12-1-2016

Recent Advancements in Regenerative Dentistry: A Review

Pouya Amrollahi
Oklahoma State University Tulsa

Brinda Shah
Marquette University

Amir Seifi
University of Oxford

Lobat Tayebi
Marquette University, lobat.tayebi@marquette.edu

Recent Advancements in Regenerative Dentistry: A Review

Pouya Amrollahi
Helmerich Advanced Technology Research Center, School of Material Science and Engineering, Oklahoma State University, Tulsa, OK
Brinda Shah
School of Dentistry, Marquette University Milwaukee, WI
Amir Seifi
School of Dentistry, Marquette University Milwaukee, WI
Lobat Tayebi
School of Dentistry, Marquette University Milwaukee, WI
Department of Engineering Science, University of Oxford, Oxford, UK

Abstract: Although human mouth benefits from remarkable mechanical properties, it is very susceptible to traumatic damages, exposure to microbial attacks, and congenital maladies. Since the human dentition plays a crucial role in mastication, phonation and esthetics, finding promising and more

Materials Science and Engineering: C, Vol 69 (December 1, 2016): pg. 1383-1390. DOI.

efficient strategies to reestablish its functionality in the event of disruption has been important. Dating back to antiquity, conventional dentistry has been offering evacuation, restoration, and replacement of the diseased dental tissue. However, due to the limited ability and short lifespan of traditional restorative solutions, scientists have taken advantage of current advancements in medicine to create better solutions for the oral health field and have coined it "regenerative dentistry." This new field takes advantage of the recent innovations in stem cell research, cellular and molecular biology, tissue engineering, and materials science *etc.* In this review, the recently known resources and approaches used for regeneration of dental and oral tissues were evaluated using the databases of Scopus and Web of Science. Scientists have used a wide range of biomaterials and scaffolds (artificial and natural), genes (with viral and non-viral vectors), stem cells (isolated from deciduous teeth, dental pulp, periodontal ligament, adipose tissue, salivary glands, and dental follicle) and growth factors (used for stimulating cell differentiation) in order to apply tissue engineering approaches to dentistry. Although they have been successful in preclinical and clinical partial regeneration of dental tissues, whole-tooth engineering still seems to be far-fetched, unless certain shortcomings are addressed.

Keywords: Regenerative dentistry, Dental tissue engineering, Stem cells, Gene therapy, 3D bio-printing

1. Introduction

After the first successful kidney transplant between two non-genetically identical patients was performed by Murray, the Nobel prize winner and scientist in the early 1960s,[1] transplantation has been the treatment for most of organ injuries and failures. However, transplantation has major drawbacks such as severe shortage in organ donors, gradual crescendo in the number of organ failure cases, indeterminate immune responses, and unreliable organ acceptability.[1] Therefore, scientists with backgrounds in cellular and molecular biology, materials science, and stem cell engineering came together and developed a new field called Tissue Engineering and Regenerative Medicine (TERM). As a rapidly growing field of research, TERM offers novel treatments for patients suffering from slight injuries to end-stage organ failure for nearly every type of human body tissue and organ. The clinically available treatments include but are not limited to strategies for urethral tissue,[2] bladder wall tissue,[3] genital tissues and organs,[4] female reproductive tissue,[5] blood vessel,[6] heart valves,[7] liver[8] and tracheal tissue.[9] In all of these cases, there are still substantial problems which need to be resolved; however, the recent advancements and their potential benefits seem to be revolutionary. In

dentistry, scientists have always placed significant emphasis on the study of novel strategies that apply TERM to the dental practice.[10]

Human teeth and orofacial tissues are responsible for phonation, mastication, esthetics, respiration, and emotional and facial expressions. Although teeth have high abrasion resistance and lifelong architectural durability, oral tissue, as one of the excessively used parts of the body, is prone to several common diseases from congenital maladies to chemical, physical, and microbial attacks.[11] While the oral cavity plays an essential role in daily life, it is severely exposed to microbial infections—therefore, any defect, induced by infections, decay or trauma and all other oral diseases including autoimmune and malignancies in the dental tissue should be addressed quickly.[12] More specifically, any large size defect that is close to pulp exposure, including moderate to advance decay, needs to be treated urgently. Often, trauma induced by mastication, accidents or even pathogens can disrupt the oral epithelium protective barrier.

The reports outline that 41% of the children aged 2–11 years (in their primary teeth), 42% of children and adolescents aged 6–19 years, and approximately 90% of human adults (in their permanent teeth) suffer from at least one of the dental diseases, such as caries,[13] which makes it important to find approaches that can restore oral tissue to normal function and form. Although the techniques used in conventional dentistry—such as restoration with filling materials, whole tooth replacement with synthetic restorative materials, and teeth removal—date back to antiquity, they have major drawbacks that necessitate exploration of more effective approaches and novel technologies in modern dentistry.[14] The current efforts are focused on the investigation of the possibility of engineering the whole tooth, as well as all of the individual dental structures separately. Both of these routes require utilization and development of stem cells, biomaterials, scaffolds, and growth factors. However, before outlining the details, grasping a better understanding of the human tooth structure and development is necessary.

Despite the presence of different morphologies, all four types of human teeth—incisor, canine, premolar, and molar—go through the same stages of morphogenesis, depicted in Fig. 1A. Tooth development is initiated as a result of the reciprocal interactions

between the ectoderm and mesoderm cells.[15] In the beginning stages, the tooth germ can be identified as the dental epithelium thickens and the tooth bud forms inside the dental mesenchyme. As proliferation continues, dental mesenchyme condenses and the odontogenic signals begin to fire. In the next step, known as the cap stage, the primary enamel knot forms and the tooth gets surrounded by the condensed mesenchyme. In this stage, epithelial cells can be categorized into three distinct regions: outer epithelium, inner epithelium, and central cell layers. Then, the continuous proliferation and cyto-differentiation in the bell stage results in odontoblast and ameloblast formations. The odontoblasts initiate dentin formation, while the ameloblasts elaborate enamel development. At last, after crown formation, root maturation and the development of cementum, alveolar bone, and periodontal ligament, the tooth eruption proceeds and is completed.[16] Fig. 1B demonstrates the structure of a mature human tooth. The soft dental pulp is surrounded by dentin, which makes a complex that builds the bulk of the tooth. The odontoblasts lining of this functional complex slowly generates dentin all throughout life. This activity can increase remarkably in the case of injury. If the odontoblasts layer, which is the last layer of dentin before the pulp, is lost, the pulp is practically exposed and the entire pulp tissue can go into necrosis. Enamel, which is 95–98% hydroxyapatite, caps the dentin and forms the tooth crown. The distinct architecture of enamel—which is hydroxyapatite nano-rods inside a microstructural matrix—gives rise to its remarkably hard and brittle tissue. Although enamel benefits from excellent mechanical properties, it is vulnerable to different elements such as poor food habits, overzealous brushing, and demineralization in the acidic environment caused by bacterial attacks. The thin tissue that covers the dentin of roots is cementum. Periodontal ligament and alveolar bone are both supporting structures that start to form in the bell stage. Periodontal ligament has fibrous tissue made from mainly collagen that intertwines into cementum and alveolar bone. Periodontal ligament, which is highly susceptible to bacterial attacks, plays an important role in supporting the tooth root. Therefore, in case of any injury or disease, it may have severe consequences.[11] The level of natural regeneration in each of the aforementioned dental tissues and structures varies from lifetime restoration to no restoration at all.[11]

Materials Science and Engineering: C, Vol 69 (December 1, 2016): pg. 1383-1390. DOI.

