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Nanodentistry: combining nanostructured materials and stem cells for dental tissue regeneration

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Summary

Regenerative dentistry represents an attractive multidisciplinary therapeutic approach that complements traditional restorative/surgery techniques and benefits from recent advances in stem cell biology, molecular biology, genomics and proteomics. Materials science is important in such advances to move regenerative dentistry from the laboratory to the clinic. The design of novel nanostructured materials such as biomimetic matrices and scaffolds for controlling cell fate and differentiation, and nanoparticles for diagnostics, imaging and targeted treatment is needed. The combination of nanotechnology, which allows the creation of sophisticated materials with exquisite fine structural detail, and stem cell biology turn out to be increasingly useful in regenerative medicine. The administration to patients of dynamic biological agents composed by stem cells, bioactive scaffolds and/or nanoparticles will certainly increase the regenerative impact of dental pathological tissues. This overview briefly describes some of the actual benefits and future possibilities of nanomaterials in the emerging field of stem cell-based regenerative dentistry.

1. Pathology and natural regenerative potential of dental tissues

Two of the hardest tissues of the body, the enamel and dentin, form as the outcome of sequential and reciprocal interactions between cells of the oral epithelium and the cranial neural crest-derived mesenchyme [1]. Mesenchymal cells give rise to the dental follicle and dental pulp, while the

oral epithelium forms the ameloblasts. A part of the dental pulp cells differentiate into odontoblasts that produce dentin matrix, whilst ameloblasts form the enamel. When the mineralization of the tooth-crown is completed, the tooth-root starts to develop and subsequently the tooth erupts in the oral cavity. Once root development and cementum deposition have been accomplished the tooth anchors to the surrounding alveolar bone through the periodontal ligament (PDL), which contains extracellular matrix and a great variety of cells such as fibroblasts, epithelial rests of Malassez, endothelial cells.

The mineralized dental tissues are vulnerable to various external harmful agents such as bacteria and acids, but also to traumatic injuries that jeopardize tooth integrity. Although the mitotic and secretory activities of dental pulp and periodontal cells are reduced in adult teeth, these biological processes can be reactivated in pathological conditions (e.g., periodontal and carious diseases) or following traumatic injury [2]. After a mild lesion such as early caries, surviving post-mitotic odontoblasts can produce new dentin through a process known as reactionary dentinogenesis. However, a severe dental injury leads to odontoblast apoptosis that activates dental pulp stem cells to differentiate into new odontoblasts, which are producing the reparative dentin [2]. Periodontal regeneration is a complex process that involves the interaction of several populations of cells that control the specific extracellular matrix components. Cell-occlusive barriers ranging from cellulose to synthetic absorbable materials, which restrict the repopulation of the periodontium from epithelial cells and favor growth of

PDL cells and cementoblasts, are commonly used for periodontium regeneration in dental clinics. These materials are often used in conjunction with biological factors to enhance the regeneration of the alveolar bone. Damaged enamel cannot be repaired naturally since ameloblasts are not present anymore in humans after tooth eruption. Thus, a common practice in dental clinics is to substitute the damaged enamel with biomaterials, ceramics and precious metals.

It is evident that the natural regenerative capacity of dental tissues is often insufficient to entirely restore damaged teeth. In such cases, stem cell biology combined with tissue engineering technology could be useful for the development of innovative strategies for cell-based dental tissue regeneration [3, 4].

2. Stem cell populations within dental and periodontal tissues

Stem cells are undifferentiated cells characterized by their ability to self-replicate throughout life and their capacity to differentiate into diverse specialized cell types [5]. Adult stem cells are found in various tissues of the human body from both epithelial and mesenchymal origin, including skin [6], adipose tissue [7], periosteum [8] and cartilage [9]. Due to their ability to give rise to every cell type in a given tissue, adult stem cells are responsible for tissue/organ homeostasis and regeneration.

Mesenchymal stem cells (MSCs) have been isolated from different locations within adult or postnatal dental tissues. Dental mesenchymal stem cells (DMSCs) have been isolated from the pulp of adult and deciduous teeth (DPSCs and SHEDs respectively) [10], apical part of dental papilla (SCAP) [11, 12], dental follicle (DFSCs) [13], and periodontal ligament (PDLSCs) [14, 15] (Figure 1). All these dental stem cell populations express typical MSCs markers such as Stromal-derived factor 1 (STRO-1), Melanoma-associated antigen (MUC-18) or Cd146, and Cd44 [16] but some of them also express other markers including Cd90, Cd73, Cd29 and Cd24 [11, 17]. However, the marker or combinations of markers that reliably recognize dental stem cells have not been established yet. Thus, DMSCs are often recognized by their ability to give rise to odontogenic [10, 16, 18-21], adipogenic [16, 22], chondrogenic [22], osteogenic [23], myogenic [24], and neurogenic [16, 25] lineages *in vitro* or to regenerate dental tissues *in vivo* [11, 17].

