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Review Article

Dental Stem Cells: The New Trend in Regenerative Medicine. An Overview

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Abstract

Dental stem cells are the new trend in regenerative medicine as well as dentistry.

The aim of this review paper is to highlight the different types and sources of dental stem cells as well as their different fields of use not only in regenerative medicine but also in regenerative dentistry.

Keywords: *Dental Stem Cells; Regenerative Medicine*

Introduction

A wide variety of stem cell niches have been identified within the human body [1] not only in bone marrow, adipose tissue and umbilical cord but also recently in teeth, which were found to be a noninvasive source of stem cells which are easy, convenient and affordable to collect. Dental stem cells hold promise for a range of very potential therapeutic applications in the field of tissue engineering which is the process of replacing or regenerating human cells, tissues or organs for therapeutic applications [2].

This concept, although not new, has significantly progressed after the discovery of stem cells and in recent times have found its application in dentistry following the identification of the dental stem cells in 1932, when Feldman was able to prove that dental pulp stem cells did grow under certain biological conditions.

Furthermore, oral tissues are expected to be not only a source but also a therapeutic target for stem cells, as tissue engineering therapies in dentistry continue to attract increasing clinical interest which is evident by the rapidly growing literature in this field as well as by the rapid and extensive development of dental stem cells banks worldwide [2,3].

The objective of this review article is to highlight the different types and sources of dental stem cells as well as the different fields of their use not only in regenerative medicine but also in regenerative dentistry.

Cellular biology of stem cells

the human body counts more than 200 different cell types which are organized into tissues and organs in order to keep their vitality and function [1,3]. In some tissues, such as the blood, and the skin two things are to be noted; the impossibility of self-renewal and the short lifespan of the differentiated cells. This led to the concept that such tissues are maintained by stem cells, which were defined by Lajtha [4] in 1979 as "cells with extensive renewal capacity and the ability to generate daughter cells that undergo further differentiation".

However, to be considered as stem cell, a cell should exhibit additional characteristics such as rarity, capacity for asymmetric division or tendency to infrequently division [5].

Weissman defined stem cells as "Stem cells are not only units of biological organization, responsible for the development and the regeneration of tissue and organ systems, but also are units in evolution by natural selection" [7].

Origins of stem cells

Currently, there are four broad categories of stem cells that are investigated in regenerative medicine: embryogenic stem cells (ESCs), stem cells derived from placenta or amniotic fluid (AFPSCs), adult stem cells (ASCs) and induced pluripotent stem cells (IPSCs) [6].

Embryonic stem cells (ESC) are stem cells isolated from an early stage embryo. these stem cells are pluripotent and have high proliferative capacity in culture since it can be expanded through far more passages _compared to adult stem cells without reaching senescence.

They are isolated from the inner cell mass of blastocysts and can be differentiated towards numerous somatic cells with varying phenotypes and therefore have a great therapeutic potential; however, their use is limited by ethical controversy as well as their potential tumorigenicity [8,9].

Amniotic fluid and placenta stem cells: These cells are derived from the amniotic fluid and placental membrane of the developing fetus and are currently being investigated for a variety of applications such as a source of immunomodulatory cells for a variety of immunotherapies [10,11].

Adult stem cells (ASC): They were first described by Friedenstein and colleagues in the 1970s and are the most well studied and most used cell type in the field of stem cell therapy. They have been identified throughout the body and thought to act as tissue-specific progenitors capable to repair the damaged tissue and to restore its function.

Multipotent adult progenitor cells are considered as a unique subset of ASCs that are often isolated from bone marrow stroma, However recent studies have demonstrated that they may also be found in other well-vascularized tissues including adipose, muscle, endometrium, and kidney [13].

Induced pluripotent stem cells (IPS): They were recently discovered by Takahashi and Yamanaka [14]. These cells can be directly generated from fibroblast cultures by the addition of defined factors and can exhibit the morphology and growth properties of ESC and express ESC marker genes. However, the time involved in resetting the cells to a pluripotent state followed by the additional time required to induce the cells to differentiate into the desired lineage is a major disadvantage. Furthermore, full transition to this new desired lineage may not occur.

Types of stem cells

The different types of stem cells are classified based on their capacity for differentiation to:

- **Totipotent stem cells:** Are cells that can generate an entire, viable organism as well as cells of the three germ layers (endoderm, ectoderm and mesoderm). We can only consider the fertilized ovocyte and the first few stages of cell division as totipotent cells [8,12]. This property is only exhibited by embryonic stem cells [1].

