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The Current Status of Stem Cell Regeneration in Intra Oral Applications—A Systematic Review

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Abstract

Aim: 1) To review the literature of various applications of stem cell regeneration in dentistry from 2010 to 2016. 2) To review these studies and to summarize the current status of stem cell regeneration in intra oral applications. 3) To present the available successful data with regard to stem cell regeneration in dentistry and to highlight the future trends. **Materials and Methods: Search Protocol:** A systematic search was made in the PubMed database with the key words gingiva, papillary reconstruction, periodontal ligament, dental pulp, salivary gland, enamel re-mineralization, dentin, cementum, bone, whole tooth, cleft palate, regeneration and stem cells. All articles from 2010 to 2016, relevant to the topic were included. After the search a total of 1826 articles were obtained which were screened and categorized by three independent reviewers as review papers, *in vitro*, animal and human studies, pertaining to stem cell regeneration in intra oral applications. On the basis of the extracted data and outcome measures, conclusions were drawn. **Results:** Maximum number of animal studies has been done to regenerate periodontal tissues, bone, dentin and pulp tissues. Few human studies have been done till date. Although clinically, researchers have been able to regenerate periodontal-like tissue, bone and pulp-like tissue, they still haven't been able to regenerate tissues that completely resemble tissues in their natural form. **Conclusion:** The future of stem cell therapy in dental applications looks promising. The predictability and efficacy of outcomes, as well as safety concerns of stem cell therapy is yet to be fully established. Ongoing research and development of newer scaffolds, understanding various signaling molecules and their cues, understanding gene expression and proteomics of stem cells are the future directions that will take us a step forward to achieving successful regeneration.

Keywords

Stem Cells, Regeneration, Dental Tissues

1. Introduction

Stem cells are defined as clonogenic cells capable of both self-renewal and multi-lineage differentiation [1]. The term "stem cell" first appeared in 1868 in the works of German biologist Haeckel [2], but was proposed for scientific use in 1908 [3]. In the 19th century, Goujon observed that transplantation of bone marrow to heterotopic anatomical sites resulted in de novo generation of ectopic bone and marrow [4]. Later, the work of Tavassoli and Crosby clearly established proof of an inherent osteogenic potential associated with the bone Marow [5]. Friedenstein *et al*. isolated Mesenchymal stem cells (MSC) from bone marrow in 1974 [6]. But, the major breakthrough in dental history was achieved by Gronthos *et al*. [7] in the year 2000.

Stem cells can be broadly classified into embryonic stem cells, adult stem cells and induced pluripotent stem cells (iPS).

Embryonic stem cells are undifferentiated pluripotent cells that are obtained from the inner cell mass of the blastocyst having the ability to form any adult cell [8].

Adult stem cells are also called somatic stem cells or postnatal stem cells, and they are found in many tissues and organs. These cells undergo self-renewal and differentiation to maintain healthy tissues and repair injured tissues [9]. Recent stem cell studies in the dental field have identified many adult stem cell sources in the oral and maxillofacial region. These cells are believed to reside in a specific area of each tissue, *i.e*., a "stem cell niche". Many types of adult stem cells reside in several mesenchymal tissues, and these cells are collectively referred to as mesenchymal stem cells or multipotent mesenchymal stromal cells (MSCs). Mesenchymal stem cells can be bone marrow derived, dental tissue-derived cells, umbilical cord derived and fat tissue derived cells that are capable of self-renewing by dividing and differentiating into multiple tissues such as bone, cartilage, muscle, fat cells, and connective tissue [10] [11]. Bone marrow-derived MSCs (BMSCs) are multipotent progenitor cells which can be harvested from sternum or iliac crest and [12] and Orofaical (maxilla and mandible) bone marrows [13]. Dental tissue-derived mesenchymal stem cells have being documented to be good, due to their easy accessibility, immunosuppressive properties, high proliferation, and the capacity to differentiate into odontoblasts, cementoblasts, osteoblasts, and other cells to be found in dental tissues [14] [15].

There are five different types of dental tissue derived stem cells which are Dental pulp stem Cells (DPSCs), Periodontal ligament stem cells (PDLSCs), Stem cells from exfoliated deciduous teeth (SHED), Stem cells from apical papilla (SCAP) and Dental follicle progenitor cells (DFPCs).

Dental pulp stem Cells (DPSCs) can differentiate into different kinds of cells and tissues [16] [17] especially bone-like tissue and their multipotency has been compared to those of bone marrow stem cells (BMSCs). It has been demonstrated that proliferation, availability, and cell number of DPSCs are greater than BMSCs [18]. DPSC have also been used to regenerate nerves [19], cornea [20], bladder and renal tissues [21] [22], skeletal muscles [23], lung tissue [24] and has

demonstrated good angiogenic [25] and neurogenic potential.

PDLSCs (Periodontal Ligament stem cells) are capable of regenerating bone, cementum, periodontal ligament-like structures and facilitates periodontal regeneration [26].

SHEDs (Stem cells from human exfoliated deciduous teeth) also have periodontal regeneration, dentin pulp-like complex and bone regenerative capacity [27] [28].

SCAPs (stem cells from apical papilla) demonstrated positive results in formation of dentin pulp-like complex and were able to form a root-like structure when seeded onto hydroxyapatite-based scaffolds and implanted in pig jaws [29] [30] [31].