Since most of dental epithelial cells disappear shortly after tooth eruption, identifying epithelial stem cells (EpSCs) within adult dental tissues constitutes a major challenge. Putative EpSC populations have been isolated from third molars [26] and, most surprisingly, dental pulp [27]. However, to date, epithelial cell rests of Malassez (ERM) located in the periodontal ligament (Figure 1), appear as the more promising source of EpSCs [28, 29].

Recently, pluripotent stem cells, named dental pulp pluripotent stem cells (DPPSCs) have been isolated from the dental pulp of third molars [30]. These cells show the ability to differentiate into tissues that derive from embryonic mesodermal, endodermal and ectodermal layers, suggesting their potential utility for the regeneration of both epithelial and mesenchymal dental tissues.

3. Stem cell-based dental tissue regeneration

Harmful agents (*e.g.*, caries) damage firstly the hard tissues of the tooth and then reach the dental pulp. The affected dental pulp is usually amputated (pulpotomy) or extracted (pulpectomy) and substituted with an artificial material after disinfection of the pulp cavity [3]. Although the tooth is preserved in its normal position, it is not vital anymore and it cannot fulfill completely its role [3]. Thus, the regeneration of the dentin-pulp complex represents the ideal solution and this process requires the revascularization and reinnervation of the pulp, as well as the deposition of newly generated dentin. As previously mentioned, DPSCs have the ability to differentiate into odontoblasts, endothelial cells, and neurons among other cell types. In mice, transplantation of DPSCs can regenerate both pulp and dentin tissues *in vivo* after pulpotomy [31]. DPSCs and SCAPs isolated from human third molars, seeded on a poly-D,L-lactide/glycolic acid scaffold, and transplanted into the empty root canal space of mouse teeth, were able to refill the empty space with a newly formed vascularized pulp [17, 32]. A continuous layer of mineralized tissue resembling dentin was deposited in the existing dental

walls of the canals [17, 32]. Although these results prove that DPSCs can regenerate the dental pulp, further studies are clearly required to investigate their potential clinical applications.

Periodontitis is one of the most common infectious diseases in humans. Periodontitis is triggered by microorganisms that attach to the teeth, cause chronic inflammation and eventually destroy the periodontal tissues [3]. Studies in immunocompromized mice have shown that transplanted PDLSCs were able to regenerate the periodontium, thus indicating their huge potential for future cell-based therapies in dental clinic [3]. However, severe damage of the periodontal tissues often results in tooth loss, so it is still necessary to develop new strategies in order to potentially use entirely regenerated teeth in clinics. This could be achieved either by generating a tooth germ *in vitro* before implanting it *in vivo*, or by grafting dental stem cells in the oral cavity. In this last case, dental stem cells could be carried on tooth-shaped biomimetic scaffolds [3]. Using different scaffolds it has been possible to induce differentiation of PDLSCs and DPSCs into the various cell types composing the root and/or the periodontal tissues both *in vitro* and *in vivo* [33, 34]. Dental pulp, cementum and PDL have been obtained by transplanting subcutaneously human DPSCs that were placed into a natural scaffold composed of human dentin matrix [33]. Regarding regeneration of dental epithelium, it has been shown that ERM derived from porcine mandible can differentiate into ameloblasts after co-culture with dental pulp cells *in vitro*. These ameloblast-like cells were positive for Keratin 14 (K14) and amelogenin. Moreover, after transplantation of ERM cells combined

with primary dental pulp cells, an enamel-like tissue was produced in the implant. Histological analysis revealed that appropriate stages of amelogenesis from initiation to maturation were present in all implants. Thick enamel-dentin structures were clearly recognized, with ameloblast-like cells expressing K14 and amelogenin were found 8 weeks post-transplantation [29].

Equally important for the development of stem cell-based therapies in dentistry is the use of signaling molecules. Several molecules involved in periodontal development are already in use in the clinical practice. Long time ago, it was shown that PDGF molecules were able to stimulate periodontal healing and regeneration [35, 36]. Since then, other molecules such as BMPs [37-39] and amelogenins [40] have been used for the stimulation of periodontal tissues regeneration.

Current studies focus on the identification of the accurate population of cells, suitable signaling molecules, and desirable scaffold materials that will be used as carriers for specific cell types.

4. Safety and efficacy issues of stem cell-based therapies in dentistry

Stem cell-based therapies are both promising and challenging. The engraftment of exogenous therapeutic cells in patients must obey strict safety rules, exclude tumor formation, and avoid or minimize rejection [41, 42]. The purity, biological activity, and quantity of the injected cells should

be optimized to ensure cell functionality. Cell functionality should be tested both *in vitro* and *in vivo* in various animal models. Defined strategies should also develop to monitor the behavior and fate of the engrafted cells before any clinical trial. There is a consensus that differentiated cells that are originated from stem cells and not undifferentiated stem cells should be used directly for transplantation in the clinics [42]. Even if stem cell injection or transplantation is successful in animal models, it is important to optimize and secure stem cell-based therapeutic strategies before clinical trials.