- **Pluripotent stem cells:** They can generate any type of cell derived from the three germ layers, but not a whole functional organism [8,12]. These cells are found in embryonic, fetal and, to some extent, in adult tissues.

- **Multipotent stem cells**: They can only produce a limited number of cell types, generally those of a closely related family of cells. For example, the hematopoietic stem cell can only generate cells of the hematopoietic system (e.g. mast cells, macrophages, neutrophils, eosinophils, platelets, erythrocytes and lymphocytes) [8,12].

- **Unipotent stem cells:** They can only differentiate into one type of cell or tissue. They have the lowest differentiation potential but still have a vast therapeutic use in treating injuries and diseases. These cells can be found in adult tissues such as muscular tissue [8,12].

Dental stem cells: History of dental stem cells

The concept of regeneration has known a significant advance following the discovery of stem cells in recent times and have found its application in dentistry after the identification of dental stem cells.

Feldman in 1932, was among the first to show evidence of regeneration of the dental pulp under certain optimal biological conditions. This work aimed to introduce the biological-aseptic principle of tooth therapy by pulp regeneration using dentine fillings as building material for stimulating pulp regeneration. Later, this work was improved by subsequent researchs until the major breakthrough in dental history when Gronthos., *et al.* in 2000 identified and isolated odontogenic progenitor population in adult dental pulp [15].

These cells were referred to as dental pulp stem cells (DPSCs). Since this discovery several researchers have reported varieties of dental stem cells, which differ in many biological features such as their growth rate, gene expression and the inclination for cell differentiation and that can be classified into following.

Dental pulp stem cells (DPSCs)

The first stem cells isolated from adult human dental pulp, called dental pulp stem cells (DPSCs) were first isolated from permanent third molars, and showed high proliferation and high frequency of colony formation by producing sporadic, but densely calcified nodules [15,16]. Additionally, DPSCs have the ability to generate functional dental tissue in the form of dentine/pulp-like complexes and to differentiate into other mesenchymal cell derivatives *in vitro* such as odontoblasts, adipocytes, chondrocytes and osteoblasts [15].

Periodontal ligament derived stem cells (PDLSCs)

These cells are capable of differentiating along mesenchymal cell lineages to produce - *in vitro* and *in vivo*- cementoblast-like cells, adipocytes and connective tissue which is rich in collagen type I. Therefore, PDLSCs are considered as the best provider of periodontal ligament-like structures [18-20].

Gingival derived mesenchymal stem cells (GMSCs)

They are isolated from the gingival lamina propria. They exhibit several stem cell-like properties such as: *in vitro* proliferation, colony-forming ability, expression of mesenchymal cell surface markers and stem cell-specific genes expression as well as multipotent differentiation into mesodermal (adipocytes, osteocytes), endodermal and neuroectodermal progenies.

These cells have the ability of self-renewal and differentiation. In addition, compared to mesenchymal stem cells derived from DPSCs and PDLSC, GMSCs express a similar profile of cell surface molecules, a higher proliferative rate and an increased population doubling. All these characteristics make GMSCs a good source for many cell-based clinical applications [17].

Stem cells from human exfoliated deciduous teeth (SHED)

Stem cells isolated from the pulp of human exfoliated deciduous teeth have the capacity to induce bone formation, to generate dentine and to differentiate into other non-dental mesenchymal cell populations *in vitro* [22].

In addition, they exhibit a higher proliferation rates, an increased population doublings an osteoinductive capacity *in vivo* and an ability to form sphere-like clusters. SHED are able to differentiate into odontoblasts in order to generate tubular dentine and angiogenic endothelial cells [23].

Immature dental pulp stem cells are a population of stem cells found in the pulp of human deciduous teeth that are able to differentiate into osteocytes, adipocytes, neurons and skeletal muscle [24].

Stem cells from the apical papilla (SCAP)

The apical papilla tissue is only present during the root development of immature teeth. At the tips of these growing tooth roots, a population of stem cells called SCAP is located. SCAP are able to differentiate into odontoblasts and adipocytes [21].