DFPCs (Dental follicle precursor cells) are observed to have potential for dentin regeneration, matrix formation in skull, formation of root like tissues with a pulp-dentin complex and a periodontal ligament connecting a cementum-like layer to host alveolar bone. These cells are isolated from follicles of human impacted third molars [32]. DPSCs, SHEDs and PDLSCs have all demonstrated bone regenerating capacity in *in-vitro* and *in-vivo* studies.

Gingiva-derived mesenchymal stem cells (G-MSC) have also been used to regenerate bone. In a comparative study of different mesenchymal stem cells, the gingiva and dental pulp stem cells proved to be putative cell sources for hard tissue regeneration [33].

In 2006, Dr. Shinya Yamanaka discovered that normal mouse adult skin fibroblasts can be reprogrammed to an embryonic state by introducing four genetic factors and the resulting cells were termed iPS cells [34]. iPS cells have been generated from various oral mesenchymal cells for dental applications.

There are numerous types of tissues that are being attempted to be regenerated with stem cells. We will limit the scope of this article to the intraoral applications of the various types of stem cells and systematically review the current status of stem cell regeneration in various intra oral hard and soft tissues.

2. Materials and Methods

A systematic search was made in the PubMed database with the key words gingiva, papillary reconstruction, periodontal ligament, dental pulp, salivary glands, Enamel re-mineralisation, dentin, cementum, bone, whole tooth, cleft palate, regeneration and stem cells. Key words pertaining to all the intraoral soft and hard tissues were used in combination with stem cells and regeneration in order to obtain all the relevant articles pertaining to various tissues.

All articles from 2010 to 2016, relevant to the topic were included. After the search a total of 1826 articles were obtained which were screened and categorized by three independent reviewers as review papers, *in vitro* and *in vivo* (animal and human) studies, pertaining to stem cell regeneration in intra oral applications.

Inclusion criteria: Animal and human studies using stem cells for intraoral hard and soft tissue regeneration were included in the review. Articles in English

language were only included.

183 articles were included but only the most relevant articles were cited in the review.

Exclusion criteria: Narrative review articles, commentaries, opinions were excluded from the study.

Figure 1 illustrates the screening process, selection and exclusion of articles after a PUBMED search.

3. Results

A total of 1826 articles were obtained after the PUBMED database search. The distribution of the articles according to intraoral hard and soft tissue regeneration based on the type of study after the initial search is depicted in Table 1. Out

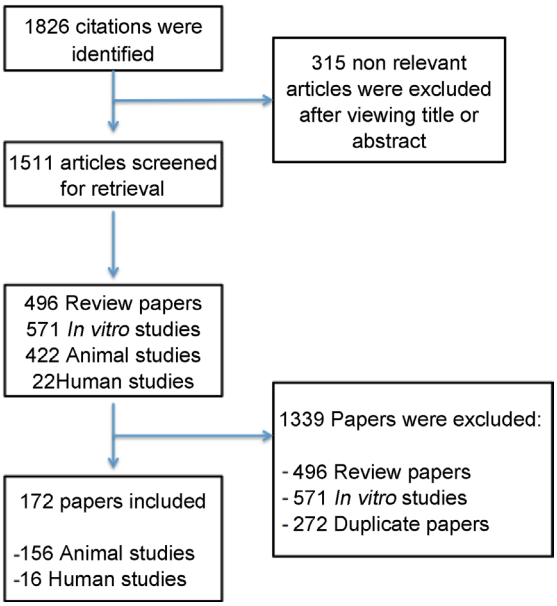

Figure 1. Flow diagram depicting article-screening process.

Table 1. Distribution of the articles pertaining to intraoral hard and soft tissue regeneration with stem cells according to the type of study after initial search.

Tissue regenerated	Total articles (n = 1826)	Review papers (n = 496)	*In vitro* studies (n = 571)	Animal studies (n = 422)	Humans studies (n = 22)
Gingiva	60	18	23	13	2
PDL	317	73	151	84	4
Pulp	474	127	194	87	6
Salivary	56	28	9	15	1
Bone	500	135	80	81	7
Dentin	222	47	83	63	1
Enamel	56	25	14	11	0
Cleft palate	16	5	1	6	0
Whole tooth	22	15	1	6	0
Cementum	103	23	15	56	1

of the total number of articles, 27% were review articles, 32% were *In vitro* studies, 23% were animal studies and only 1% were human studies (Figure 2). Articles that were not in English language and overlapping articles that appeared due to similar key words were excluded and only animal and human studies pertaining to the topic were further analyzed. A detailed distribution of the number of studies done in animals and humans to regenerate various intraoral soft and hard tissues has been presented in Table 2. A total of 156 animal studies and 16 human studies were included in this review.

After comparative evaluation of all type of articles between 2010 to 2016 pertaining to stem cell therapy application in intraoral hard and soft tissue application it was observed that till date maximum number of animal studies have been done to regenerate Periodontal tissues, Bone, Dentin and pulp tissues. Very few human studies have been done till date (Figure 3).

4. Discussion

The use of stem cells for tissue engineering has great potential to solve clinical and surgical problems related to tissue loss and organ functional failures. For the ease of data analysis, we classified the intraoral applications of stem cell therapy according to soft and hard tissue regeneration.