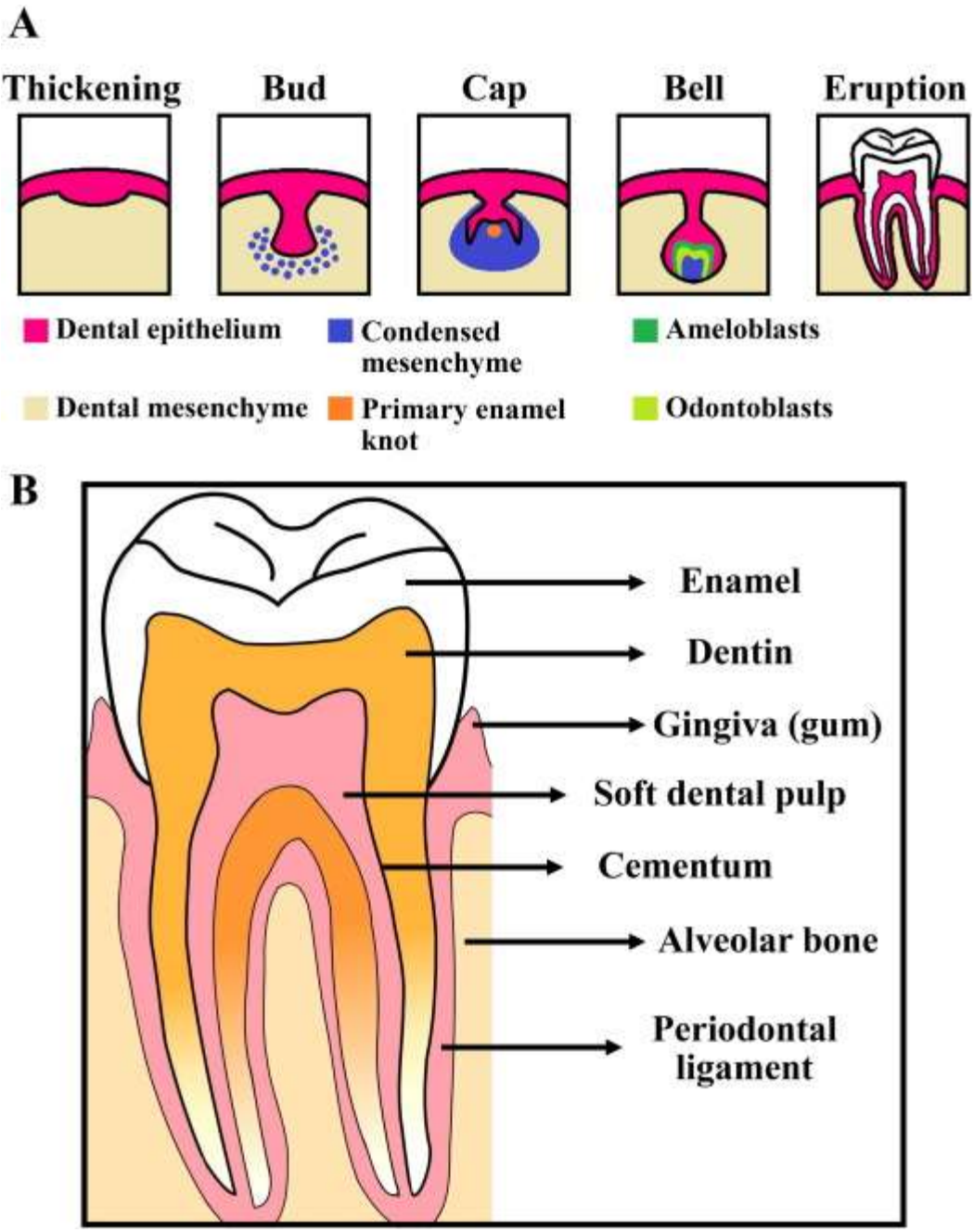

Fig. 1. (A) Tooth development process and (B) human tooth structure.

In this review, the recent advancements in regenerative dentistry (RD) are investigated and discussed extensively. First, the prospects of using stem cells derived from various sources to regenerate different oral tissues are being explored. Next, a wide range of biomaterials and scaffolds used in this field are introduced.

Materials Science and Engineering: C, Vol 69 (December 1, 2016): pg. 1383-1390. DOI.

Then, gene therapy techniques, microscale technologies, and three-dimensional (3D) bio-printing are presented as novel regenerative approaches in modern dentistry. Finally, after explaining the major obstacles and drawbacks that scientists face today in pushing boundaries of RD, the future directions that may lead us to more feasible clinical treatments will be stated.

2. Materials and methods

An electronic literature study of scientific articles was conducted using the databases Scopus and Web of Science. The key search terms used in combinations were "regenerative dentistry", "dental tissue engineering," and "dental stem cells". The search strategy was specific to each database. The related terms were combined using "OR" and "AND" operators. None of the search results were excluded based on the year of publication. In most cases the articles that did not explicitly made a link between "dental stem cells" and any of the two are key search terms were excluded. Next, the search results were merged and duplicates were removed both manually and electronically. After the initial screening of the titles and the abstracts, the articles were categorized according to the sections of the present review. At last, the full text of the articles were studied and reviewed, in order to provide the readers with the most recent and relevant information on "Regenerative Dentistry".

3. Current approaches in regenerative dentistry

The recent scientific advancements in reprogramming and guided-differentiation of human embryonic and adult stem cells, producing biocompatible materials, and scaffolding systems that support cell growth have convinced scientists to apply these technologies to modern dentistry. Here, the major approaches recently used are discussed.

3.1. Dental stem cells and growth factors

In the past few decades, a lot of progress has been made in understanding, extracting and utilizing human embryonic and adult stem cells.[17] Self-renewal, programmability, and the potential to

produce various cell types are the main factors which make these cell types attractive for any field of medicine.[18] In RD, scientists have used both pluripotent and adult stem cells derived from embryo, bone marrow, dental tissues, oral tissues, and glands. They have also used induced pluripotent stem (iPS) cells.[19] After discovery of the potential of extracting stem cells from dental pulp by Gronthos et al.,[20] dental pulp stem cells (DPSCs) and stem cells from human exfoliated deciduous teeth (SHED) were the first cell lines derived from human dental pulp.[20,21] They are both favored because of their non-invasive harvest and potential for multi-lineage differentiation.[22] Shi et al.[23] compared human dental pulp stem cells and bone marrow mesenchymal stromal stem cells (BMSCs) and showed distinct gene expression patterns for DPSCs.[23] In 2008 stem cells derived from apical papilla (SCAP), which were harvested from wisdom teeth, were shown to have potential in dentin regeneration.[24,25] Although the abundant presence of progenitor cells in the periodontal ligament was proven a long time ago,[26] Seo et al.[27] investigated the stem cells harvested from the periodontal ligament (PDLSCs) of the third molar and found out that these stem cells are capable of developing a tissue similar to their extraction site.[27] Morsczeck et al.[28] isolated precursor cells from the dental follicle (DFSCs) of wisdom teeth and developed them into a mature periodontium.[28] Honda et al.[29] studied DFSCs and showed their osteogenic potential.[29] The progenitor cells extracted from the tooth germ of the third molar during the bell stage (TGPCs), by Ikeda et al.[30] demonstrated the ability to differentiate into osteoblasts, hepatocytes, and neural cells.[30] Scientists have also reported the extraction of stem cells from human dental epithelium tissue. Oral epithelial stem cells,[19] gingiva-derived mesenchymal stromal cells (GMSCs),[31] and periosteum-derived stem cells (PSCs)[32] have shown the potential to differentiate into lineages of all three germ layers. On the other hand, stem cells derived from human salivary glands have not shown the potential to proliferate into all forms of epithelial cells.[33] Fig. 2[19] depicts the various oral and dental sources of adult stem cells.

Materials Science and Engineering: C, Vol 69 (December 1, 2016): pg. 1383-1390. DOI.

