carp, J.; Sethi, A.; Bartol, S.; Craig, J.; Les, C.M. Complications of anterior cervical discectomy and fusion using recombinant human bone morphogenetic protein-2. *Eur. Spine J.* **2007**, *16*, 1257–1265. [CrossRef] [PubMed]

98. Kawasaki, K.; Aihara, M.; Honmo, J.; Sakurai, S.; Fujimaki, Y.; Sakamoto, K.; Fujimaki, E.; Wozney, J.M.; Yamaguchi, A. Effects of recombinant human bone morphogenetic protein-2 on differentiation of cells isolated from human bone, muscle, and skin. *Bone* **1998**, *23*, 223–231. [CrossRef] [PubMed]

99. Ulusoy, A.T.; Turedi, I.; Cimen, M.; Cehreli, Z.C. Evaluation of Blood Clot, Platelet-rich Plasma, Platelet-rich Fibrin, and Platelet Pellet as Scaffolds in Regenerative Endodontic Treatment: A Prospective Randomized Trial. *J. Endod.* **2019**, *45*, 560–566. [CrossRef] [PubMed]

100. De Jong, O.G.; Van Balkom, B.W.; Schiffelers, R.M.; Bouten, C.V.; Verhaar, M.C. Extracellular vesicles: Potential roles in regenerative medicine. *Front. Immunol.* **2014**, *5*, 608. [CrossRef] [PubMed]

101. Teixeira, F.G.; Salgado, A.J. Mesenchymal stem cells secretome: Current trends and future challenges. *Neural. Regen. Res.* **2019**, *15*, 75–77. [CrossRef] [PubMed]

102. Katagiri, W.; Osugi, M.; Kawai, T.; Hibi, H. First-in-human study and clinical case reports of the alveolar bone regeneration with the secretome from human mesenchymal stem cells. *Head Face Med.* **2016**, *12*, 5. [CrossRef]

103. Lin, H.; Chen, H.; Zhao, X.; Chen, Z.; Zhang, P.; Tian, Y.; Wang, Y.; Ding, T.; Wang, L.; Shen, Y. Advances in mesenchymal stem cell conditioned medium-mediated periodontal tissue regeneration. *J. Transl. Med.* **2021**, *19*, 456. [CrossRef] [PubMed]

104. Katagiri, W.; Osugi, M.; Kawai, T.; Ueda, M. Novel cell-free regeneration of bone using stem cell-derived growth factors. *Int. J. Oral Maxillofac. Implant.* **2013**, *28*, 1009–1016. [CrossRef] [PubMed]

105. Osugi, M.; Katagiri, W.; Yoshimi, R.; Inukai, T.; Hibi, H.; Ueda, M. Conditioned Media from Mesenchymal Stem Cells Enhanced Bone Regeneration in Rat Calvarial Bone Defects. *Tissue Eng. Part A* **2012**, *18*, 1479–1489. [CrossRef] [PubMed]

106. Inukai, T.; Katagiri, W.; Yoshimi, R.; Osugi, M.; Kawai, T.; Hibi, H.; Ueda, M. Novel application of stem cell-derived factors for periodontal regeneration. *Biochem. Biophys. Res. Commun.* **2013**, *430*, 763–768. [CrossRef] [PubMed]

107. Kawai, T.; Katagiri, W.; Osugi, M.; Sugimura, Y.; Hibi, H.; Ueda, M. Secretomes from bone marrow-derived mesenchymal stromal cells enhance periodontal tissue regeneration. *Cytotherapy* **2015**, *17*, 369–381. [CrossRef]

108. Cornish, J.; Grey, A.; Callon, K.E.; Naot, D.; Hill, B.L.; Lin, C.Q.X.; Balchin, L.M.; Reid, I.R. Shared pathways of osteoblast mitogenesis induced by amylin, adrenomedullin, and IGF-1. *Biochem. Biophys. Res. Commun.* **2004**, *318*, 240–246. [CrossRef]

109. Li, Y.; Yu, X.; Lin, S.; Li, X.; Zhang, S.; Song, Y.-H. Insulin-like growth factor 1 enhances the migratory capacity of mesenchymal stem cells. *Biochem. Biophys. Res. Commun.* **2007**, *356*, 780–784. [CrossRef]

110. Han, X.; Amar, S. Role of insulin-like growth factor-1 signaling in dental fibroblast apoptosis. *J. Periodontol.* **2003**, *74*, 1176–1182. [CrossRef]