5. Soft Tissue Regeneration

5.1. Gingiva

5.1.1. Animal Studies

Mucogingival defects

Mucogingival defects have been treated successfully in animal models with amniotic membranes used as 3D scaffolds and bone marrow stem cells. The defects were completely closed on the seventh day, colour matching was good and scaffold was well tolerated by the gingival tissues [35]. Amniotic membranes boost angiogenesis and increase reparative regeneration of damaged tissues.

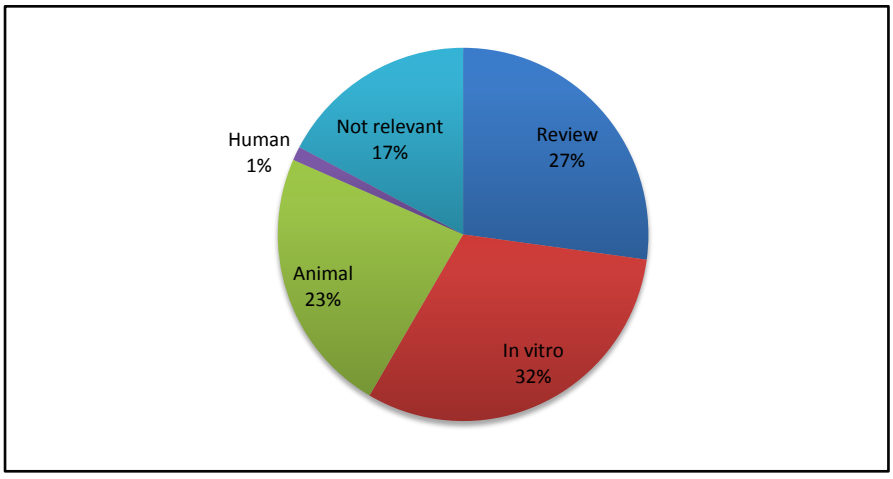

Figure 2. Percentage of the type of studies in intraoral hard and soft tissue regeneration with stem cells.

Table 2. Number of animal and human studies done to regenerate various intraoral hard and soft tissues.

	Number of Animal studies (n = 156)		Number of Human studies (n = 16)	
Gingiva	Mucogingival defects	1	Root coverage	2
	Oral mucositis	1	Papillary reconstruction	1
	Peri-implant mucosa	1		
Dental Pulp	Pulp regeneration	10	Immature teeth	1
	Pulp-like tissue	7	Pulp revascularisation	1
	Dentin/pulp complex	5		
Dentin	Dentin	6		
	Dentin-like tissue	4		
	Odontogenic differentiation	14		
Periodontal complex	Periodontal regeneration	25	Intrabony defects	4
	Furcation defects	4		
	Periodontium like structure	2		
	Cementum/PDL complex	4		
	Cementum/cementum like	5		
	Cementum and bone	1		
	Periodntal defect model	8		
	Furcation perforation defect	1		
Bone	Craniofacial/alveolar bone regeneration	8	Alveolar bone regeneration	2
	Mandibular bone defect	2	Mandible defects	1
	Bone/bone-like tissue	13	Craniofacial bone regeneration	2
	Maxillary alveolar defects (Cleft palate)	4	Alveolar cleft defects	1
	Osteonecrosis	1		
	Peri-implant bone defect	2		
	Osseointegration of implants	3		
Enamel	Enamel	4		
	Enamel + Dentin	1		
Whole tooth	Whole tooth	2		
	Bio-root	1		
	Tooth-like structure	1		
Salivary gland	Salivary gland regeneration	6	Sjogrens syndrome	1
	Radiation induced hyposalivation	3		
	Radiation induced xerostomia	1		
	Radiation damaged gland	5		

Mucositis

Spheroid GMSCs are capable of enhanced multipotency and augmented secretion of several chemokines and cytokines relevant to cell migration, survival, and angiogenesis. Using an *in vivo* murine model of chemotherapy-induced oral mucositis, Moshaverinia *et al*. demonstrated that spheroid-derived GMSCs

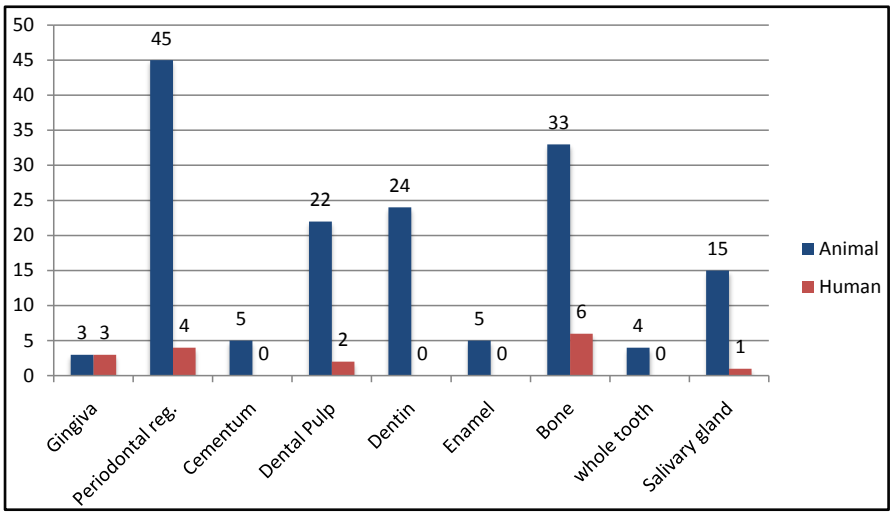

Figure 3. Comparison of the number of animal and human studies done to regenerate various intraoral hard and soft tissues.

promoted the regeneration of disrupted epithelial lining of the mucositic tongues, therefore, suggesting that 3D spheroid culture allows early stemness preservation and potentially precondition GMSCs for enhanced mitigation of oral mucositis [36].