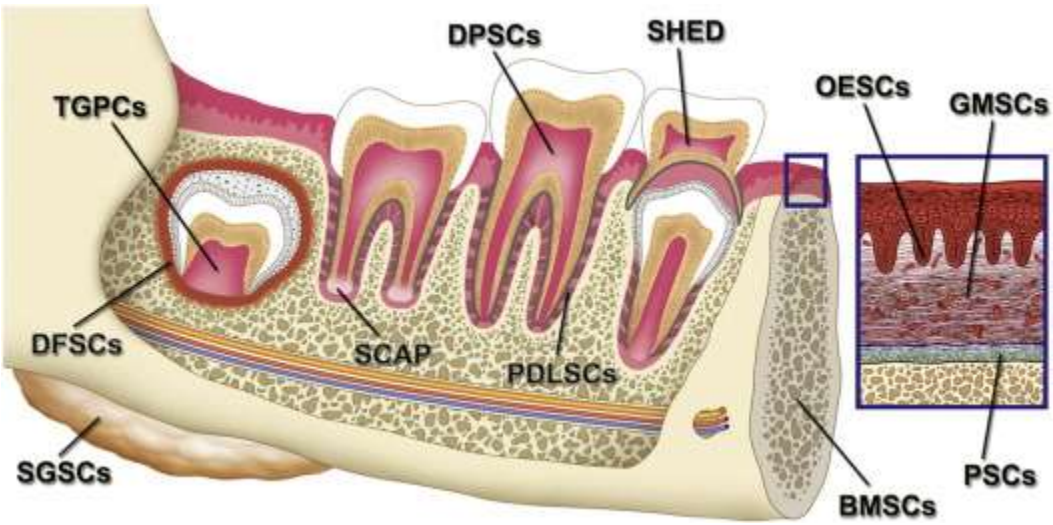

Fig. 2. Dental and oral sources for human adult stem cells [19].

Adipose is loose, connective tissue which controls body energy resources in order to keep the body warm against cold environments.[34] Adipose-derived stromal cells (ADSCs), which can be harvested through relatively less-invasive procedures, have shown the capacity for multi-lineage differentiation.[35] One-hundredth of white adipose cells are ADSCs with mesenchymal properties.[36] Tobita et al.[37] has shown the possibility of periodontal tissue regeneration using ADSCs.[37] Before the discovery of iPS by Takahashi et al.,[38] embryonic stem cells were the only available pluripotent cells used in dentistry. Research on human and mouse embryonic stem cells led to great accomplishments in differentiation of stem cells into oral tissues and organs.[39,40] Dental iPS cells have shown to be readily accessible from various dental stem cells[41,42] and fibroblasts.[43,44]

The aim in using stem cells is to explore the possibility of craniofacial, tooth, pulp, periodontal ligament, enamel, and dentin regeneration.[45] So far extensive studies on cementum matrix by Handa et al.,[46] periodontal ligament by Lin et al.,[47] soft dental pulp regeneration by Cordeiro et al.[48] and Huang et al.,[48,49] and enamel regeneration by Honda et al.[50] have shown great promise in the future of stem cells in RD. Recently, Iglesias-Linares et al.[51] have investigated the revascularization and apexogenesis induced by stem cells and demonstrated the latest advancements in apical regeneration.[51] Although the scientists have established the preclinical safety, efficacy and feasibility of pulp regeneration derived from dental

stem cells,[52] clinical trials cannot be launched, until certain challenges, such as the difficulty to handle critically-sized defects, are addressed.[53]

Growth factors can help tissue regrowth by regulating the signaling between the cells, their environment, and their neighbors.[54] Through making an information-conducive and extra-cellular matrix, growth factors play a crucial role in the regeneration of dental tissues.[55] Tayalia et al. demonstrated how scientists can take advantage of growth factors to improve specifically guided differentiation of cells.[56] It is extremely important to know which growth factors are suitable for specific types of cells and have the ability to orchestrate the cell type's proliferation and differentiation into the anticipated cell lineage.[57] A wide spectrum of growth factors is required in order to control each step of tissue regeneration and the fate of the stem cell.[58] The growth factors entrapped in the dentin matrix, which are actively protected in the dentin matrix, are responsible for the stimulation of processes—such as odontoblast differentiation—that lead to dentin formation.[59] Dental growth factors are also in charge of differentiation of adult pulp stem cells[60] and dentin bridging.[61] Although important prerequisite steps for utilizing growth factors—delivery, immobilization, and release—are currently undergoing active investigations, preclinical and stage I/II clinical trials have demonstrated how growth factors can accelerate and improve periodontal and bone regeneration.[62,63]

3.2. Biomaterials and scaffolds

Scientists have used three major categories of materials in TERM: namely, naturally derived materials (such as chitosan, elastin, and collagen), acellular tissue matrices, and synthetic materials.[1] Since application of natural materials is limited, FDA-approved synthetic polymers, such as polylactic acid (PLA), polyglycolic acid (PGA), and poly(lactic-co-glycolic acid) (PLGA) have wide applications in many TERM fields including RD.[64,65] Pre-clinical studies on animal models using all of the aforementioned categories have shown promising results in dental tissue regeneration.[66] Besides the conventional mechanical and chemical routes for synthesizing materials with biomedical applications,[67,68,69,70,71,72] scientists have always tried to explore innovative biomaterial synthesis techniques, such as green in

situ synthesis of silver particle encapsulated gelatin-based scaffolds, in situ encapsulation of iron nanoparticles in hydroxyapatite/chitosan matrix, and particulate sol–gel and cellulose templating of nanostructured zirconium titanate fibers.[73,74,75,76,77] Recently, novel biomaterials with more sophisticated designs that can be reinforced by bioactive elements have appealed to scientists.[78,79,80,81] Some examples include coating of bone scaffolds with fluoridated hydroxyapatite,[80] adding various ion substitutes to bioactive glasses,[79] and incorporation of bone morphogenetic protein into various bio-matrices to enhance osteogenesis.[82] Moreover, biodegradable hydrogels that profit from their tissue-like properties and cross-linking potential can also be used for efficient incorporation of biological agents.[83,84] In general, biomaterials that are used in RD are artificial and must be able to promote the epithelial and mesenchymal interactions.[85] Trombelli and Farina[86] demonstrated how using calcium phosphate bone substitutes and collagen derivatives can encourage alveolar bone tissue rebuilding.[86] Marine biomaterials have also started to attract a lot of attention in TERM and RD. A broad spectrum of biomaterials with high bio-availability can be extracted from marine products. In 2011, Addad et al. isolated collagen from jellyfish.[87] Two years later, Wysokowski et al. extracted chitin from marine sponges.[88] Marine biomimetics can be put into action in RD through either deployment without cellular content or *in vitro* culturing of mature tissues inside their matrices.[89] Another approach in delivering bioactive factors is called small molecule technique, which involves utilizing carbon-based compounds comprising only a small sequence of natural protein ligands.[90] In the past fifteen years, several small molecules have been designed and investigated for their osteoblast-promoting and osteoclast-inhibiting properties.[91,92,93] Due to their relatively smaller molecular size (< 1000 Da), these molecules neither induce unwanted immune responses, nor necessitate structural integrity for bioactivity.[94] Researchers have performed a number of preclinical animal studies on bone defects in order to reduce the nonspecific adverse effect of small molecules.[95,96,97,98]

Scaffolds provide 3-D support for cells, biological agents, and biomaterials in order to accomplish different missions—such as cell adhesion, stem cell differentiation, guided tissue regeneration, and permanent mechanical support.[99,100,101] For decades, metallic implants have been widely used for medical and dental applications and have

Materials Science and Engineering: C, Vol 69 (December 1, 2016): pg. 1383-1390. DOI.

been tailored for specific reconstruction of small or large hard-tissue defects.[102,103] However, scaffolds are suggested to be made of biodegradable materials with a degradation rate close to the tissue generation rate.[11,104,105,106] Therefore, the utilization of metallic scaffolds can be limited as they are mostly non-degradable, and thus may require second surgery to be removed from the body. Scaffolds can be used in RD as structural templates for stem cell differentiation and proliferation. Recently, Song et al. demonstrated the successful production of hard dental tissues on the periphery of macro-porous biphasic calcium phosphate scaffolds. Synthetic polymeric scaffolds have shown great potential in promoting dental pulp tissue regeneration.[107] Recent experiments on platelet-rich plasma scaffolds have demonstrated that they can effectively improve the healing induction and tissue regeneration in regenerative endodontic treatments.[108,109] Useful implications of these scaffolds have been shown on a group of pediatric patients clinically or radiographically, although not significantly better than conventional blood clot scaffolds.[110] These studies can justify the investigations that are currently being carried out for the design and improvement of dental scaffolds. Marine sponge skeletons,[111] diatom skeletons,[112] and *Foraminifera* micro-skeletons[113] have been used as scaffolds, bioactive molecule delivery devices, and bone substitutes. These biomimetic structures can be interesting options for dental bone regeneration. Several pre-clinically successful collagen-base periodontal tissue regeneration strategies and also clinically available scaffold materials have shown promising results to be used in RD.[114,115,116]