111. Kaigler, D.; Krebsbach, P.H.; West, E.R.; Horger, K.; Huang, Y.C.; Mooney, D.J. Endothelial cell modulation of bone marrow stromal cell osteogenic potential. *FASEB J.* **2005**, *19*, 665–667. [CrossRef]

112. Morishita, R.; Nakamura, S.; Hayashi, S.; Taniyama, Y.; Moriguchi, A.; Nagano, T.; Taiji, M.; Noguchi, H.; Takeshita, S.; Matsumoto, K.; et al. Therapeutic angiogenesis induced by human recombinant hepatocyte growth factor in rabbit hind limb ischemia model as cytokine supplement therapy. *Hypertension* **1999**, *33*, 1379–1384. [CrossRef]

113. Bostrom, M.P.; Asnis, P. Transforming growth factor beta in fracture repair. *Clin. Orthop. Relat. Res.* **1998**, *355S*, S124–S131. [CrossRef] [PubMed]

114. Gao, J.; Symons, A.L.; Bartold, P.M. Expression of transforming growth factor-beta 1 (TGF-beta1) in the developing periodontium of rats. *J. Dent. Res.* **1998**, *77*, 1708–1716. [CrossRef] [PubMed]

115. Ozaki, Y.; Nishimura, M.; Sekiya, K.; Suehiro, F.; Kanawa, M.; Nikawa, H.; Hamada, T.; Kato, Y. Comprehensive Analysis of Chemotactic Factors for Bone Marrow Mesenchymal Stem Cells. *Stem Cells Dev.* **2007**, *16*, 119–130. [CrossRef] [PubMed]

116. Chen, L.; Jiang, W.; Huang, J.; He, B.-C.; Zuo, G.-W.; Zhang, W.; Luo, Q.; Shi, Q.; Zhang, B.-Q.; Wagner, E.R.; et al. Insulin-like Growth Factor 2 (IGF-2) Potentiates BMP-9-Induced Osteogenic Differentiation and Bone Formation. *J. Bone Miner. Res.* **2010**, *25*, 2447–2459. [CrossRef] [PubMed]

117. Dissanayaka, W.L.; Zhan, X.; Zhang, C.; Hargreaves, K.M.; Jin, L.; Tong, E.H. Coculture of dental pulp stem cells with endothelial cells enhances osteo-/odontogenic and angiogenic potential in vitro. *J. Endod.* **2012**, *38*, 454–463. [CrossRef]

118. Venugopal, C.; Shobha, K.; Rai, K.S.; Dhanushkodi, A. Neurogenic and cognitive enhancing effects of human dental pulp stem cells and its secretome in animal model of hippocampal neurodegeneration. *Brain Res. Bull.* **2022**, *180*, 46–58. [CrossRef]

119. Kumar, A.; Kumar, V.; Rattan, V.; Jha, V.; Bhattacharyya, S. Secretome Cues Modulate the Neurogenic Potential of Bone Marrow and Dental Stem Cells. *Mol. Neurobiol.* **2017**, *54*, 4672–4682. [CrossRef]

120. Huang, C.-C.; Narayanan, R.; Alapati, S.; Ravindran, S. Exosomes as Biomimetic Tools for Stem Cell Differentiation: Applications in Dental Pulp Tissue Regeneration. *Biomaterials* **2016**, *111*, 103–115. [CrossRef]

121. Liu, H.; Li, D.; Zhang, Y.; Li, M. Inflammation, mesenchymal stem cells and bone regeneration. *Histochem. Cell Biol.* **2018**, *149*, 393–404. [CrossRef]

122. Li, W.; Ren, G.; Huang, Y.; Su, J.; Han, Y.; Li, J.; Chen, X.; Cao, K.; Chen, Q.; Shou, P.; et al. Mesenchymal stem cells: A double-edged sword in regulating immune responses. *Cell Death Differ.* **2012**, *19*, 1505–1513. [CrossRef]

123. Peng, Q.; Yang, J.-y.; Zhou, G. Emerging functions and clinical applications of exosomes in human oral diseases. *Cell Biosci.* **2020**, *10*, 68. [CrossRef] [PubMed]

124. Zheng, Y.; Dong, C.; Yang, J.; Jin, Y.; Zheng, W.; Zhou, Q.; Liang, Y.; Bao, L.; Feng, G.; Ji, J.; et al. Exosomal microRNA-155-5p from PDLSCs regulated Th17/Treg balance by targeting sirtuin-1 in chronic periodontitis. *J. Cell Physiol.* **2019**, *234*, 20662–20674. [CrossRef] [PubMed]