Peri-implant mucosa

MSC transplantation accelerated the formation of the peri-implant epithelium-mediated mucosa around the implants at an early stage after implantation, hence reinforcing the epithelial seal around the dental implants [37].

5.1.2. Human Studies

Root coverage procedures

Predictable root coverage is possible for single-tooth and multiple-tooth recession defects, with subepithelial connective tissue graft (SCTG) procedures [38]. However, autogenous graft tissue procurement increases patient morbidity and duration of surgery. To avoid these complications stem cells in combination with membranes have been used clinically in the treatment of multiple gingival recession defects. Zanwar *et al*. compared the clinical efficacy between stem cells in combination with PLA/PGA membranes and subepithelial connective tissue graft (SCTG) in the treatment of multiple gingival recession defects and found that stem cells in combination with bioresorbable PLA/PGA membrane was effective for root coverage and resulted in a significant reduction in gingival recession and greater gain in CAL than SCTG [39]. Recently, various researchers have used resorbable amniotic membranes for root coverage procedures with successful outcomes [40]. Amniotic membranes not only maintain the structural and anatomical configuration of regenerated tissues, but also enhance gingival wound healing by providing a rich source of stem cells.

Papilla reconstruction

Currently, the available techniques to treat black triangles are to either augment the papilla itself or graft autogenous tissue surgically. However, these

available techniques haven't been able to demonstrate predictable results. Yamada *et al*. in 2015 investigated the potential of a tissue-engineered method for soft tissue augmentation with mesenchymal stem cells (MSC), platelet-rich plasma (PRP) and Hyaluronic acid (HA) as the scaffold and achieved predictable results with aesthetic improvements in the black triangle. This technique could emerge as a novel option for periodontal regenerative therapy in the near future [41].

5.2. Periodontal Complex

Periodontal disease is one of the most common conditions affecting humans, and the prevalence of advanced periodontitis in adults is about 15% [42]. According to the American Academy of Periodontology in 2005 [43], the formation of new bone and cementum with supportive periodontal ligament is the ultimate objective that current periodontal-regenerative therapies are incapable of fulfilling. Most of the current regenerative procedures, used either alone or in combination, have limitations in attaining complete regeneration, especially in deep periodontal defects [44]. Stem cell therapy has shown some promising results to regenerate the periodontal apparatus.

5.2.1. Animal Studies

A total of 43 studies have been done in animals to regenerate periodontal tissues using different types of stem cells. 38 out of the 43 included studies demonstrated a statistically significant improvement in periodontal tissue regeneration.

Periodontal regeneration

Out of the 38 successful studies, 25 studies showed statistically significant improvements in periodontal regeneration.

Various types of stem cells have been used to regenerate the periodontal complex. Periodontal ligament stem cells and Bone marrow derived stem cells have been used successfully in most of the studies to regenerate the periodontium [45] [46] [47].

According to a systematic review, PDL-derived cells showed a favorable effect on new PDL formation, although there was no statistically significant difference between PDL-derived or Bone marrow derived stem cells [48]. Other cell types like DPSC [49], GMSC [50], DFAT [51], SHED [52] have also been able to successfully regenerate periodontal tissue.

2 studies demonstrated regeneration of periodontium-like tissue [53] [54]. Wang *et al*. isolated human PDL stem cells (PDLSCs) and jaw-bone mesenchymal stem cells (JBMSCs) and then induced them to form cell sheets. Platelet-rich fibrin (PRF) derived from human venous blood was then fabricated into bioabsorbable fibrin scaffolds containing various growth factors. Eight weeks after implantation, the PDLSC sheets tended to develop into PDL-like tissues, while the JBMSC sheets tended to produce predominantly bone-like tissues [54].

The enhancement of PDL derived cells in PDL regeneration is probably caused by the fact that PDL-derived cells contain several subpopulations of cells,

including osteoblasts, fibroblasts, and cementoblasts, and the combined subpopulations of cells which can simultaneously synthesize both hard and soft periodontal tissues [55].

Furcation defects

A total of 4 studies have been done to treat furcation defects with stem cells. 2 studies showed promising results. They were able to improve periodontal regeneration in class II furcation defects and treated class III furcation defects with PDLSC and GTR [56]. Another study compared Autogeous cortical bone (ACB), ACB/PRP and MSC/PRP. Complete filling of class II furcation defects with cementum, alveolar bone and PDL were obtained in 8 weeks, but the efficacy of none of the groups was higher than the other [57].

Periodontal defect model

8 studies have successfully been able to regenerate bone and periodontal structures in a critical size periodontal defect model. BMSC were used in 3 studies [58] [59], one of which was a one walled intra bony defect [60], PDLSC in 2 studies [61] [62], DPSC in 1 study [49] and adipose derived stem cell in one study [63] successfully. PDLSC were also used to treat periodontal fenestration defects and was able to form bone, PDL and cementum successfully [64].

5.2.2. Human Studies

In a pilot study, Feng *et al*. examined the feasibility and safety of reconstructing periodontal intrabony defects with autologous periodontal ligament progenitor (PDLP) implantation in three patients. All treated patients showed no adverse effects during the entire course of follow up. They also found that PDLPs were analogous to PDLSCs in terms of high proliferation, expression of mesenchymal surface molecules, multipotent differentiation, and *in vivo* tissue regain. However, PDLPs failed to express scleraxis, a marker of tendon, as seen in PDLSCs [65].