3.3. Other approaches

There are over 700 genetic syndromes that cause approximately 75% of the congenital defects occurring in the United States. Beside the significant impact of these genetic disorders on the quality of life, the estimated yearly treatment cost for these patients is more than $750 million.[117] For a long time, transferring manipulated genes for clinical applications has been a dream, but nowadays with the recent advances in biotechnology, gene therapy has shown promising pre-clinical results in curing non-hereditary and hereditary diseases.[118] In gene therapy, by either using a viral or non-viral vector as a carrier molecule, functional genes replace the abnormal and malfunctioning

mutant alleles after the insertion into the patient's cells.[119] Unlike somatic gene therapy, in which functional genes are inserted into the patient's somatic cells, germ line gene therapy targets genetic modification of sperm and egg and would be heritable to the offspring.[120,121] When using viral vectors, namely retroviruses, adenoviruses, adeno-associated viruses, or herpes simplex virus—even though the forms of the genetic materials are different—the transportation takes place after the virus infects the host cell. For non-viral gene transfection, scientists have tried direct transfer of naked DNA.[122] inactivation of diseased genes using oligonucleotides,[123] liposomal delivery of plasmid DNA,[124] application of cationic dendrimers and endocytosis,[125] and the combination of two or more techniques.[126] Over the past two decades, scientists have passionately worked on applying gene therapy to dentistry and as a result they have made tremendous progress in periodontal bone regeneration.[127] The salivary gland is another target of gene therapy and this area has shown promising results in both curing salivary gland diseases and even serious systemic pathologies.[128] Recently, genetically modified cell therapy, by combining the benefits of direct gene delivery and cell therapy,[129] has been explored for periodontal ligament in rabbits.[130,131] Showing good patient specific adaptability, this novel therapy has potential for a bright future in this field.

Dental tissues have complex architecture, anisotropic mechanical properties, and heterogeneous cell distribution; hence, it is hard to mimic their complex 3-D structure using the conventional techniques. To overcome this challenge, recently 3-D bio-printing of dental and craniofacial tissues has been proposed.[132,133] 3-D bio-printed scaffolds can be designed for each individual patient and have shown remarkable controllability over cell and biomaterial positioning, while maintaining great accuracy in internal and external details.[134] In general, printers use computed designs and follow the basic concept of layer-by-layer deposition of materials to produce 3-D volumetric structures. Based on the type of their ink dispenser, bio-printers can be grouped into three categories: Inkjet 3-D printers (capable of applying low-viscosity bio-inks using thermal or piezoelectrical controlling system), laser-assisted printers (capable of using cell and biomaterial sources with various viscosities for pulse laser deposition of 3-D structures), and extrusion printers (capable of extruding high-viscosity and stiff polymeric sources at relatively high

temperatures).[133] Due to their excellent biocompatibility and outstanding tenability, polymeric hydrogels have been the best nominees to be used as materials for 3-D bio-printing.[135] As the bio-printing process usually involves high-temperature steps, cells and growth factors (temperature susceptible materials) are not initially amalgamated in the polymeric mixture.[136] Both ceramic (such as hydroxyapatite)[137] and composite materials (such as polymer composite hydrogels)[138] are considered as alternative bio-ink materials. Applying this novel technique to TERM, Reichert et al.[139] used 3-D bio-printed scaffolds to study the bone formation in a sheep model in which the sheep was suffering from a critically-sized bone defect and eventually showed significant bone formation improvement.[139] In RD, scientists have made several attempts to mimic the intricate architecture of the periodontium in order to improve the regeneration of the periodontal complex.[140,141] In an investigation on scaffolds for cartilage regeneration, Schek et al.[142] used composite bio-printed scaffolds seeded with fibroblasts and reported a remarkable growth of cartilaginous tissue in the craniofacial region.[142] Kim et al.[143] made a 3-D printed tooth replica to perform *in vitro* and *in vivo* experiments on the whole-tooth regeneration process.[143] Other groups have also tried the same route of research and have narrowed down the fundamentals of whole-tooth regeneration *via* 3-D bio-printing.[144,145] All of these technological advances show promise for a hopeful future in 3-D bio-printing of the whole tooth and other oral tissues for future generations.

In the last fifteen years, scientists have started to apply their knowledge of micro-electronics and achievements in semiconducting materials to cellular differentiation and its microenvironment.[146] These technologies can potentially solve some of the challenges that other TERM approaches face—for example, they can shed light on the reconstruction of ectodermal and mesodermal interactions. They can provide nano-resolution for building patterns to develop various cell types; hence, making these technologies useful for producing scaffolds carrying several stem cells. Moreover, microscale technologies provide the possibility of isolating, seeding, and combining various cell types, which makes them suitable for *in vitro* assessments of cell behaviors in well-controlled environments.[147] This can enable rapid evaluation of the effects of biomaterials, drugs, and biological agents as a result of performing patterned single or multi-culturing *in vitro* experiments.

Flaim et al.[148] investigated the potential synergistic effects of the simultaneous utilization of growth factors and extracellular matrix proteins on stem cell activity.[148] These novel technologies have improved growth factor delivery by offering precise cell control and regulation. For example, Ennett et al.[149] performed long-lasting growth factor release using PLGA micro-spheres *in vitro* and *in vivo*.[149] The so called "microscale technology approach" can be carried out through either soft lithography or photolithography.[15] Kane et al.[150] and Rozkiewicz et al.[151] used soft lithography to mold templates and pattern selective cells.[150,151] Zhang et al.[152] and Kim et al.[153] used photolithography to fabricate 3-D micro-vascularized scaffolds and structures.[152,153] These techniques' ability to form 3-D micro-channels can help in supporting the cell metabolism.[154] This advantage can play a crucial role in achieving a reliable technique for tooth regeneration. Hydrogels, with approximately 99% water content, are the best materials to use in the microscale approach.[155] These materials can provide controllability in the structural formation with great detail. Microscale technologies can fabricate micro-structures, provide open channels, support vascularization, enhance diffusion, help regulate the cell activity, and facilitate high-throughput approaches; hence, they have a huge potential for both the *in vitro* and *in vivo* constructions of tooth-like structures.[156]

Even after reaching the advanced technology of building patient-specific tooth substructures, the major challenges of the application of TE in dentistry range from the cost-efficiency of these approaches to their availability to public (in terms of well-equipped health centers and institutes). Moreover, RD inherits the controversial ethical challenge of choosing which cell source (patient's own or donors') and cell type (adult or fetal) for TE. However, the ongoing research on TE and RD opens the venue to future investigations toward the development of whole-tooth structure during the next decades; which furtherly can shed light on the regeneration of other organs.

4. Conclusion

Although a lot of advancements in RD have revolutionized modern dentistry, there are still several steps left to take before declaring RD as a reliable alternative to conventional dentistry. RD

owes plenty to stem cell science and growth factor engineering. However, a good source of totipotent stem cells is not yet readily accessible and extracting human embryonic stem cells is a problematic and controversial issue. Moreover, it is not easy to control the stem cell differentiation. Delivery of active growth factor to the desired site is challenging and might provoke side effects. Biomaterials and scaffolds have played fundamental roles in facilitating partial dental tissue regeneration, but until today, none of the materials have met all the mechanical and biological standards required for RD. Gene therapy has opened up new directions to curing dental congenital diseases in individuals and their offspring; however, viral vectors used in this technique might trigger immune responses and side effects with irreversible damage. These genes live for a short period of time, which makes them ineffective in some cases. Furthermore, 3-D bio-printing and microscale technologies are pushing the boundaries of RD, but both are costly and are still in their early developmental stages. Though there is much work left, these are areas with great promise for the future of RD. The future of dentistry is in the hands of cellular biologists, geneticists, biomedical engineers, and materials scientists that strive to find and perfect novel approaches and techniques to address the aforementioned issues. Although the partial regeneration of human dental tissues and structures seems to be attainable near, considering the obstacles ahead, whole-tooth regeneration may be achievable in the farther future.