An optimal engraftment strategy must avoid (or minimize) immune response in the host. Grafted or injected stem cells are recognized as foreign material by the immune system of the host, thus generating a cascade of events that results in the destruction and rejection of the transplanted cells. This process can compromise the immune status of the recipient. Immunosuppressive treatments that increase graft survival are not desired, since it has been shown that a correlation exists between the length of exposure to immunosuppressive drugs and the risk of malignancy after stem cell transplantation. Recent results have shown that mesenchymal stem cells from umbilical cord blood, dental pulp, periodontal ligament and bone marrow have immunosuppressive properties *in vitro* [43-45]. Moreover, clonogenic nature of adult stem cells represents an advance over heterogeneous stem cells populations resulting in a more reproducible, potent immunosuppressive effect between patients [46]. Autologous cell-based therapies are advantageous because there is a minimal risk of immunological rejection and disease transmission. However, the outcome of

all tissue engineering approaches using autologous stem cell transplantation is subjective to the patient since the patient is at the same time the source and the recipient of the cells that will be used for his/her own treatment. Factors related to the age, general health status of the patient, health condition of the dental pulp and periodontium at the moment of surgery, as well as the size and site of the injury may influence the efficacy of stem cell-based dental treatments. The influence of these factors on the efficacy of cell preparations for cell-based dental treatments has not been investigated exhaustively.

The generation of iPSCs by reprogramming somatic cells via a cocktail of transcription factors [47, 48] could be advantageous towards cell-based regenerative therapies. Somatic cells have been reprogrammed and turned into pluripotent cells by the overexpression of a cocktail of 4 transcription factors (Oct4, Sox2, c-Myc and Klf4) [47]. It has been recently shown that mouse iPSCs can give rise to neural crest-like cells that can be further differentiated into odontogenic mesenchymal cells [49]. iPSCs could be generated from somatic cells of the patient, who will be donor and recipient simultaneously, thus overcoming the problems of an allogeneic immune rejection [50]. Although tempting, this potential has not been proven, since there is no yet a clear understanding of the effects that the reprogrammed cells could have to the immune system [51]. For example, studies in animal models have shown immunoreactivity toward grafted iPSCs of the same genetic background [52]. Both adult dental stem cells and induced pluripotent stem cells (iPSCs) represent an attractive source of cells for

regenerative dentistry. Nevertheless, there are still safety and immunogenicity issues that should be overcome before using them in clinics.

Although promising advances have been made in dental stem cell isolation and expansion, it is still necessary to refine these procedures. It is noteworthy that stem cells from every individual patient should be considered as specific pools and be quality controlled accordingly. It is obvious that there is no yet an ideal and unique approach for cell-based repair of dental tissues. However, rapid progress in stem cell biology and biomaterial sciences might allow the development of new methods and protocols for personalized dental treatments.

5. Nanomedicine: a giant leap forward disease diagnosis and treatment

Nanomedicine represents a subfield of nanotechnology that uses particles in the size range 1-1,000 nm for the treatment, diagnosis, monitoring, and control of various diseases [53, 54]. Nanoparticles, which are similar in scale to biological macromolecules such as DNA and proteins [53], can be used for targeted therapy through DNA, protein and drug delivery, *in vivo* imaging, diagnostics, as well as for the creation of active scaffolds and implants [55-57]. Nanoparticles can be composed of organic (e.g., lipids), inorganic materials (e.g., iron oxide, gold), or combinations of both types. Novel and improved nanostructured materials can be tailored by engineering their characteristics such as structure, stability, size, shape and surface properties in order to be selectively delivered to precise sites (target

regions) of the body [58]. This can be achieved through passive or active targeting mechanisms: passive targeting is enabled by the enhanced vasculature permeability during neo-angiogenesis of injured or pathological body sites, while active targeting benefits from the overexpression in the infectious or damaged areas of several cell surface molecules that can bind specifically to pre-coated nanoparticle ligands [58, 59]. Recently, a dual modular system that mimics the communication dependent recruitment of inflammatory cells to regions of disease has been developed to improve tissue target efficiency of nanoparticles [60]. Another more recent study has demonstrated the programming and assembly of DNA-based nanorobots that are able to carry molecular loads, transport chemical ingredients to target cells and stimulate their intracellular alterations [61].