In a recent randomized clinical trial, Chen *et al*. assessed the safety and feasibility of using autologous periodontal ligament stem cells (PDLSCs) as an adjuvant to grafting materials in guided tissue regeneration (GTR) to treat periodontal intrabony defects. Each group showed a significant increase in the alveolar bone height over time. The efficacy of cell-based periodontal therapy requires further validation by multicenter, randomized controlled clinical studies with an increased sample size [66].

Dhote *et al*. performed a randomized controlled clinical trial to evaluate the effectiveness of mesenchymal stem cells cultured on beta tricalcium phosphate (β-TCP) in combination with rh-PDGF-BB in treatment of intrabony defect in humans and concluded added benefit in terms of CAL gains, PPD reductions greater radiographic defect fill and improvement in Linear bone growth (LBG) compared to the OFD alone [67].

Shunsuke *et al*. [68] evaluated the safety and efficacy of autologous mesenchymal stem cells (MSCs) with a biodegradable three-dimensional (3D) woven-fabric composite scaffold and platelet-rich plasma (PRP) in ten patients with intrabony defects. The trial suggested that MSCs-PRP/3D scaffold constituted a

novel safe and effective regenerative treatment option for periodontitis.

The predictability and efficacy of outcomes, as well as safety concerns and the cost-to-benefit ratio of various treatment options are key factors to be considered for any new emerging technology. At this time, there is insufficient evidence on emerging periodontal regenerative technologies to warrant definitive clinical recommendations [69].

5.3. Dental Pulp

For treating pulpal pathological conditions, pulpal regeneration through transplanted stem/progenitor cells might be an alternative to conventional root canal treatment. A number of animal studies demonstrate beneficial effects of stem/progenitor cell transplantation for pulp-dentin complex regeneration.

5.3.1. Animal Studies

Out of a total of 22 animal studies, 15 studies demonstrated efficacy of DPSC in formation of vascularized pulp [70] [71] [72], pulp-like tissue [73] [74] or formation of pulp-dentin like complex [75] [76] in the root canal space and 1 study was able to form pulp tissue with BMSC [77].

Ishikazi *et al*. in their study demonstrated that pulp stem cells have higher angiogenic, neurogenic and regenerative potential and may therefore be superior to bone marrow and adipose stem cells for cell therapy [78].

DPSCs have rapid proliferation, multiple differentiation capacity, and development potential. Wang *et al*. transplanted these cells into the pulpless root canal with Gelfoam as the scaffold and they were capable of generating pulp-like tissues containing blood vessels and dentin-like tissue. Thickening of the root canal wall was also observed [79]. This study demonstrates the feasibility of using stem cell-mediated tissue engineering to realize pulp regeneration in immature teeth.

On the other hand, Zhu *et al*. transplanted DPSCs and/or PRP into root canals and showed no enhancement in new tissue formation compared with inducement of a blood clot into the root canals alone [80].

Many studies have found clinical success following revascularization procedures, for example, no symptoms and no periapical lesions [81] [82]. However, the histological observations from animal experiments have revealed that the tissues formed in the root canal do not reflect the regeneration of pulp-dentin but are rather formed of periodontal tissues, such as cementum, periodontal ligament, and bone [83]. The revascularization procedure has its own clinical advantages in the treatment of immature teeth, but it does not result in pulp-dentin complex regeneration in the true sense.

Another important aspect worth noting is the role of scaffolds in stem cell regeneration. Conde *et al*. [84] stated that the relationship between the scaffolds, the environment and the growth factors released from dentine are critical for de novo pulp tissue regeneration.

Misako *et al*. assessed the efficacy and safety of pulp stem cell transplantation as a prelude before the initiation of clinical trials. Granulocyte-colony stimulat-

ing factor (G-CSF) induces subsets of dental pulp stem cells to form mobilized dental pulp stem cells (MDPSCs). Therefore, the standardization and establishment of regulatory guidelines for stem cell therapy in clinical endodontics is now a reality [85].

5.3.2. Human Studies

Current applications of stem cells in endodontic research have revealed their potential to continue root development in necrotic immature teeth and transplanted/replanted teeth. In a clinical case report Surendran *et al*. [86] have demonstrated successful application of pulp revascularization and have highlighted the role of dental stem cells as a promising tool for regeneration of individual tissue types like dentine, pulp and even an entire functional tooth.

Shiehzadeh *et al*. [87] used MSC to treat necrotic/immature teeth with periradicular periodontitis, which was not treated with conventional apexification techniques. All cases developed mature apices and bone healing after 3 to 4 months after the initial treatment without complications, and faster than traditional treatments. Their clinical observations support a shifting paradigm toward a biologic approach by providing a favorable environment for tissue regeneration.

In summary, although vascularized pulp-like tissues have been formed in some experimental and clinical studies, little has been known on the function of the regenerated tissue. Studies on the cell sources of pulp regeneration via cell homing strategy might reveal more information on the signaling molecular selection. The proper signaling molecules for pulp regeneration should facilitate the recruitment of stem cells with the vasculogenic and neurogenic differentiation potential, while inhibiting cells with osteogenic or cementogenic potential (e.g., periodontal ligament cells).