Acknowledgment

The Authors would like to thank the financial supports from Delta Dental, Marquette Innovation Fund, and NSF (CMMI-1363485).

References

[1]A. Atala. Regenerative medicine strategies. *J. Pediatr. Surg.,* 47 (1) (2012), pp. 17–28

[2]A. Raya-Rivera, D.R. Esquiliano, J.J. Yoo, E. Lopez-Bayghen, S. Soker, A. Atala. Tissue-engineered autologous urethras for patients who need reconstruction: an observational study. *Lancet*, 377 (9772) (2011), pp. 1175–1182

[3]F. Oberpenning, J. Meng, J.J. Yoo, A. Atala. De novo reconstitution of a functional mammalian urinary bladder by tissue engineering. *Nat. Biotechnol.,* 17 (2) (1999), pp. 149–155

[4]T.G. Kwon, J.J. Yoo, A. Atala. Autologous penile corpora cavernosa replacement using tissue engineering techniques. *J. Urol.,* 168 (4) (2002), pp. 1754–1758

[5]R.E. De Philippo, C.E. Bishop, L. Freitas Filho, J.J. Yoo, A. Atala. Tissue engineering a complete vaginal replacement from a small biopsy of autologous tissue. *Transplantation*, 86 (2) (2008), pp. 208–214

[6]T. Shin'oka, Y. Imai, Y. Ikada. Transplantation of a tissue-engineered pulmonary artery. *N. Engl. J. Med.,* 344 (7) (2001), pp. 532–533

[7]P. Amrollahi, L. Tayebi. Bioreactors for heart valve tissue engineering: a review. *J. Chem. Technol. Biotechnol.* (2015)

[8]R.N. Bhandari, L.A. Riccalton, A.L. Lewis, J.R. Fry, A.H. Hammond, S.J. Tendler, *et al.* Liver tissue engineering: a role for co-culture systems in modifying hepatocyte function and viability. *Tissue Eng.,* 7 (3) (2001), pp. 345–357

[9]S. Baiguera, M.A. Birchall, P. Macchiarini. Tissue-engineered tracheal transplantation. *Transplantation*, 89 (5) (2010), pp. 485–491

[10]M.K. Marei. Regenerative dentistry. *Synth. Lect. Tissue Eng.,* 2 (1) (2010), pp. 1–178

[11]K.M. Galler, R.N. D'Souza. Tissue engineering approaches for regenerative dentistry. *Regen. Med.,* 6 (1) (2011), pp. 111–124

[12]K. Singh, N. Mishra, L. Kumar, K.K. Agarwal, B. Agarwal. Role of stem cells in tooth bioengineering. *J. Oral Biol. Craniofacial Res.,* 2 (1) (2012), pp. 41–45

[13]E.D. Beltrán-Aguilar, L. Barker, M. Canto, B. Dye, B. Gooch, S. Griffin, *et al.* Centers for Disease Control and Prevention (CDC). Surveillance for dental caries, dental sealants, tooth retention, edentulism, and enamel fluorosis—United States, 1988–1994 and 1999–2002. *MMWR Surveill. Summ.,* 54 (3) (2005), pp. 1–43

[14]S.K. Majumdar. History of dentistry: an overview. *Bull. Indian Inst. Hist. Med. (Hyderabad),* 32 (1) (2001), pp. 31–42

[15]S. Hacking, A. Khademhosseini. Applications of microscale technologies for regenerative dentistry. *J. Dent. Res.,* 88 (5) (2009), pp. 409–421

[16]S.E. Duailibi, M.T. Duailibi, J.P. Vacanti, P.C. Yelick. Prospects for tooth regeneration. *Periodontol.,* 41 (1) (2006), pp. 177–187

[17]C.-G. Fan, Z. Q-j, Z. J-r. Therapeutic potentials of mesenchymal stem cells derived from human umbilical cord. *Stem Cell Rev. Rep.,* 7 (1) (2011), pp. 195–207

[18]Y. Sakaguchi, I. Sekiya, K. Yagishita, T. Muneta. Comparison of human stem cells derived from various mesenchymal tissues: superiority of synovium as a cell source. *Arthritis Rheum.,* 52 (8) (2005), pp. 2521–2529

[19]H. Egusa, W. Sonoyama, M. Nishimura, I. Atsuta, K. Akiyama. Stem cells in dentistry–part I: stem cell sources. *J. Prosthodont. Res.,* 56 (3) (2012), pp. 151–165

[20]S. Gronthos, M. Mankani, J. Brahim, P.G. Robey, S. Shi. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. *Proc. Natl. Acad. Sci.,* 97 (25) (2000), pp. 13625–13630

[21]M. Miura, S. Gronthos, M. Zhao, B. Lu, L.W. Fisher, P.G. Robey, *et al.* SHED: stem cells from human exfoliated deciduous teeth. *Proc. Natl. Acad. Sci.,* 100 (10) (2003), pp. 5807–5812

[22]S. Gronthos, J. Brahim, W. Li, L. Fisher, N. Cherman, A. Boyde, *et al.* Stem cell properties of human dental pulp stem cells. *J. Dent. Res.,* 81 (8) (2002), pp. 531–535

[23]S. Shi, P. Robey, S. Gronthos. Comparison of human dental pulp and bone marrow stromal stem cells by cDNA microarray analysis. *Bone*, 29 (6) (2001), pp. 532–539

[24]W. Sonoyama, Y. Liu, T. Yamaza, R.S. Tuan, S. Wang, S. Shi, *et al.* Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: a pilot study. *J. Endod.,* 34 (2) (2008), pp. 166–171

[25]G.T.-J. Huang, W. Sonoyama, Y. Liu, H. Liu, S. Wang, S. Shi. The hidden treasure in apical papilla: the potential role in pulp/dentin regeneration and bioroot engineering. *J. Endod.,* 34 (6) (2008), pp. 645–651

[26]C. McCulloch. Progenitor cell populations in the periodontal ligament of mice. *Anat. Rec.,* 211 (3) (1985), pp. 258–262

[27]B.-M. Seo, M. Miura, S. Gronthos, P.M. Bartold, S. Batouli, J. Brahim, *et al.* Investigation of multipotent postnatal stem cells from human periodontal ligament. *Lancet*, 364 (9429) (2004), pp. 149–155

[28]C. Morsczeck, W. Götz, J. Schierholz, F. Zeilhofer, U. Kühn, C. Möhl, *et al.* Isolation of precursor cells (PCs) from human dental follicle of wisdom teeth. *Matrix Biol.,* 24 (2) (2005), pp. 155–165

[29]M.J. Honda, M. Imaizumi, H. Suzuki, S. Ohshima, S. Tsuchiya, K. Satomura. Stem cells isolated from human dental follicles have osteogenic potential. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.,* 111 (6) (2011), pp. 700–708

[30]E. Ikeda, K. Yagi, M. Kojima, T. Yagyuu, A. Ohshima, S. Sobajima, *et al.* Multipotent cells from the human third molar: feasibility of cell-based therapy for liver disease. *Differentiation*, 76 (5) (2008), pp. 495–505

[31]Q. Zhang, S. Shi, Y. Liu, J. Uyanne, Y. Shi, S. Shi, *et al.* Mesenchymal stem cells derived from human gingiva are capable of immunomodulatory functions and ameliorate inflammation-related tissue destruction in experimental colitis. *J. Immunol.,* 183 (12) (2009), pp. 7787–7798