Compared to DPSCs, they showed *in vitro* a higher proliferative rate. When co-transplanted into baby pigs' tooth sockets SCAP and PDLSC gave rise to dentin as well as periodontal ligament. These findings suggest that SCAP, together with PDLSC, could be used, in the future, to create a biological root that can be capped with an artificial dental crown as replacement to missing teeth, instead of using a metal dental implant [25].

Dental follicle stem cells (DFSCs)

The dental follicle is a soft tissue that surrounds the developing tooth germ. It contains stem cells that can differentiate into cementoblasts, osteoblasts and periodontal ligament cells. Similar to other dental stem cells, dental follicle stem cells express similar cell surface marker and have a relatively higher proliferation rate. DFSCs have an osteogenic differentiation ability under appropriate conditions. The neural differentiation potential of DFSCs has also been explored [26].

Tooth germ progenitor cells (TGPCs)

Human tooth germ stem cells display numerous mesenchymal stem cells properties and can differentiate into several cell types. These cells are usually isolated from the pulp of the teeth germs and are considered to be an alternative source to adult stem cells [17].

Recent researches found that TGSCs can successfully differentiate into osteogenic and odontogenic cell lineages *in vitro*. However, they may not differentiate into all cell lineages necessary for the dental regeneration [26].

Fields of use of dental stem cells

The therapeutic use of dental stem cells has been explored all around the world using *in vitro* and *in vivo* models by several clinical groups. It has been proved that these cells possess tremendous potential in regenerative medicine as well as in dentistry.

Fields of use of dental stem cells in regenerative medicine

Neural differentiation of dental pulp stem cells

The dental pulp stem cells extracted from both rats' and humans' teeth expressed neuronal phenotype and produced neurotrophic factors, including nerve growth factor, brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor) [28]. These factors promoted the survival of the sensory and the dopaminergic neurons and collateral sprouting. Besides, the DPSCs display several multipotency markers that indicate a spontaneous neural differentiation [29]. Moreover, it has been proved that DPSCs protected primary neurons and helped in the cell viability. In addition, conditioned media from dental stem cells can enhance the growth rate of Schwann cells and induce the neurite outgrowth *in vitro*. In a model of a rat's sciatic nerve injury, dental stem cells had the ability to induce axon regeneration. However, among all the dental stem cells, SCAPs have shown the greatest potential for neurotrophic effect [30]. Appel., *et al.* reported that DPSCs' exosomes have the ability to penetrate the blood-brain barrier and to reduce or replace neuronal loss in Parkinson's disease [32].

In another experimental study, the DPSCs were transplanted into rats with completely severed spinal cords. It was demonstrated that DPSCs promoted the regeneration of transacted axons by directly inhibiting multiple axon growth inhibitors and preventing the apoptosis of neurons, astrocytes and oligodendrocytes. The DPSCs also differentiated into mature oligodendrocytes to replace cells that were lost.

Mead., *et al.* assessed the therapeutic benefit of implanted rat DPSCs into the vitreous body of the eye after a surgically induced optic nerve crush injury and proved that DPSCs promoted the neurotrophic-mediated survival of the rat ganglion cells as well as the axon regeneration [28].

Role of dental pulp stem cells in myocardial infarction

Gandia., *et al.* evaluated the therapeutic potential of DPSCs with regards to myocardial infarction. To examine whether these cells could have therapeutic potential in the repair of myocardial infarction (MI), DPSC were infected with a retrovirus encoding the green fluorescent protein (GFP) and expanded *ex vivo*. Seven days after induction of myocardial infarction by coronary artery ligation, 1.5 x 10(6) GFP-DPSC were injected intramyocardially in nude rats. At 4 weeks, cell-treated animals showed an improvement in cardiac function, observed by percentage changes in anterior wall thickening left ventricular fractional area change, in parallel with a reduction in infarct size. These data suggest that DPSC could provide a novel alternative cell population for cardiac repair, at least in the setting of acute myocardial infraction [35].

Differentiation of dental pulp stem cells into muscular tissue

Yang., *et al*. discovered that clones of mononucleated stem cells of human tooth pulp fused into multinucleated myotubes that robustly expressed myosin heavy chain *in vitro* with or without co-culture with mouse skeletal myoblasts (C2C12 cells) [36].

furthermore, DPSCs were able to differentiate into dystrophin-producing muscle cells in muscles that were paralyzed by cardiotoxin in a mouse model.