125. Zhao, M.; Dai, W.; Wang, H.; Xue, C.; Feng, J.; He, Y.; Wang, P.; Li, S.; Bai, D.; Shu, R. Periodontal ligament fibroblasts regulate osteoblasts by exosome secretion induced by inflammatory stimuli. *Arch. Oral Biol.* **2019**, *105*, 27–34. [CrossRef] [PubMed]

126. Wang, Z.; Maruyama, K.; Sakisaka, Y.; Suzuki, S.; Tada, H.; Suto, M.; Saito, M.; Yamada, S.; Nemoto, E. Cyclic Stretch Force Induces Periodontal Ligament Cells to Secrete Exosomes That Suppress IL-1β Production Through the Inhibition of the NF-κB Signaling Pathway in Macrophages. *Front. Immunol.* **2019**, *10*, 1310. [CrossRef]

127. Wang, M.; Li, J.; Ye, Y.; He, S.; Song, J. SHED-derived conditioned exosomes enhance the osteogenic differentiation of PDLSCs via Wnt and BMP signaling in vitro. *Differentiation* **2020**, *111*, 1–11. [CrossRef]

128. Wakayama, H.; Hashimoto, N.; Matsushita, Y.; Matsubara, K.; Yamamoto, N.; Hasegawa, Y.; Ueda, M.; Yamamoto, A. Factors secreted from dental pulp stem cells show multifaceted benefits for treating acute lung injury in mice. *Cytotherapy* **2015**, *17*, 1119–1129. [CrossRef]

129. Kou, X.; Xu, X.; Chen, C.; Sanmillan, M.L.; Cai, T.; Zhou, Y.; Giraudo, C.; Le, A.; Shi, S. The Fas/Fap-1/Cav-1 complex regulates IL-1RA secretion in mesenchymal stem cells to accelerate wound healing. *Sci. Transl. Med.* **2018**, *10*, eaai8524. [CrossRef]

130. Shi, H.Z.; Zeng, J.C.; Shi, S.H.; Giannakopoulos, H.; Zhang, Q.Z.; Le, A.D. Extracellular Vesicles of GMSCs Alleviate Aging-Related Cell Senescence. *J. Dent. Res.* **2021**, *100*, 283–292. [CrossRef]

131. Shi, Q.; Qian, Z.; Liu, D.; Sun, J.; Wang, X.; Liu, H.; Xu, J.; Guo, X. GMSC-Derived Exosomes Combined with a Chitosan/Silk Hydrogel Sponge Accelerates Wound Healing in a Diabetic Rat Skin Defect Model. *Front. Physiol.* **2017**, *8*, 904. [CrossRef]

132. Koh, B.; Ab Rahman, F.H.; Matlan, N.A.; Rajan, M.; Musta'ain, A.Y.; Mohd Jeffry Lee, M.R.; Ramli, R.; Mohd Yunus, S.S.; Binti Hj Idrus, R.; Yazid, M.D. Potential role of dental pulp stem cells conditioned medium for odontoblastic differentiation. *Biol. Res.* **2022**, *55*, 11. [CrossRef]

133. Sarra, G.; Machado, M.E.d.L.; Caballero-Flores, H.V.; Moreira, M.S.; Pedroni, A.C.F.; Marques, M.M. Effect of human dental pulp stem cell conditioned medium in the dentin-pulp complex regeneration: A pilot in vivo study. *Tissue Cell* **2021**, *72*, 101536. [CrossRef] [PubMed]

134. Xian, X.; Gong, Q.; Li, C.; Guo, B.; Jiang, H. Exosomes with Highly Angiogenic Potential for Possible Use in Pulp Regeneration. *J. Endod.* **2018**, *44*, 751–758. [CrossRef] [PubMed]

135. Yu, S.; Chen, H.; Gao, B. Potential Therapeutic Effects of Exosomes in Regenerative Endodontics. *Arch. Oral Biol.* **2020**, *120*, 104946. [CrossRef] [PubMed]

136. Sicari, B.M.; Rubin, J.P.; Dearth, C.L.; Wolf, M.T.; Ambrosio, F.; Boninger, M.; Turner, N.J.; Weber, D.J.; Simpson, T.W.; Wyse, A.; et al. An acellular biologic scaffold promotes skeletal muscle formation in mice and humans with volumetric muscle loss. *Sci. Transl. Med.* **2014**, *6*, 234ra258. [CrossRef] [PubMed]

137. Smith, E.E.; Zhang, W.; Schiele, N.R.; Khademhosseini, A.; Kuo, C.K.; Yelick, P.C. Developing a biomimetic tooth bud model. *J. Tissue Eng. Regen. Med.* **2017**, *11*, 3326–3336. [CrossRef]