[32]C. De Bari, F. Dell'Accio, J. Vanlauwe, J. Eyckmans, I.M. Khan, C.W. Archer, *et al.* Mesenchymal multipotency of adult human periosteal cells

demonstrated by single-cell lineage analysis. *Arthritis Rheum.,* 54 (4) (2006), pp. 1209–1221

[33]A. Sato, K. Okumura, S. Matsumoto, K. Hattori, S. Hattori, M. Shinohara, *et al.* Isolation, tissue localization, and cellular characterization of progenitors derived from adult human salivary glands. *Cloning Stem Cells,* 9 (2) (2007), pp. 191–205

[34]L.E. Kokai, K. Marra, J.P. Rubin. Adipose stem cells: biology and clinical applications for tissue repair and regeneration. *Transl. Res.,* 163 (4) (2014), pp. 399–408

[35]N. Arceo, J.J. Sauk, J. Moehring, R.A. Foster, M.J. Somerman. Human periodontal cells initiate mineral-like nodules in vitro. *J. Periodontol.,* 62 (8) (1991), pp. 499–503

[36]W.K. Ong, S. Sugii. Adipose-derived stem cells: fatty potentials for therapy. *Int. J. Biochem. Cell Biol.,* 45 (6) (2013), pp. 1083–1086

[37]M. Tobita, A.C. Uysal, R. Ogawa, H. Hyakusoku, H. Mizuno. Periodontal tissue regeneration with adipose-derived stem cells. *Tissue Eng. A,* 14 (6) (2008), pp. 945–953

[38]K. Takahashi, S. Yamanaka. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell,* 126 (4) (2006), pp. 663–676

[39]Y. Shamis, K.J. Hewitt, M.W. Carlson, M. Margvelashvilli, S. Dong, C.K. Kuo, *et al.* Fibroblasts derived from human embryonic stem cells direct development and repair of 3D human skin equivalents. *Stem Cell Res. Ther.,* 2 (10) (2011)

[40]F. Ning, Y. Guo, J. Tang, J. Zhou, H. Zhang, W. Lu, *et al.* Differentiation of mouse embryonic stem cells into dental epithelial-like cells induced by ameloblasts serum-free conditioned medium. *Biochem. Biophys. Res. Commun.,* 394 (2) (2010), pp. 342–347

[41]X. Yan, H. Qin, C. Qu, R.S. Tuan, S. Shi, G.T.-J. Huang. iPS cells reprogrammed from human mesenchymal-like stem/progenitor cells of dental tissue origin. *Stem Cells Dev.,* 19 (4) (2010), pp. 469–480

[42]Y. Oda, Y. Yoshimura, H. Ohnishi, M. Tadokoro, Y. Katsube, M. Sasao, *et al.* Induction of pluripotent stem cells from human third molar mesenchymal stromal cells. *J. Biol. Chem.,* 285 (38) (2010), pp. 29270–29278

[43]K. Miyoshi, D. Tsuji, K. Kudoh, K. Satomura, T. Muto, K. Itoh, *et al.* Generation of human induced pluripotent stem cells from oral mucosa. *J. Biosci. Bioeng.,* 110 (3) (2010), pp. 345–350

[44]N. Wada, B. Wang, N.H. Lin, A.L. Laslett, S. Gronthos, P.M. Bartold. Induced pluripotent stem cell lines derived from human gingival fibroblasts and periodontal ligament fibroblasts. *J. Periodontal Res.,* 46 (4) (2011), pp. 438–447

[45]M. Sethi, A. Dua, V. Dodwad. Stem cells: a window to regenerative dentistry. *Int. J. Pharm. Biomed. Res.,* 3 (2012), pp. 175–180

[46]K. Handa, M. Saito, A. Tsunoda, M. Yamauchi, S. Hattori, S. Sato, *et al.* Progenitor cells from dental follicle are able to form cementum matrix in vivo. *Connect. Tissue Res.,* 43 (2–3) (2002), pp. 406–408

[47]N.H. Lin, S. Gronthos, P. Bartold. Stem cells and periodontal regeneration. *Aust. Dent. J.,* 53 (2) (2008), pp. 108–121

[48]M.M. Cordeiro, Z. Dong, T. Kaneko, Z. Zhang, M. Miyazawa, S. Shi, *et al.* Dental pulp tissue engineering with stem cells from exfoliated deciduous teeth. *J. Endod.,* 34 (8) (2008), pp. 962–969

[49]G.T.-J. Huang, T. Yamaza, L.D. Shea, F. Djouad, N.Z. Kuhn, R.S. Tuan, *et al.* Stem/progenitor cell-mediated de novo regeneration of dental pulp with newly deposited continuous layer of dentin in an in vivo model. *Tissue Eng. A,* 16 (2) (2009), pp. 605–615

[50]M.J. Honda, Y. Shinmura, Y. Shinohara. Enamel tissue engineering using subcultured enamel organ epithelial cells in combination with dental pulp cells. *Cells Tissues Organs,* 189 (1–4) (2009), pp. 261–267

[51]A. Iglesias-Linares, R.-M. Yáñez-Vico, E. Sánchez-Borrego, A.M. Moreno-Fernández, E. Solano-Reina, A. Mendoza-Mendoza. Stem cells in current paediatric dentistry practice. *Arch. Oral Biol.,* 58 (3) (2013), pp. 227–238

[52]M. Nakashima, K. Iohara. Mobilized dental pulp stem cells for pulp regeneration: initiation of clinical trial. *J. Endod.,* 40 (4) (2014), pp. S26–S32

[53]G.T.J. Huang, M. Al-Habib, P. Gauthier. Challenges of stem cell-based pulp and dentin regeneration: a clinical perspective. *Endod. Top.,* 28 (1) (2013), pp. 51–60

[54]D.E. Discher, D.J. Mooney, P.W. Zandstra. Growth factors, matrices, and forces combine and control stem cells. *Science,* 324 (5935) (2009), pp. 1673–1677

[55]F.-M. Chen, Y. Jin. Periodontal tissue engineering and regeneration: current approaches and expanding opportunities. *Tissue Eng. B Rev.,* 16 (2) (2010), pp. 219–255

[56]P. Tayalia, D.J. Mooney. Controlled growth factor delivery for tissue engineering. *Adv. Mater.,* 21 (32 − 33) (2009), pp. 3269–3285

[57]F.-M. Chen, Y. An, R. Zhang, M. Zhang. New insights into and novel applications of release technology for periodontal reconstructive therapies. *J. Control. Release,* 149 (2) (2011), pp. 92–110

[58]F.J. Hughes, W. Turner, G. Belibasakis, G. Martuscelli. Effects of growth factors and cytokines on osteoblast differentiation. *Periodontol.,* 41 (1) (2006), pp. 48–72

[59]A. Smith, P. Murray, A. Sloan, J. Matthews, S. Zhao. Trans-dentinal stimulation of tertiary dentinogenesis. *Adv. Dent. Res.,* 15 (1) (2001), pp. 51–54

[60]K. Iohara, L. Zheng, M. Ito, A. Tomokiyo, K. Matsushita, M. Nakashima. Side population cells isolated from porcine dental pulp tissue with self-renewal and multipotency for dentinogenesis, chondrogenesis, adipogenesis, and neurogenesis. *Stem Cells,* 24 (11) (2006), pp. 2493–2503

[61]H. Lovschall, O. Fejerskov, A. Flyvbjerg. Pulp-capping with recombinant human insulin-like growth factor I (rhIGF-I) in rat molars. *Adv. Dent. Res.,* 15 (1) (2001), pp. 108–112

[62]R. Rutherford, C. Niekrash, J. Kennedy, M. Charette. Platelet-derived and insulin-like growth factors stimulate regeneration of periodontal attachment in monkeys. *J. Periodontal Res.,* 27 (4) (1992), pp. 285–290

[63]T.H. Howell, J.P. Fiorellini, D.W. Paquette, S. Offenbacher, W.V. Giannobile, S.E. Lynch. A phase I/II clinical trial to evaluate a combination of recombinant human platelet-derived growth factor-BB and recombinant human insulin-like growth factor-I in patients with periodontal disease. *J. Periodontol.,* 68 (12) (1997), pp. 1186–1193