5.4. Salivary Glands

Stem cell therapy has been applied to treat radiation induced damage, injury or diseases of the salivary glands. We found a total of 15 animal studies and one human study wherein stem cell therapy was used to treat a patient with Sjogren's syndrome.

5.4.1. Animal Studies

Salivary gland regeneration

Ono H *et al*. [88] attempt to regenerate salivary glands using induced pluripotent stem (iPS) cells. Glandular tissues that were similar to the adult submandibular glands (SMGs) and sublingual glands could be partially produced by the transplantation of iPS cells into mouse salivary glands. The coculture of embryonic SMG cells and iPS cells had a better-developed epithelial structure and fewer undifferentiated specific markers than monoculture of embryonic SMG cells. Their results suggest that iPS cells have a potential ability to accelerate differentiation for salivary gland development and regeneration.

Bone marrow cell extract (termed as BM Soup) has been demonstrated to repair irradiated salivary glands (SGs) and restore saliva secretion. Misuno *et al*. in

their study showed that BM Soup treatment is effective to restore the function of damaged SGs in NOD mice [89].

Radiation damaged salivary glands

Various studies have shown that local transplantation of Adipose tissue derived Mesenchymal stem cells (AdMSC) improves tissue-remodeling following radiation damage in SG tissue, and that use of a carrier enhances the protective effects of AdMSC-mediated cellular protection against irradiation induced damage via paracrine secretion and suggest that hAdMSC administration should be viewed as a candidate therapy for the treatment of radiation-induced SG damage [90] [91] [92].

Hyposalivation

Jeong *et al*. [93] isolated tissue-specific stem cells from the human submandibular salivary gland (hSGSCs). Transplantation of hSGSCs to radiation-damaged rat salivary glands rescued hyposalivation and body weight loss, restored acinar and duct cell structure, and decreased the amount of apoptotic cells. These data suggests that the isolated hSGSCs, which may have characteristics of mesenchymal-like stem cells, could be used as a cell therapy agent for the damaged salivary gland.

Xerostomia

Salivary gland hypofunction, also known as xerostomia, occurs as a result of radiation therapy for head cancer, Sjögren's syndrome or aging, and can cause a variety of critical oral health issues, including dental decay, bacterial infection, mastication dysfunction, swallowing dysfunction and reduced quality of life. Ogawa *et al*. [94] demonstrated the full functional regeneration of a salivary gland that reproduces the morphogenesis induced by reciprocal epithelial and mesenchymal interactions through the orthotopic transplantation of a bioengineered salivary gland germ as a regenerative organ replacement therapy. The bioengineered germ developed into a mature gland through acinar formations with a myoepithelium and innervation. The bioengineered submandibular gland produced saliva in response to the administration of pilocarpine and gustatory stimulation by citrate, protected against oral bacterial infection and restored normal swallowing in a salivary gland-defective mouse model. Their study thus provides a proof-of-concept for bioengineered salivary gland regeneration as a potential treatment of xerostomia.

5.4.2. Human Studies

Sjögren syndrome (SS) is a systemic autoimmune disease characterized by dry mouth and eyes, and the cellular and molecular mechanisms for its pathogenesis are complex. Xu *et al*. for the first time showed that bone marrow mesenchymal stem cells in SS-like NOD/Ltj mice and human patients were defective in immunoregulatory functions. Importantly, treatment with mesenchymal stem cells (MSCs) suppressed autoimmunity and restored salivary gland secretory function in both mouse models and SS patients. They suggested that immunologic regulatory functions of MSCs play an important role in SS pathogenesis, and allogeneic MSC treatment may provide a novel, effective, and safe therapy for pa-

tients with SS [95].

6. Hard Tissue Regeneration

6.1. Enamel

Currently a lot of investigators are interested in developing cell-based strategies to regenerate enamel. Like other tissues, regenerative treatments for enamel fundamentally require stem cells, scaffold and growth factors [96].

Cell based strategies

Huang *et al*., studied the possibility of using synthetic and bioactive nanostructures that are known to self-assemble in physiologic environments into nanofibers network, in order to mimic the extracellular matrix that surrounds the ameloblasts [97] Ameloblast-like cells and primary enamel organ epithelial (EOE) cells were cultured within Polyacrylamide (PA) hydrogels, and the PA was injected into the enamel organ epithelia of mouse embryonic incisors and transplanted under the kidney capsules in host mice for long-term culture [97].

Further study was done to elucidate the coupling response of integrin receptors to the biomaterial and gene expression profiles. These cues provide an insight into molecular mechanisms involved in enamel formation, which helps in designing synthetic regenerative approaches and to manipulate pathways to control enamel regeneration [98].

Enamel tissue engineering

Developing a technique to manipulate EOE cells for enamel replacement and attempts are made to generate enamel based on sub-cultured EOE cells using tissue-engineering technology.

Honda *et al*. examined the enamel-forming capability of sub-cultured EOE cells, by transplanting cells onto a biodegradable scaffold *in vivo* [99]. Four weeks after transplantation of EOE cells combined with dental pulp cells in scaffolds, several phenomena related to amelogenesis were distinguished in the implants [99]. In the most mature structures, enamel was readily found in the implants. Furthermore, amelogenin immunoreactivity was detected in tall columnar epithelial cells on the surface of the dentin or enamel, indicating that the tissue-engineered enamel contains well-developed ameloblasts. Together, these results indicate that the sub-cultured EOE cells have the potential to generate enamel and provide a promising step towards a new therapy for reforming enamel.