These sophisticated biomaterials are increasingly being incorporated into the stem cell biology field. The combination of stem cells with innovative nanotechnology products holds great promise for applications in the biomedical arena. Fundamental challenges include stem cell expansion *in vitro* without using feeder layers, enhancement of stem cells survival after transplantation and reproducible regulation of their fate in the body [55]. The development of nanomaterials could be helpful in detecting and manipulating stem cells that will be used for tissue repair in the clinic. Nanomaterials are being used to define precisely the stem cell microenvironment through the provision of morphogenetic gradients and cell adhesion molecules, to direct stem cell fates, and to provide a template for stem cells for the formation of new tissues and organs. Furthermore,

internalization of nanoparticles, previously labeled with chelated ions, small molecules, metals and nanocrystals, by stem cells enables their detection by imaging. The physical, chemical and biological properties of nanomaterials can be exploited to influence proliferation, attachment, fate and differentiation of stem cells [62]. This multidisciplinary approach allowed scientists to create a fully synthetic organ for transplantation after soaking a porous polymer nanocomposite tracheobronchial replica in a solution of bone marrow stem cells [63]. Although these new developments are encouraging, long-term studies are necessary before exploitation of such synthetic nanosystems in the clinics. For example, it is important to verify the non-toxicity, exclude the tumorigenic potential [64] and adverse side effects on a systemic level of nanoparticles and study their interference with the self-renewal ability of stem cells. In addition, the pharmaceutical industry has been reticent to engage with the cell-based regenerative medicine industry probably because of the complex regulatory and ethical issues [65]. This leads to uncertainty regarding the cost and time that will be required to successfully gain market approval for the nanomedicine.

5.1. Monitoring of stem cells after transplantation: magnetic nanoparticles and quantum dots

Stem cell-based regenerative therapies necessitate thorough testing firstly in animals and finally in humans. For the evaluation of the therapeutic efficacy of the transplanted stem cells it is important to track their survival, migration, fate and regenerative impact *in vivo*. Transplanted stem cells can

be assessed for long-term periods using non-invasive imaging techniques [66, 67]. Stem cells can be tracked *in vivo* after their transplantation using different strategies: initial labeling of stem cells with fluorescent dyes or magnetic nanoparticles such as the superparamagnetic iron oxide (SPIO), and stem cell transfection with several reporter genes such as the LacZ and Green Fluorescence Protein (GFP) [68, 69]. The visualization of the labeled stem cells requires either simple or complex and sophisticated imaging systems such as the magnetic resonance imaging (MRI) [66, 70, 71], computed tomography (CT) imaging [72], positron emission tomography (PET) and single-photon emission computed tomography (SPECT) imaging [73]. SPIO nanoparticles (60-150 nm in diameter) are composed of biodegradable and recyclable iron and are coated with dextran or carboxydextran to prevent aggregation and ensure aqueous solubility [74, 75]. Magnetic nanoparticles can attach to the stem cell surface, but are also capable to be internalized by phagocytosis or, more often, by endocytosis [76], a process that is often facilitated by the use of coating and membrane receptor-binding agents [69, 77]. Endocytosis of magnetic nanoparticles does not affect stem cells viability, growth, fate and differentiation [78].

Quantum dots (QDs) are cadmium selenide semiconductor fluorescence-emitting nanostructures (less than 10 nm in diameter) that are used for long-term labeling of stem cells [79-81]. QDs present a number of advantages over conventional organic dyes (*e.g.*, Dil) and fluorescence proteins (*e.g.*, GFP): high brightness, superb photostability and a single excitation wavelength for multiple colors [82]. Due to their excellent optical properties,

the detection of QDs-labeled stem cells relies on imaging systems that are less sophisticated and complex than MRI. QDs have been used to monitor in real time the dynamics of various cell components [83]. Information concerning participation and clustering of multiple cell-surface molecules involved in stem cell migration and differentiation might be useful for the design of innovative scaffolds for homing stem cells before transplantation. QDs are also internalized by endocytosis, which is improved by the use of specific peptides such RGD, phospholipids, and cholera toxin [84-86]. A number of internalized QDs are transported via endosomes to the perinuclear region [87], while QDs that will not be used by the cells display an oxidative degradation [68] that could lead to mitochondria dysfunction and ultimately cell death [88]. It is possible that the composition and physical properties of QDs and magnetic nanoparticles lead to unique toxic responses [53, 85, 87]. To date there is no conclusive evidence of known human toxic responses that are exclusively caused by nanomaterials [53]. Furthermore, most of the studies demonstrated no interference of these nanoparticles in stem cell differentiation [84, 85]. However, variations in the composition, structure, size, and surface coating of nanoparticles might influence stem cell behavior and fate [88, 89].

Magnetic nanoparticles and QDs may provide valuable information about stem cell migration, anchorage, fate and differentiation in the context of dental pathology (*e.g.*, periodontitis, pulpitis, traumatic injury) and repair [3, 5, 90]. For example, internalized nanoparticles would allow monitoring kinetics and fate of the labeled stem cells that were injected into the

periodontal space or pulp chamber following dental injury (Figure 2). These approaches are necessary to evaluate the therapeutic effects of stem cells when exposed to a specific microenvironment before their application in dental clinics.