[64]R. Langer, D.A. Tirrell. Designing materials for biology and medicine. *Nature,* 428 (6982) (2004), pp. 487–492

[65]M. YazdiMamaghani, S.M. Davachi, P. Amrollahi, D. Vashaee, L. Tayebi. Conducting polymers: developments. *Encyclopedia of Biomedical Polymers and Polymeric Biomaterials,* Taylor & Francis (2016), pp. 1997–2010

[66]F.-M. Chen, H.-H. Sun, H. Lu, Q. Yu. Stem cell-delivery therapeutics for periodontal tissue regeneration. *Biomaterials,* 33 (27) (2012), pp. 6320–6344

[67]P. Amrollahi, A. Ataie, A. Nozari, E. Seyedjafari, A. Shafiee. Cytotoxicity evaluation and magnetic characteristics of mechano-thermally synthesized CuNi nanoparticles for hyperthermia. *J. Mater. Eng. Perform.,* 24 (3) (2015), pp. 1220–1225

[68]P. Amrollahi, J.S. Krasinski, R. Vaidyanathan, L. Tayebi, D. Vashaee. Electrophoretic deposition (EPD): fundamentals and applications from nano-to microscale structures. *Handbook of Nanoelectrochemistry: Electrochemical Synthesis Methods, Properties, and Characterization Techniques* (2016), pp. 561–591

[69]P. Amrollahi, A. Ataie, A. Nozari, S. Sheibani. Synthesis and characterization of CuNi magnetic nanoparticles by mechano-thermal route. *J. Supercond. Nov. Magn.,* 27 (2) (2014), pp. 481–485

[70]E. Salahinejad, M. Hadianfard, D. Macdonald, I. Karimi, D. Vashaee, L. Tayebi. Aqueous sol–gel synthesis of zirconium titanate ($ZrTiO_4$)

nanoparticles using chloride precursors. *Ceram. Int.,* 38 (8) (2012), pp. 6145–6149

[71]E. Salahinejad, M. Hadianfard, D. Macdonald, M. Mozafari, D. Vashaee, L. Tayebi. Zirconium titanate thin film prepared by an aqueous particulate sol–gel spin coating process using carboxymethyl cellulose as dispersant. *Mater. Lett.,* 88 (2012), pp. 5–8

[72]E. Salahinejad, M. Hadianfard, D. Macdonald, M. Mozafari, D. Vashaee, L. Tayebi. Multilayer zirconium titanate thin films prepared by a sol–gel deposition method. *Ceram. Int.,* 39 (2) (2013), pp. 1271–1276

[73]M. Yazdimamaghani, D. Vashaee, S. Assefa, M. Shabrangharehdasht, A.T. Rad, M.A. Eastman, *et al.* Green synthesis of a new gelatin-based antimicrobial scaffold for tissue engineering. *Mater. Sci. Eng. C,* 39 (2014), pp. 235–244

[74]M. Yazdimamaghani, T. Pourvala, E. Motamedi, B. Fathi, D. Vashaee, L. Tayebi. Synthesis and characterization of encapsulated nanosilica particles with an acrylic copolymer by in situ emulsion polymerization using thermoresponsive nonionic surfactant. *Materials,* 6 (9) (2013), pp. 3727–3741

[75]F. Heidari, M.E. Bahrololoom, D. Vashaee, L. Tayebi. In situ preparation of iron oxide nanoparticles in natural hydroxyapatite/chitosan matrix for bone tissue engineering application. *Ceram. Int.,* 41 (2) (2015), pp. 3094–3100

[76]E. Salahinejad, M. Hadianfard, D. Macdonald, M. Mozafari, D. Vashaee, L. Tayebi. A new double-layer sol–gel coating to improve the corrosion resistance of a medical-grade stainless steel in a simulated body fluid. *Mater. Lett.,* 97 (2013), pp. 162–165

[77]P. Rouhani, E. Salahinejad, R. Kaul, D. Vashaee, L. Tayebi. Nanostructured zirconium titanate fibers prepared by particulate sol–gel and cellulose templating techniques. *J. Alloys Compd.,* 568 (2013), pp. 102–105

[78]N. Huebsch, D.J. Mooney. Inspiration and application in the evolution of biomaterials. *Nature,* 462 (7272) (2009), pp. 426–432

[79]S.M. Rabiee, N. Nazparvar, M. Azizian, D. Vashaee, L. Tayebi. Effect of ion substitution on properties of bioactive glasses: a review. *Ceram. Int.,* 41 (6) (2015), pp. 7241–7251

[80]M. Razavi, M. Fathi, O. Savabi, D. Vashaee, L. Tayebi. Biodegradable magnesium alloy coated by fluoridated hydroxyapatite using MAO/EPD technique. *Surf. Eng.,* 30 (8) (2014), pp. 545–551

[81]M. Yazdimamaghani, M. Razavi, D. Vashaee, V.R. Pothineni, J. Rajadas, L. Tayebi. Significant degradability enhancement in multilayer coating of polycaprolactone-bioactive glass/gelatin-bioactive glass on magnesium scaffold for tissue engineering applications. *Appl. Surf. Sci.,* 338 (2015), pp. 137–145

[82]B.D. Ratner, S.J. Bryant. Biomaterials: where we have been and where we are going. *Annu. Rev. Biomed. Eng.,* 6 (2004), pp. 41–75

[83]G.D. Nicodemus, S.J. Bryant. Cell encapsulation in biodegradable hydrogels for tissue engineering applications. *Tissue Eng. B Rev.,* 14 (2) (2008), pp. 149–165

[84]V. Shabafrooz, M. Mozafari, G.A. Köhler, S. Assefa, D. Vashaee, L. Tayebi. The effect of hyaluronic acid on biofunctionality of gelatin–collagen intestine tissue engineering scaffolds. *J. Biomed. Mater. Res. Part A,* 102 (9) (2014), pp. 3130–3139

[85]T. Ohara, T. Itaya, K. Usami, Y. Ando, H. Sakurai, M.J. Honda, *et al.* Evaluation of scaffold materials for tooth tissue engineering. *J. Biomed. Mater. Res. Part A,* 94 (3) (2010), pp. 800–805

[86]L. Trombelli, R. Farina. Clinical outcomes with bioactive agents alone or in combination with grafting or guided tissue regeneration. *J. Clin. Periodontol.,* 35 (s8) (2008), pp. 117–135

[87]S. Addad, J.-Y. Exposito, C. Faye, S. Ricard-Blum, C. Lethias. Isolation, characterization and biological evaluation of jellyfish collagen for use in biomedical applications. *Mar. Drugs,* 9 (6) (2011), pp. 967–983

[88]M. Wysokowski, M. Motylenko, V.V. Bazhenov, D. Stawski, I. Petrenko, A. Ehrlich, *et al.* Poriferan chitin as a template for hydrothermal zirconia deposition. *Front. Mater. Sci.,* 7 (3) (2013), pp. 248–260

[89]D.W. Green, W.-F. Lai, H.-S. Jung. Evolving marine biomimetics for regenerative dentistry. *Mar. Drugs,* 12 (5) (2014), pp. 2877–2912

[90]H. Egusa, Y. Kaneda, Y. Akashi, Y. Hamada, T. Matsumoto, M. Saeki, *et al.* Enhanced bone regeneration via multimodal actions of synthetic peptide SVVYGLR on osteoprogenitors and osteoclasts. *Biomaterials,* 30 (27) (2009), pp. 4676–4686

[91]H. Egusa, M. Saeki, M. Doi, S. Fukuyasu, T. Matsumoto, Y. Kamisaki, *et al.* A small-molecule approach to bone regenerative medicine in dentistry. *J. Oral Biosci.,* 52 (2) (2010), pp. 107–118

[92]C.T. Laurencin, K.M. Ashe, N. Henry, H.M. Kan, K.W.-H. Lo. Delivery of small molecules for bone regenerative engineering: preclinical studies and potential clinical applications. *Drug Discov. Today,* 19 (6) (2014), pp. 794–800