Role of dental pulp stem cells in tendon and cartilage regeneration

Tendons have a very limited ability for self-repair when injured. Given the similarity between the tendons and the periodontal ligament by the fact that they both have the potential to absorb mechanical forces of stress and strain, PDLSCs can be used for tendon regeneration. Gronthos., *et al.* reported that ovine PDLSCs express scleraxis, a tendon-specific transcription factor, *in vitro* [18]. Moreover, Cell sheet technology has achieved important advancement with regards to the regeneration of injured cartilage, in animal models, using PDLSCs and GMSCs.

Therapeutic role of dental pulp stem cells in ischemia, angiogenesis and vasculogenesis

Stem cells and endothelial progenitor cells can be used to stimulate vasculogenesis as a potential treatment for ischaemic disease.

Iohora., *et al.* have successfully isolated a highly vasculogenic DPSCs -similar to endothelial progenitor cells- from porcine dental pulp [37].

Besides, DPSCs showed successful engraftment and an increase in the blood flow, including high density of capillary formation in a rat model suffering from hind limb ischemia.

Furthermore, Bronckaers., *et al.* proved, the suitability of DPSCs for the treatment of pathologies correlated with inadequate angiogenesis, such as coronary artery disease [38].

Differentiation of dental pulp stem cells into bone cells

According to Laino., *et al.* DPSC can be differentiated into osteoblasts, as assessed by a large positivity to several specific antibodies started to secrete an extracellular mineralized matrix, which, 2 weeks later, built a considerable number of 3D woven bone samples called living autologous bone (LAB). These bone samples, after *in vivo* transplantation into immunosuppressed rats, were remodeled in a lamellar bone containing entrapped osteocytes, providing strong evidence that human deciduous teeth are a source of stromal stem cells that can be considered as an ideal source of osteoblasts, as well as of mineralized tissue, ready for bone regeneration, transplantation, and tissue-based clinical therapies [39].

In addition to that, it has been shown that there were no differences when comparing stem cells and differentiated cells obtained from young and older subjects teeth, regarding their expansion rate and the LAB nodules obtained.

Graziano., *et al.* and Aquino., *et al.* assessed bone regeneration by DPSCs both clinically and radiographically using a collagen scaffold and showed that within three months of colonization on the scaffold, a complete radiographic bone regeneration which dimensions were equal to the grafted scaffolds could be observed and that the bone differentiation and its maturation were almost complete. Moreover, Haver's channels containing blood vessels and surrounded by bone arranged in a lamellar configuration with an evident and functionally efficient vascularization took place [40,41].

Costa., *et al.* and Chadipiralla., *et al.* compared the osteogenic differentiation of the PDLSCs and the SHED and concluded that PDLSC had a better osteogenic potential because of its higher cell proliferation rate [43,44].

Dental pulp stem cells considerations for diabetes

Diabetes is a chronic degenerative endocrinal disease in which the autoimmune destruction of pancreatic β-cells or the decreased insulin sensitivity leads to a persistent hyperglycaemia.

The potential of DPSCs to differentiate into pancreatic cell lineage resembling islet-like cell aggregates has been explored by Govindsamy., *et al.* who showed that *in vitro* islet-like cell aggregates glucose-dependently released insulin and C peptide [45].

Besides, Carnevale., *et al.* demonstrated that human DPSCs under appropriate stimuli, expressed genes related to pancreatic β-cell development and function [46].

Dental stem cells considerations for autoimmune diseases

Le Blanc., *et al.* and Sun., *et al.* proved that mesenchymal stem cells have immunomodulatory properties that can be used clinically to treat some autoimmune diseases, such as graft-vs.-host disease, systemic lupus erythematosus and systemic sclerosis [47,48].

Yamaza., *et al.* demonstrated that SHED were shown to possess an immunomodulatory function that leads to successful therapies for immune diseases and that SHED transplantation is effectively capable of reverse the systemic lupus erythematosus-associated renal disorders such as nephritis with glomerular basal membrane disorder and mesangial proliferation in a systemic lupus erythematosus-like mice [49].

Moreover, Ding., *et al.* investigate the immunological properties of DPSCs and the underlying mechanisms and came to the conclusion that DPSCs are low immunogenic, could inhibit the proliferation of lymphocytes, regulate the production of cytokines *in vitro* [50].