5.2. Targeting therapy: gene, protein and drug intracellular delivery

One of the most attractive concepts in manipulating the fate of stem cells, directing thus their differentiation into specific cell populations, is the use of nanomaterials for intracellular gene delivery (e.g., RNAi, DNA) [91, 92]. Generally, viral (e.g., adenoviruses, lentiviruses, retroviruses) and non-viral vectors (e.g., lipids, polymers) can be used for cellular transfection and/or nucleofection, thus offering durable gene expression within stem cells [93-96]. Non-viral carriers have a number of advantages over viral vectors, since they exhibit low-risk immunogenicity and insertional mutagenesis, controllable toxicity, and great gene-carrying capacity [95]. Many efforts for the improvement of non-viral vectors are focused on cationic polymers that interact with negatively charged DNA or RNAi. Polymers, including poly(L-lysine)-palmitic acid, poly(L-lysine), and polyethylenimine, condense the genetic material into particles of 200-300 nm in diameter, protect them from enzymes, and facilitate cellular entrance [94, 97]. These complexes of polymers with genetic material (called “polyplexes”) have a transfection efficiency that is equivalent to the adenoviral vectors [97].

Nanoparticles, carbon nanotubes and silicon nanowire arrays have also been used for gene delivery [98, 99]. The apatite particles coated with E-cadherin and fibronectin, ensure high gene delivery capacity in stem cells [100].

Polymeric biodegradable nanoparticles of 100-300 nm in diameter could also serve as platforms to incorporate and deliver proteins and chemicals within stem cells. It has been shown that after internalization these nanoparticles accumulate in the perinuclear region and have a minimal effect on the viability and proliferation of stem cells, but a high impact on their differentiation [68].

The cytotoxicity of “polyplexes”, nanoparticles, and nanotubes has been evaluated in stem cells and the results showed that in general the toxicity correlates with the chemistry, concentration, size, shape and coating of the nanomaterials [97, 98].

5.3. Nanobiomimetics: design of bioactive scaffolds and artificial stem cell niches

The behavior of stem cells is tightly controlled by a specialized microenvironment called the “stem cell niche” [5]. Thus, this microenvironment regulates the survival, proliferation and differentiation of stem cells.

Injection of stem cells into the injured or pathological tissue limits their spreading and, in addition, does not ensure their good engraftment [101]. Injected cells could die due to the absence of trophic factors, oxygen, or lack of a suitable extracellular matrix (ECM) for their adhesion. This can be avoided by placing stem cells in biocompatible and biodegradable nanofiber scaffolds that recreate temporarily the fibrous three-dimensional (3D) network of ECM and mimic the structural aspects of the stem cell niche. Hence, stem cells are anchored to the nanofibers of the scaffolds that behave as artificial stem cell niches, and then transplanted to the lesion site. This will improve stem cell survival, migration and differentiation potentials, and finally their 3D organization [101]. Stem cells cultured on nanofiber scaffolds exhibit high viability and lower mobility, and differ in morphology when compared to cells cultured on conventional substrates (e.g., polystyrene) [102, 103]. Nanofibers with controlled diameter (e.g., 300-1,000 nm) are composed by either natural polymers, such as collagen and silk, or synthetic polymers including poly(lactic acid) and poly(amide) [102, 104, 105]. The 3D organization, surface and chemistry of these scaffolds result in stem cell self-renewal, migration, and differentiation. Nanofiber scaffolds have high porosity and specific surface that offer an ideal environment for stem cell homing.

Identifying the appropriate stem cell populations and providing the suitable microenvironment that allows them to repair or regenerate an injured tissue is the key for a successful cell-based therapy. Nanotechnology can be used to create artificial microenvironments that will direct stem cells or progenitor

cells towards a precise fate and function. A big challenge is to engineer materials that resemble the structural complexity of stem cell niches, which represent specific anatomic locations homing stem cells and prevented them from exiting the mitotic cycle [106]. ECM molecules such as collagen, fibronectin, laminin, and proteoglycans represent the non-cellular components of the niches and are important for the creation of a particular microenvironment (e.g., tooth, bone, heart). ECM provides nanoscale structures such as the 15-300 nm in diameter collagen fibrils that allow cell adhesion (via integrins) and immobilization of signaling molecules, thus influencing the fate and behavior (*i.e.*, proliferation, migration, differentiation) of stem cells [107]. The concentration, size, spacing, surface chemistry and shape (e.g., ridges, grooves, pores, pits) of the artificial nanostructures (e.g., nanotubes, nanolines) are important parameters for the development of cell adhesion sites that monitor stem cell behavior [108-110]. For example, it has been shown that surface irregularity (e.g., nanoline grating) and diverse surface chemistries (e.g., silica, poly[methyl methacrylate]) are capable to enhance adhesion, alignment, growth, and differentiation of stem cells [108, 109].