138. Karp, J.M.; Leng Teo, G.S. Mesenchymal stem cell homing: The devil is in the details. *Cell Stem Cell* **2009**, *4*, 206–216. [CrossRef]

139. Galler, K.M.; Widbiller, M.; Schmalz, G. Cell-Free Approaches for Dental Pulp Tissue Engineering. *J. Endod.* **2020**, *46*, S143–S149. [CrossRef]

140. Schmalz, G.; Widbiller, M.; Galler, K.M. Signaling Molecules and Pulp Regeneration. *J. Endod.* **2017**, *43*, S7–S11. [CrossRef]

141. Duncan, H.F.; Kobayashi, Y.; Shimizu, E. Growth Factors and Cell Homing in Dental Tissue Regeneration. *Curr. Oral Health Rep.* **2018**, *5*, 276–285. [CrossRef]

142. Widbiller, M.; Driesen, R.B.; Eidt, A.; Lambrichts, I.; Hiller, K.-A.; Buchalla, W.; Schmalz, G.; Galler, K.M. Cell Homing for Pulp Tissue Engineering with Endogenous Dentin Matrix Proteins. *J. Endod.* **2018**, *44*, 956–962. [CrossRef]

143. Song, J.S.; Takimoto, K.; Jeon, M.; Vadakekalam, J.; Ruparel, N.B.; Diogenes, A. Decellularized Human Dental Pulp as a Scaffold for Regenerative Endodontics. *J. Dent. Res.* **2017**, *96*, 640–646. [CrossRef] [PubMed]

144. Torabinejad, M.; Alexander, A.; Vahdati, S.A.; Grandhi, A.; Baylink, D.; Shabahang, S. Effect of Residual Dental Pulp Tissue on Regeneration of Dentin-pulp Complex: An In Vivo Investigation. *J. Endod.* **2018**, *44*, 1796–1801. [CrossRef] [PubMed]

145. Koh, B.; Sulaiman, N.; Ismadi, S.N.S.W.; Ramli, R.; Yunus, S.S.M.; Idrus, R.B.H.; Ariffin, S.H.Z.; Wahab, R.M.A.; Yazid, M.D. Mesenchymal stem cells: A comprehensive methods for odontoblastic induction. *Biol. Proced. Online* **2021**, *23*, 18. [CrossRef] [PubMed]

146. Schulte, W. Implants and the periodontium. *Int. Dent. J.* **1995**, *45*, 16–26.

147. Ivanovski, S.; Lee, R. Comparison of peri-implant and periodontal marginal soft tissues in health and disease. *Periodontol. 2000* **2018**, *76*, 116–130. [CrossRef]

148. Zhan, C.; Huang, M.; Yang, X.; Hou, J. Dental nerves: A neglected mediator of pulpitis. *Int. Endod. J.* **2021**, *54*, 85–99. [CrossRef]

149. Zhao, H.; Feng, J.; Seidel, K.; Shi, S.; Klein, O.; Sharpe, P.; Chai, Y. Secretion of Shh by a Neurovascular Bundle Niche Supports Mesenchymal Stem Cell Homeostasis in the Adult Mouse Incisor. *Cell Stem Cell* **2018**, *23*, 147. [CrossRef]

150. Bakopoulou, A.; Leyhausen, G.; Volk, J.; Tsiftsoglou, A.; Garefis, P.; Koidis, P.; Geurtsen, W. Comparative analysis of in vitro osteo/odontogenic differentiation potential of human dental pulp stem cells (DPSCs) and stem cells from the apical papilla (SCAP). *Arch. Oral Biol.* **2011**, *56*, 709–721. [CrossRef]

151. Sosnik, A.; Seremeta, K.P. Polymeric Hydrogels as Technology Platform for Drug Delivery Applications. *Gels* **2017**, *3*, 25. [CrossRef]

152. Chatterjee, S.; Hui, P.C.-l.; Kan, C.-w. Thermoresponsive Hydrogels and Their Biomedical Applications: Special Insight into Their Applications in Textile Based Transdermal Therapy. *Polymers* **2018**, *10*, 480. [CrossRef]

153. Zhu, X.; Zhang, C.; Huang, G.T.; Cheung, G.S.; Dissanayaka, W.L.; Zhu, W. Transplantation of dental pulp stem cells and platelet-rich plasma for pulp regeneration. *J. Endod.* **2012**, *38*, 1604–1609. [CrossRef]