Future trend may be the application of genes for enamel formation.

6.2. Dentin Regeneration

Out of a total of 24 animal studies 10 studies demonstrated regeneration of dentin or dentin-like tissue and 14 studies demonstrated the odontogenic potential of stem cells.

Animal studies

Regeneration of dentin tissues in the pulp space of teeth serves as the ultimate goal of preserving teeth via endodontic approaches. In recent times, many stu-

dies suggested that human dentin scaffolds combined with dental stem cells was a potential strategy for the complete dentin tissue regeneration. Tran *et al*. found that DPSCs was able to regenerate dentin-like tissues, which expressed specific dentin markers such as dentin sialophosphoprotein and dentin matrix protein 1 [100].

Reparative dentin formation is essential for maintaining the integrity of dentin structure during disease or trauma. Zheng *et al*. investigated stem/progenitor cell-based tissue engineering for dentin regeneration in a large animal model. At 16 weeks after transplantation, the PDPSCs mixed with β-TCP significantly regenerated the dentin-like structures and nearly completely restored the pulp chamber roof defects. This study demonstrated that the PDPSC/scaffold construct was useful in direct pulp-capping and provides pre-clinical evidence for stem/progenitor cell-based dentin regeneration [101].

In a comparative study of human dental follicle cells and human periodontal ligament cells it was observed that although PDLCs could form the dentin-like tissues, the structure of dentin tissues generated by DFCs was more complete [102].

Autologous mesenchymal BMSCs were able to promote hard-tissue formation after direct pulp capping procedures [103].

6.3. Cementum Regeneration

A total of 10 animal studies were relevant with regards to cementum regeneration with stem cells. Out of which 5 studies were regarding regeneration of cementum or cementum like tissue [104], 4 studies attempted regeneration of the cementum-PDL complex [105] and 1 study cementum and bone [106].

Animal studies

These results suggest that the mixed-type hPDLSC pellet could mimic the microenvironment of PDL and enhance the reconstruction of physiologic architecture of a dental cementum/PDL-like complex. This tissue mimicking may also be a promising alternative to promote periodontal defect repair for additional clinical applications [105].

Lee *et al*. in their study stated that colocalization of biomolecules at zones of the PDL adjacent to attachment sites may be essential for the formation of pre-cementum and osteoid interfaces at a load-bearing bone-PDL-tooth fibrous joint. Biophysical cues resulting from development and function can regulate recruitment and differentiation of stem cells potentially from a vascular origin toward osteo- and cemento-blastic lineages at the PDL-bone and PDL-cementum entheses [107].

The regenerative abilities of cementum with inserted PDL are important for the prevention of tooth loss. Jin *et al*. investigated the effects of recombinant human plasminogen activator inhibitor-1 (rhPAI-1) on cementogenic differentiation of human PDLSCs (hPDLSCs) and found that rhPAI-1 induced hPDLSCs to regenerate cementum-like tissue with PDL fibers inserted into newly formed cementum-like tissue suggesting that rhPAI-1 may play a key role in cemento-

genic differentiation of hPDLSCs and may be a good candidate for future clinical applications in periodontal tissue regeneration and possibly in tooth root bioengineering [108].

Local activation of canonical Wnt signaling resulted in *in vitro* cementogenic differentiation of hPDLCs and significant new cellular cementum deposition and the formation of well-organized periodontal ligament fibers *in vivo* [109].

6.4. Bone Regeneration

Numerous studies have been done in an attempt to regenerate bone. A total of 35 animal studies were successful in regenerating bone and bone like tissues [110] [111]. 5 clinical studies were identified wherein stem cells were used to regenerate functional bone in alveolar deficiencies [112] [113], Mandible defects [114] and craniofacial bone regeneration [115] [116].

Animal studies

Nakajima *et al*. collected granulation tissue from dog dental socket 3 days after tooth extraction. The ability of dDSCs to regenerate periodontal tissue in a one-wall defect model resulted in cementum-like and periodontal ligament-like tissues and alveolar bone, whereas only bony tissue was observed in the control group (β-TCP/PGA) [117].

Morad *et al*., reported *in vivo* use of DPSC for bone regeneration by using different experimental models and types of scaffolds with different results [118]. Cavalcanti *et al*., and Moshaverinia *et al*. reported successful bone regeneration using DPSC in alginate or Pura matrix in *in vitro* and *in vivo* studies [119] [120].

Peri-Implant bone defect

During an immediate implant placement the large bone defect peri-implant has a negative influence on the process of bone healing. Various researchers have been able to successfully promote new bone formation and accelerate bone formation in the peri-implant defect with PRF/Umbilical cord mesenchymal stem cells [121], HA-based scaffolds previously seeded with Adipose derived stem cells [122] and BMMSC [123].

6.5. Clinical Studies

Using cell therapy, Rajan *et al*. reported the upper jaw reconstruction of a patient who lost teeth and 75% of the supporting jawbone following injury. Clinical, radiographic, and histological analyses confirmed that by 4 months, the cell therapy regenerated 80% of the original jawbone deficiency with vascularized, mineralized bone sufficient to stably place oral implants. Functional and aesthetic rehabilitation of the patient was successfully completed with installation of a dental prosthesis 6 months following implant placement. This proof-of-concept clinical report used an evidence-based approach for the cell transplantation protocol and is the first to describe a cell therapy for craniofacial trauma reconstruction [116].