In vivo transplantation of stem cells anchored to nanofiber-based scaffolds is a technique successfully used in regenerative medicine [111, 112]. Transplanted biodegradable scaffolds act as temporary niches that guide, by controlling stem cell behavior, the formation of a new specific ECM for tissue repair. The design of tissue-specific artificial niches offers new perspectives to stem cell-based applications in dentistry for the treatment of

peculiar anatomic sites (e.g., alveolar bone, dentin-pulp complex, enamel, periodontium). Furthermore, nanomaterials could be successfully used for the generation of new nanotextured “osteogenic coating” dental implants that may lead to direct bone-material contact and also bone healing in those cases in which bone is compromised. The variety of adult stem cell populations within dental tissues indicates that their differentiation potential and response to nanoscale materials may be different. However, there are not yet methodical comparative studies that will allow the assessment of nanomaterials on the various dental stem cell lines. The lack of this crucial information delays the application of stem cell-based therapies in dental clinics.

Conclusion

There is no doubt that nanotechnology offers enormous benefits and a plethora of exciting perspectives to cell-based regenerative medicines. Recent advances in nanoscale materials increase the potential to control stem cell fate, improve DNA and drug delivery, modulate the immune response to implanted cells, and create advanced scaffolds for treatment of various diseases. Nanomaterials and cell-based products must be regulated and manufactured at a low cost scale to ensure their successful application in clinics. Dentists could benefit from the use of nanoparticles to label stem cells, which after being placed on scaffolds could be further implanted into damaged dental tissues in order to regenerate them (Figure 2). The application of nanotechnology for dental purposes (nanodentistry) holds

great promise as a type of personalized medicine for the management of target-specific treatment and imaging of dental tissues.

Future perspective

Dental clinics could benefit in the near future from the combinatorial use of stem cells and nanostructures (e.g., creation of specific scaffolds). These devices that will contain cells could be implanted into damaged dental sites in order to regenerate them. However, there are serious issues concerning standardization of techniques, nanoparticles and stem cells that have to be solved before their clinical application in humans.

Executive summary

1. Pathology and natural regenerative potential of dental tissues

The mineralized dental tissues are vulnerable to various external harmful agents such as bacteria and acids, but also to traumatic injuries that jeopardize tooth integrity. The natural regenerative capacity of dental tissues is often insufficient to entirely restore damaged teeth.

2. Stem cell populations within dental and periodontal tissues

Dental mesenchymal stem cells (DMSCs) have been isolated from the pulp of adult and deciduous teeth (DPSCs and SHEDs respectively), apical part of dental papilla (SCAP), dental follicle (DFSCs), and periodontal ligament (PDLSCs).

Putative epithelial stem cell populations (EpSCs) have been isolated from dental pulp. However, epithelial cell rests of Malassez (ERM) located in the periodontal ligament, appear as the more promising source of EpSCs.

3. Stem cell-based dental tissue regeneration

Studies in animals have shown that transplanted dental mesenchymal stem cells (DMSCs) were able to regenerate the periodontium and dental pulp *in vivo* thus indicating their huge potential for future cell-based therapies in dental clinics.

4. Safety and efficacy issues of stem cell-based therapies in dentistry

There are still safety and efficacy issues that need to be solved before the application of stem cell-based therapies in clinics. Immunogenicity of the transplanted cells is one example. The potential use of iPSCs in regenerative dentistry is discussed.

5. Nanomedicine: a giant leap forward disease diagnosis and treatment

Nanomedicine represents a subfield of nanotechnology that uses particles in the size range 1-1,000 nm for the treatment, diagnosis, monitoring, and control of various diseases. Different sophisticated biomaterials are increasingly being incorporated into the stem cell biology field.

5.1. Monitoring of stem cells after transplantation: magnetic nanoparticles and quantum dots

For the evaluation of the therapeutic efficacy of the transplanted stem cells it is important to track their survival, migration, fate and regenerative impact *in vivo*. Stem cells can be tracked *in vivo* after their transplantation using different types of nanoparticles such as superparamagnetic iron oxide (SPIO) or quantum dots (QD).

5.2. Targeting therapy: gene, protein and drug intracellular delivery

Polyplexes, nanoparticles, carbon nanotubes and silicon nanowire arrays can be used for gene delivery to stem cells before they transplanting them *in vivo*.

5.3. Nanobiomimetics: design of bioactive scaffolds and artificial stem cell niches

Biocompatible and biodegradable nanofiber scaffolds constitute artificial stem cell niches that influence the survival, self-renewal and differentiation of stem cells.

Conclusion

The use of nanotechnology for dental purposes (nanodentistry) holds great promise as a type of personalized medicine for the management of target-specific treatment and imaging of dental tissues. However, there are still certain safety issues to be solved before any clinical application.