154. Han, Y.; Chen, Q.; Zhang, L.; Dissanayaka, W.L. Indispensable Role of HIF-1α Signaling in Post-implantation Survival and Angio-/Vasculogenic Properties of SHED. *Front. Cell Dev. Biol.* **2021**, *9*, 655073. [CrossRef] [PubMed]

155. Rashid, I.; Pathak, A.K.; Kumar, R.; Srivastava, P.; Singh, M.; Murali, S.; Kushwaha, B. Genome-Wide Comparative Analysis of HIF Binding Sites in Cyprinus Carpio for In Silico Identification of Functional Hypoxia Response Elements. *Front. Genet.* **2019**, *10*, 659. [CrossRef] [PubMed]

156. Chen, S.; Fang, L.; Guo, W.; Zhou, Y.; Yu, G.; Li, W.; Dong, K.; Liu, J.; Luo, Y.; Wang, B.; et al. Control of T(reg) cell homeostasis and immune equilibrium by Lkb1 in dendritic cells. *Nat. Commun.* **2018**, *9*, 5298. [CrossRef]

157. Zhang, Q.; Shi, S.; Liu, Y.; Uyanne, J.; Shi, Y.; Shi, S.; Le, A.D. Mesenchymal stem cells derived from human gingiva are capable of immunomodulatory functions and ameliorate inflammation-related tissue destruction in experimental colitis. *J. Immunol.* **2009**, *183*, 7787–7798. [CrossRef] [PubMed]

158. Zhao, J.; Chen, J.; Huang, F.; Wang, J.; Su, W.; Zhou, J.; Qi, Q.; Cao, F.; Sun, B.; Liu, Z.; et al. Human gingiva tissue-derived MSC ameliorates immune-mediated bone marrow failure of aplastic anemia via suppression of Th1 and Th17 cells and enhancement of CD4+Foxp3+ regulatory T cells differentiation. *Am. J. Transl. Res.* **2019**, *11*, 7627–7643, Erratum in *Am. J. Transl. Res.* **2020**, *12*, 1167.

159. Luo, Y.; Wu, W.; Gu, J.; Zhang, X.; Dang, J.; Wang, J.; Zheng, Y.; Huang, F.; Yuan, J.; Xue, Y.; et al. Human gingival tissue-derived MSC suppress osteoclastogenesis and bone erosion via CD39-adenosine signal pathway in autoimmune arthritis. *EBioMedicine* **2019**, *43*, 620–631. [CrossRef]

160. Bermudez, M.A.; Sendon-Lago, J.; Eiro, N.; Treviño, M.; Gonzalez, F.; Yebra-Pimentel, E.; Giraldez, M.J.; Macia, M.; Lamelas, M.L.; Saa, J.; et al. Corneal epithelial wound healing and bactericidal effect of conditioned medium from human uterine cervical stem cells. *Investig. Ophthalmol. Vis. Sci.* **2015**, *56*, 983–992. [CrossRef]

161. Bermudez, M.A.; Sendon-Lago, J.; Seoane, S.; Eiro, N.; Gonzalez, F.; Saa, J.; Vizoso, F.; Perez-Fernandez, R. Anti-inflammatory effect of conditioned medium from human uterine cervical stem cells in uveitis. *Exp. Eye Res.* **2016**, *149*, 84–92. [CrossRef]

162. Sendon-Lago, J.; Rio, L.G.; Eiro, N. Tailored Hydrogels as Delivery Platforms for Conditioned Medium from Mesenchymal Stem Cells in a Model of Acute Colitis in Mice. *Pharmaceutics* **2021**, *13*, 1127. [CrossRef] [PubMed]

163. Xu, Z.; Shi, L.; Wang, Y.; Zhang, J.; Huang, L.; Zhang, C.; Liu, S.; Zhao, P.; Liu, H.; Zhu, L.; et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir. Med.* **2020**, *8*, 420–422. [CrossRef]

164. Eiró, N.; Sendon-Lago, J.; Seoane, S.; Bermúdez, M.A.; Lamelas, M.L.; Garcia-Caballero, T.; Schneider, J.; Perez-Fernandez, R.; Vizoso, F.J. Potential therapeutic effect of the secretome from human uterine cervical stem cells against both cancer and stromal cells compared with adipose tissue stem cells. *Oncotarget* **2014**, *5*, 10692–10708. [CrossRef] [PubMed]

165. Fernández-Francos, S.; Eiro, N. Mesenchymal Stem Cells as a Cornerstone in a Galaxy of Intercellular Signals: Basis for a New Era of Medicine. *Int. J. Mol. Sci.* **2021**, *22*, 3576. [CrossRef] [PubMed]