Dental pulp stem cells differentiation to hepatocyte

Ishkitieve., *et al.* were among the first researchers who differentiated hepatocyte like cells from deciduous tooth pulp stem and extracted third molar pulp stem cells with a protocol that used fetal bovine serum. But it showed high contaminations of nondifferentiated cells and concluded that without serum both cell types differentiated into high-purity hepatocyte-like cells. These cells offer a source for hepatocyte lineage differentiation for transplantation in the future.

However, a difference in the markers' expression between SHED and DPSCs was found suggesting that SHED might be a better hepatic progenitor source than DPSCs [51].

Fields of use of dental stem cells in dentistry

The use of dental stem cells in the dentin-pulp regeneration

Gronthos., *et al*. first isolated human DPSCs from dental pulp of impacted third molars. When *in vitro* expanded DPSCs were mixed with hydroxyapatite/tricalcium phosphate ceramic powder and then transplanted into immunocompromised mice, a dentin–pulp-like complex was observed within 6 weeks [17].

Other dental mesenchymal stem cells, such as SHED and SCAP, also generated the dentin-pulp-like complex after their subcutaneous transplantation. In 2003, Batouli., *et al.* [52] loaded DPSCs onto a human dentin surface and then subcutaneously transplanted them into immunocompromised mice. eight weeks after the transplantation, DPSCs were able to form a reparative dentin-like structure on the human dentin surface. Inside the dentin-like structure, blood vessels and connective tissue were observed, indicating that a dentin–pulp complex was formed.

Further study showed that the pretreatment of the dentin could influence the cellular behavior of the subcutaneously transplanted DPSCs at the cell–dentin interface. On EDTA-treated dentin cylinders, DPSCs could form a vascularized soft connective tissue similar to the dental pulp, however, on the NaOCl-treated dentin cylinders, DPSCs did not organize well. These data suggested that dental mesenchymal stem cells are able to regenerate dental pulp on calcific carriers under certain conditions [53].

Nevertheless, regenerating dental pulp inside the pulp chamber by transplanting dental mesenchymal stem cells remains a major challenge because of the lack of an abundant blood supply, most transplanted stem cells undergo a necrotic or an apoptotic cell death. In this perspective, Huang., *et al*. in 2010 showed that SHED and DPSCs could regenerate a pulp-like tissue in an emptied root canal space (6 - 7 mm in length) with an enlarged diameter (2 mm) after their subcutaneous transplantation [54]. The use of the granulocyte-colony stimulating factor showed stimulative effects on the angiogenesis and the stem cell mobilization [55].

In 2013, Iohara., *et al.* [56] and Murakami., *et al.* [57] showed that the autologous transplantation of DPSCs and granulocyte-colony stimulating factor into a dog's pulpectomized tooth resulted in the formation of a pulp-like tissue with a good vascularization and a good innervation. The DPSCs differentiated into odontoblasts-like cells that were attached to the dentinal wall in the root canal. No infiltration of the inflammatory cells was observed in the periapical region 30 days after the transplantation. In addition, there was no internal or external

resorption of the tooth. The three-dimensional images of the vascularization in the regenerated tissue were also similar in their density, as well as their orientation, to those of a normal pulp. Nerve fibers positively stained by Protein Gene Product 9.5 antibody invaded the newly regenerated tissues, then formed a dense subodontoblastic plexus, and finished in the odontoblastic layer proving that the neurogenesis and the reinnervation of the regenerated tissue really took place. In the apical part of the root canal, the diameter of the physiological apical foramen gradually decreased by the formation of additional dentin and cementum.

The use of dental stem cells in root formation

In a study led by Huang., *et al.* in 2008, a continued root tip formation was observed in an injured tooth with preserved apical papilla despite the surgical removal of the pulp. This finding suggested that the continued apical development is due to dentin formation from the apical papilla. However, to verify whether the evidence of continued apical development were not only due to cementum formation, the authors used mini pigs model in which a surgical removal of the apical papilla at an early stage of the root development was performed. This resulted in the alteration of the root development despite the pulp tissue being intact, while the other roots of the same tooth containing apical papilla showed normal growth and development. Although the finding suggested that the apical papilla is likely to play a pivotal role in the root formation, further researches are needed to verify that this halted root development in the mini pig was not due to damage of Hertwig's epithelial root sheath during the removal of the apical papilla of that particular root apex [21].

The use of dental stem cells in the bioroot engineering and the tooth reconstruction

Using the animal study models, cells isolated from tooth buds seeded onto scaffolds were able to form ectopic teeth *in vivo*.