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Conflicts of interest

Conflicts of interest (personal or financial) do not exist. The authors declare that they have not received writing assistance.

Figures

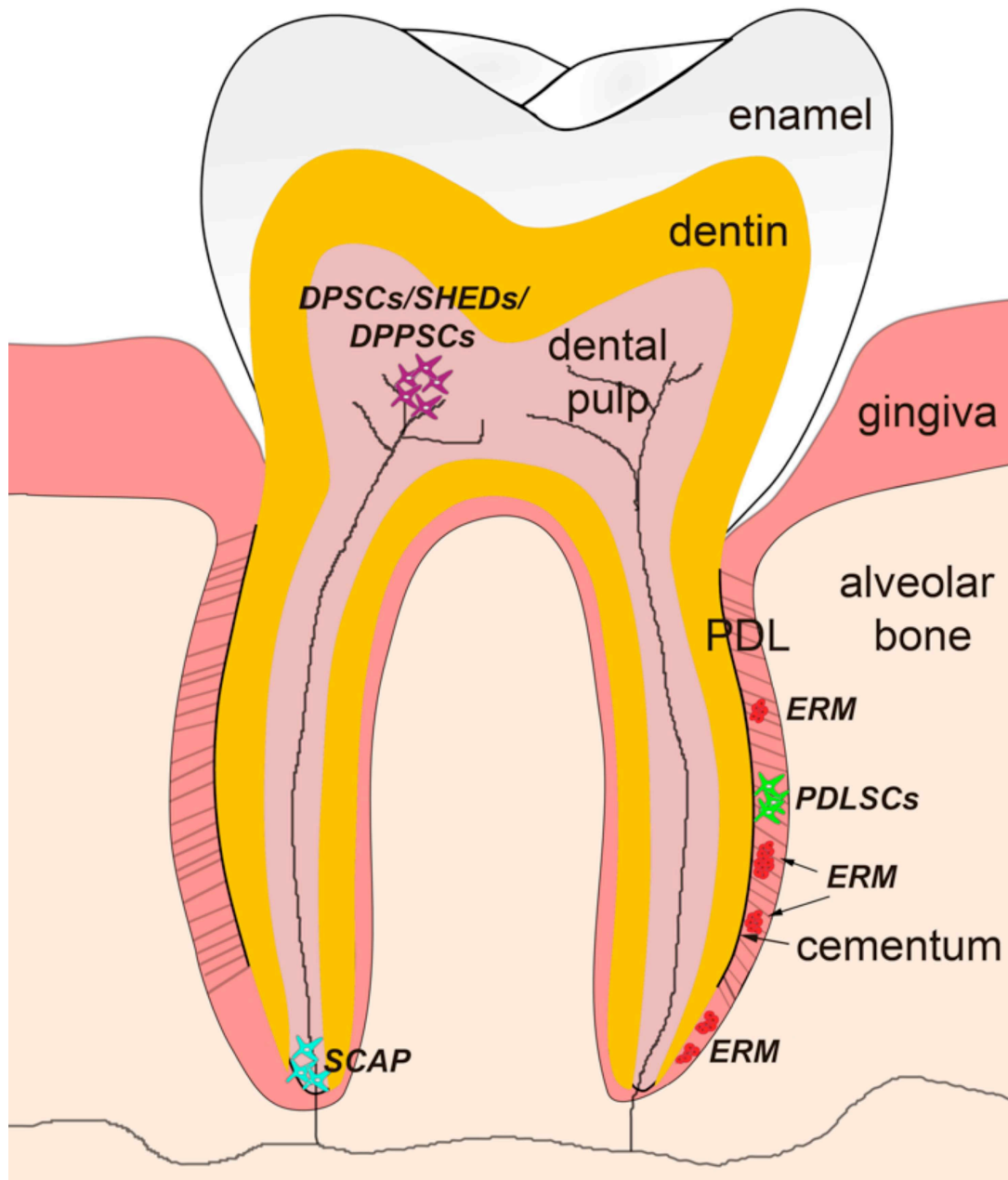
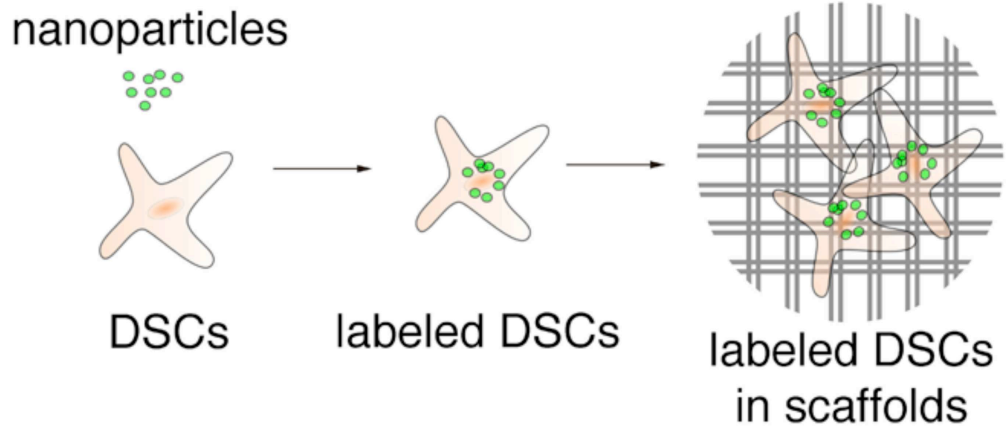