A clinical trial was performed on seven patients with mandibular bone defects by transplant of DPSC in a collagen scaffold. A 3 years of follow-up, (Giuliani *et*

al., 2013) reported good clinical outcomes. The regenerated tissue from the graft sites was composed of a fully compact bone with a higher matrix density than control human alveolar spongy bone from the same patient. Thus, the regenerated bone, being entirely compact, is completely different from normal alveolar bone [114].

In a randomized controlled trial, transplantation of Tissue repair cells (TRCs) from bone marrow for treatment of alveolar bone defects appeared safe and accelerated bone regeneration, enabling jawbone reconstruction [115]. Secreted growth factors and cytokines in the conditioned medium from bone marrow-derived mesenchymal stem cells (MSC-CM) have several effects on cell behavior. Katagiri *et al*. in their study showed that MSC-CM had great osteogenic potential for regeneration of bone [113]. Injectable tissue-engineered bone (TEB) composed of mesenchymal stem cells (MSCs) and platelet-rich plasma was able to regenerate functional bone in alveolar deficiencies and was able to restore masticatory function in patients [112].

6.6. Cleft Palate

Scaffolds are presently employed for cleft palate repair. Scaffolds induce cell growth, host cell migration, cell attachment and absorbs at the rate of bone formation. It allows cell-to-cell interaction and diffusion of nutrients and metabolites [124]. Materials like Collagen [125], Hyaluronic acid [126], Hydroxyapatite [127], Mesoporous bioactive glass [128], Poly (epsilon-caprolactone)/nano-fluoridated hydroxyapatite (PCL-FHA) [129], Platelet rich fibrin (PRF) etc. are used as scaffolds.

4 studies reported success of alveolar defect repair using DPSC [132], BMSC/PRF [130], Adipose derived stem cells [131] and mesenchymal stem cells derived from iliac bone [133].

Both bone marrow-derived mesenchymal stem cells and platelet-rich fibrin are capable of improving the repair of dog alveolar cleft, and the mixture of them is more potent than each one of them used singly for enhancing new bone regeneration [131]. In another study, autografts and tissue-engineered bone were used for bone regeneration. Bone formation on the autograft sides was higher than on the stem cell sides, demonstrating that autograft is still the gold standard for bone regeneration and tissue engineered bone may provide an acceptable alternative [132].

Perfect closure of the jaw cleft in dogs was achieved six months after the transplantation of MSC. The X-ray and histological examination revealed that the regenerated bone on the experimental side was almost equivalent to the original bone adjoining the jaw cleft. It was suggested the application of MSCs with Carbonated Hydroxyapatite (CAP) particles can become a new treatment modality for bone regeneration for cleft lip and palate patients [133].

Human Studies

Behnia *et al*. evaluated the effect of recombinant platelet derived growth factor and *in vitro* osteogenic differentiated human bone marrow mesenchymal stem

cells (hBMMSCs) in alveolar cleft defects. They reported significant improvement in bone regeneration three months after the operation, suggesting an enhancement effect of recombinant platelet derived growth factor with hMSCs on regeneration capacity of the cells [134].

7. Whole Tooth Regeneration

Ikeda *et al*. reported that Suji T and team formed a fully functional complete tooth by combining isolated mouse dental epithelial and MSC [135]. In 2011, Oshima *et al*. reported that the same research group developed a complete tooth unit consisting of a mature tooth, periodontal ligament and alveolar bone, which were transplanted into toothless mouse jaw regions *in vivo*, and erupted correctly and in occlusion [136]. Angelova Volponi *et al*. reported success using adult gingival epithelial cells, which upon recombination with mesenchymal cells generate mature teeth and form enamel and dentin [137]. Similarly, Yang *et al*. generated a complete tooth by implantation of a tooth germ like structure in animal models [138].

In tissue engineering of teeth with stem cells, platelet-rich fibrin (PRF) that is rich in growth factors and cytokines, may improve regeneration. PRF added into fibrin glue to enrich the microenvironment with growth factors shows promising results when transplanted with Dental bud cells. One animal developed a complete tooth with crown, root, pulp, enamel, dentin, odontoblast, cementum, blood vessels, and periodontal ligaments in indiscriminate shape. This study demonstrated that DBCs seeded into fibrin glue-PRF could regenerate a complete tooth [139]. Other authors have employed iPS-derived cells to generate mature bioengineered teeth by similar recombination methods [140] [141].

Although many animal model studies have been reported a complete human study is yet to be documented.

8. Conclusions

Stem cell therapy applications are observed to show promising results as future trends points out to successful clinical outcomes.

The present review concludes

1) On comparison between dental hard and soft tissue regeneration and repair via stem cell therapy it has been observed that more number of clinical case reports is seen with dental hard tissue repair than soft tissue.

2) The majority of the studies are still *in vivo* animal studies in both dental hard and soft tissues.

3) The ultimate aim of stem cell therapy is to regenerate or repair dental tissues without much of surgical hazards.

4) Whole tooth regenerations are still in the animal model research phase.

5) More number of successful clinical follow-ups of more than five years is required to establish credibility of stem cell therapy in oral application.

6) Development of newer scaffolds, understanding cues of various signaling molecules and understanding gene expression and proteomics of stem cells are

future challenges that will have to be addressed for successful regeneration through stem cell therapy.

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