In 2006, Nakao., *et al.* [58] bioengineered teeth by we developing a three-dimensional organ-germ culture method. The bioengineered tooth germ generated a structurally correct tooth, after both *in vitro* organ culture as well as transplantation under a tooth cavity *in vivo*, showing penetration of blood vessels and nerve fibers.

Honda., *et al.* [59] proved that cultured rat tooth bud cells, when seeded onto biodegradable scaffolds then implanted into the jaws of adult rat hosts for 12 weeks, were able to form small, organized, bioengineered tooth crowns that contained dentin, enamel, pulp as well as periodontal ligament tissues, similar to identical cell-seeded scaffolds implanted and grown in the omentum. In the other hand, the bioengineered teeth consisted of organized dentin, enamel, and pulp tissues proved by radiographic, histological, and immunohistochemical analyses. This study advanced practical applications for dental tissue engineering by demonstrating that bioengineered tooth tissues can be regenerated at the site of previously lost teeth.

In the study of Kuo., *et al.* [60] tooth-like structures, including well-organized dentin-pulp complex, cementum, and periodontal ligament, were evident in the original alveolar socket, 36-week post-transplantation of the swine dental bud cells (DBCs).

The DBCs were first isolated from the developing mandibular teeth, expanded *in vitro* and cultured onto cylinder scaffold gelatin-chondroitin-hyaluronan-tri-copolymer (GCHT). After culturing *in vitro*, the DBCs/GCHT scaffold was autografted back into the original alveolar socket. These findings provided a technical advance for tooth regeneration.

Sonoyama., *et al.* [61] transplanted both human SCAP and periodontal ligament stem cells (PDLSCs) to generate a root/periodontal complex capable of supporting a porcelain crown, to restaure a normal tooth function. Using a minipig model, autologous SCAP and PDLSCs were loaded onto tricalcium phosphate/hydroxyapatite and gel foam scaffolds and implanted into sockets of the lower jaw. Three months later, the bioroot was exposed, and a porcelain crown was inserted. The bioroot was different from the natural root in that the root structure is developed by SCAP in a random manner. Nevertheless, the bioroot was encircled with a periodontal ligament tissue and appeared to have a natural relationship with the surrounding bone.

The use of dental stem cells in periodontal regeneration

The challenge for cell-based replacement of a functional periodontium is to form a new ligament and a new bone, and to ensure that the appropriate connections are made between these tissues, as well as between the bone and the tooth root. One aim of current research is to use different populations of dental stem cells to replicate the key events in periodontal development both temporally and spatially, so that the healing can occur in a sequential manner to regenerate the periodontium [62].

A conceptually simpler approach to the periodontal regeneration methods involves engineered cell sheets to facilitate the human periodontal ligament cell transplantation [63]. The periodontal ligament cells isolated from a human third molar tooth were cultured on poly N-isopropylacryl-amide-grafted dishes that induced spontaneous detachment of the cells as viable cell sheets upon low temperature treatment. The human periodontal ligament cells sheets were implanted into athymic rats that had the periodontium and the cementum removed from their first molars. Fibril anchoring resembling native periodontal ligament fibres, together with an acellular cementum-like layer, were observed, indicating that this technique could be applicable to future periodontal regeneration [63].

Although promising, this approach does not take into account any replacement of the bone that might be required. The outstanding issue with these approaches is the extent to which any reconstituted periodontium can maintain integrity and function during mastication over long periods of time.

Conclusion

Here may still be a long way to go before the dental stem cells become more and more of a reality. These stem cells have multiple applications, nevertheless there are certain limitations as well. Starting with the difficulty to identify, isolate, purify and grow these cells consistently in labs which is considered to be a complicated, expensive and time consuming procedure. Other major limitations is the oncogenic potential as well as the immune rejection of these cells which are still to be determined in long-term clinical studies. Moreover, the researchs are mainly confined to animal models and their extensive clinical application is yet to be tested. Last and not least, stem/progenitor cells are comparatively less potent than the embryonic stem cells. Teeth-like structures cannot replace actual teeth, thus a considerable research and development efforts is required to advance the dental regenerative therapeutics. Researchers still need to grow blood and nerve supply of teeth to make them fully functional. Although not currently available, these approaches may one day be used as biological alternatives to the synthetic materials currently used.

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