Figure 1. Schematic representation showing the various dental stem populations within an adult human tooth. Abbreviations: DPSCs, dental pulp stem cells; DPPSCs, dental pulp pluripotent stem cells; ERM, epithelial cell rests of Mallassez; PDL, periodontal ligament; PDLSCs, periodontal ligament stem cells; SCAP, stem cells from the apical papilla; SHED, stem cells from human exfoliated deciduous teeth.

A



B

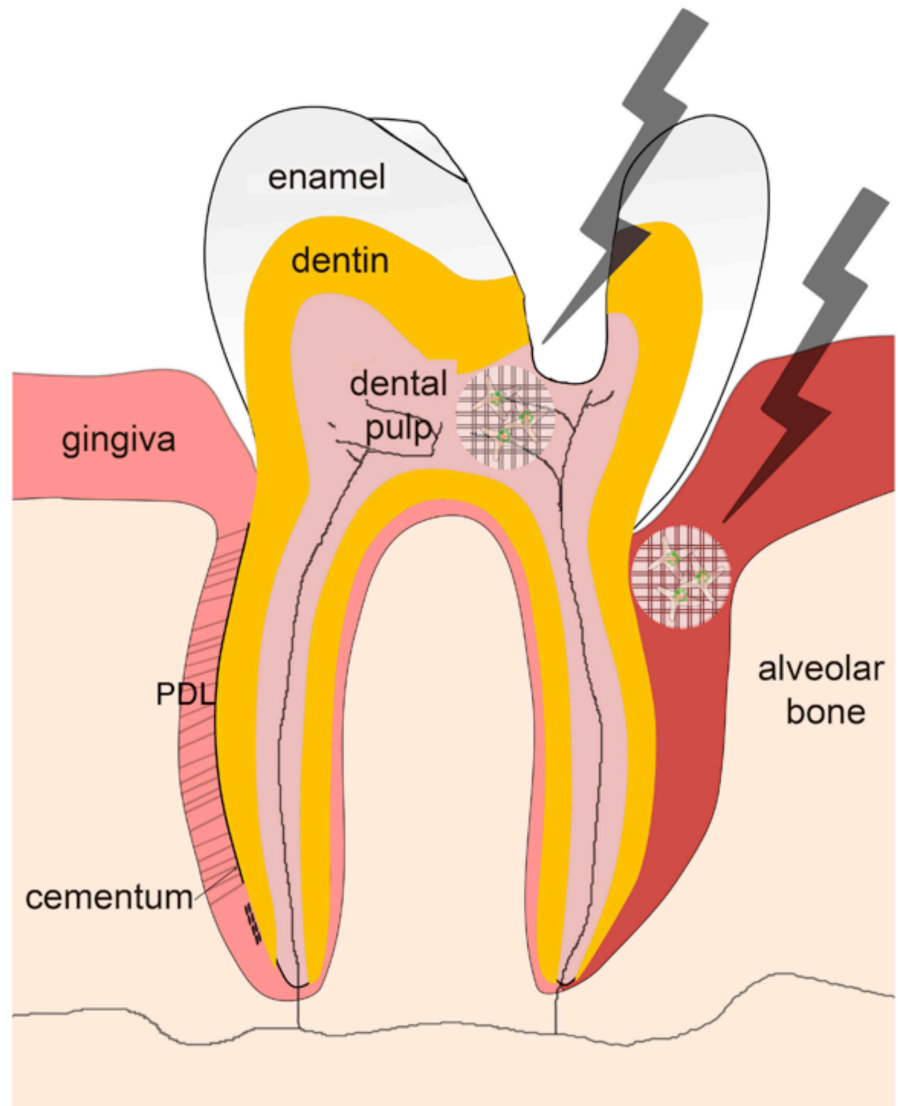


Figure 2. Nanotechnology in regenerative dentistry. Dental stem cells (DSCs) can be labeled with nanoparticles before placing them into biomimetic scaffolds (A). Afterwards, those scaffolds that contain labeled DSCs could be transplanted to repair dental damaged tissues. Tooth crown, pulp and periodontium are the most commonly affected dental tissues.

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