[93]C.-Y.E. Han, Y. Wang, L. Yu, D. Powers, X. Xiong, V. Yu, *et al.* Small molecules with potent osteogenic-inducing activity in osteoblast cells. *Bioorg. Med. Chem. Lett.,* 19 (5) (2009), pp. 1442–1445

[94]K.W. Lo, K.M. Ashe, H.M. Kan, C.T. Laurencin. The role of small molecules in musculoskeletal regeneration. *Regen. Med.,* 7 (4) (2012), pp. 535–549

[95]T. Ito, M. Takemasa, K. Makino, M. Otsuka. Preparation of calcium phosphate nanocapsules including simvastatin/deoxycholic acid assembly, and their therapeutic effect in osteoporosis model mice. *J. Pharm. Pharmacol.,* 65 (4) (2013), pp. 494–502

[96]K. Gellynck, R. Shah, M. Parkar, A. Young, P. Buxton, P. Brett. Small molecule stimulation enhances bone regeneration but not titanium implant osseointegration. *Bone,* 57 (2) (2013), pp. 405–412

[97]Y. Qi, T. Zhao, W. Yan, K. Xu, Z. Shi, J. Wang. Mesenchymal stem cell sheet transplantation combined with locally released simvastatin enhances bone formation in a rat tibia osteotomy model. *Cytotherapy,* 15 (1) (2013), pp. 44–56

[98]B. Bostan, T. Güneş, M. Aşçı, C. Sen, M. Keleştemur, M. Erdem, *et al.* Simvastatin improves spinal fusion in rats. *Acta Orthop. Traumatol. Turc.,* 45 (4) (2010), pp. 270–275

[99]M. Mozafari, D. Vashaee, L. Tayebi, M. Mehraien. Electroconductive Nanocomposite Scaffolds: A New Strategy into Tissue Engineering and Regenerative Medicine. *INTECH Open Access Publisher* (2012)

[100]M. Yazdimamaghani, M. Razavi, D. Vashaee, L. Tayebi. Surface modification of biodegradable porous Mg bone scaffold using polycaprolactone/bioactive glass composite. *Mater. Sci. Eng. C,* 49 (2015), pp. 436–444

[101]M. Yazdimamaghani, M. Razavi, D. Vashaee, L. Tayebi. Microstructural and mechanical study of PCL coated Mg scaffolds. *Surf. Eng.,* 30 (12) (2014), pp. 920–926

[102]K. Alvarez, H. Nakajima. Metallic scaffolds for bone regeneration. *Materials,* 2 (3) (2009), pp. 790–832

[103]W.-E. Yang, M.-L. Hsu, M.-C. Lin, Z.-H. Chen, L.-K. Chen, H.-H. Huang. Nano/submicron-scale TiO_2 network on titanium surface for dental implant application. *J. Alloys Compd.,* 479 (1) (2009), pp. 642–647

[104]M. Razavi, M.H. Fathi, O. Savabi, D. Vashaee, L. Tayebi. Biodegradation, bioactivity and in vivo biocompatibility analysis of plasma electrolytic oxidized (PEO) biodegradable Mg implants. *Phys. Sci. Int. J.,* 4 (5) (2014), p. 708

[105]M. Razavi, M. Fathi, O. Savabi, S.M. Razavi, F. Heidari, M. Manshaei, *et al.* In vivo study of nanostructured diopside ($CaMgSi_2O_6$) coating on magnesium alloy as biodegradable orthopedic implants. *Appl. Surf. Sci.,* 313 (2014), pp. 60–66

[106]A. Tahmasbi Rad, N. Ali, H.S.R. Kotturi, M. Yazdimamaghani, J. Smay, D. Vashaee, *et al.* Conducting scaffolds for liver tissue engineering. *J. Biomed. Mater. Res. A,* 102 (11) (2014), pp. 4169–4181

[107]L. Casagrande, M.M. Cordeiro, S.A. Nör, J.E. Nör. Dental pulp stem cells in regenerative dentistry. *Odontology,* 99 (1) (2011), pp. 1–7

[108]M. Torabinejad, M. Turman. Revitalization of tooth with necrotic pulp and open apex by using platelet-rich plasma: a case report. *J. Endod.,* 37 (2) (2011), pp. 265–268

[109]T. Bezgin, A. Yılmaz, B. Celik, H. Sönmez. Concentrated platelet-rich plasma used in root canal revascularization: 2 case reports. *Int. Endod. J.,* 47 (1) (2014), pp. 41–49

[110]T. Bezgin, A.D. Yilmaz, B.N. Celik, M.E. Kolsuz, H. Sonmez. Efficacy of platelet-rich plasma as a scaffold in regenerative endodontic treatment. *J. Endod.,* 41 (1) (2015), pp. 36–44

[111]S. Heinemann, H. Ehrlich, C. Knieb, T. Hanke. Biomimetically inspired hybrid materials based on silicified collagen. *Int. J. Mater. Res.,* 98 (7) (2007), pp. 603–608

[112]M.S. Aw, S. Simovic, J. Addai-Mensah, D. Losic. Silica microcapsules from diatoms as new carrier for delivery of therapeutics. *Nanomedicine,* 6 (7) (2011), pp. 1159–1173

[113]J. Chou, J. Hao, H. Hatoyama, B. Ben-Nissan, B. Milthorpe, M. Otsuka. The therapeutic effect on bone mineral formation from biomimetic zinc containing tricalcium phosphate (ZnTCP) in zinc-deficient osteoporotic mice. *PLoS One,* 8 (8) (2013)

[114]T. Nakahara, T. Nakamura, E. Kobayashi, K.-I. Kuremoto, T. Matsuno, Y. Tabata, *et al.* In situ tissue engineering of periodontal tissues by seeding with periodontal ligament-derived cells. *Tissue Eng.,* 10 (3–4) (2004), pp. 537–544

[115]Y. Liu, Y. Zheng, G. Ding, D. Fang, C. Zhang, P.M. Bartold, *et al.* Periodontal ligament stem cell-mediated treatment for periodontitis in miniature swine. *Stem Cells,* 26 (4) (2008), pp. 1065–1073

[116]M. Taba, Q. Jin, J. Sugai, W. Giannobile. Current concepts in periodontal bioengineering. *Orthod. Craniofacial Res.,* 8 (4) (2005), pp. 292–302

[117]R.J. Gorlin, M.M. Cohen, R.C. Hennekam. *Syndromes of the Head and Neck*. Oxford University Press, New York (1990)

[118]K. Gupta, S. Singh, K.N. Garg. Gene therapy in dentistry: tool of genetic engineering. Revisited. *Arch. Oral Biol.,* 60 (3) (2015), pp. 439–446

[119]T. Friedmann, R. Roblin. Gene therapy for human genetic disease? *Science*, 175 (4025) (1972), pp. 949–955

[120]A. Bank. Human somatic cell gene therapy. *BioEssays,* 18 (12) (1996), pp. 999–1007

[121]Q.L. Matthews, D.T. Curiel. Gene therapy: human germline genetic modifications--assessing the scientific, socioethical, and religious issues. *Gene Ther.,* 100 (1) (2007)

[122]J.A. Wolff, R.W. Malone, P. Williams, W. Chong, G. Acsadi, A. Jani, *et al.* Direct gene transfer into mouse muscle in vivo. *Science,* 247 (4949) (1990), pp. 1465–1468

[123]E. Pierce, Q. Liu, O. Igoucheva, R. Omarrudin, H. Ma, S. Diamond, *et al.* Oligonucleotide-directed single-base DNA alterations in mouse embryonic stem cells. *Gene Ther.,* 10 (1) (2003), pp. 24–33

[124]N.J. Caplen, E.W. Alton, P.G. Mddleton, J.R. Dorin, B.J. Stevenson, X. Gao, *et al.* Liposome-mediated CFTR gene transfer to the nasal epithelium of patients with cystic fibrosis. *Nat. Med.,* 1 (1) (1995), pp. 39–46

[125]S.P. Chaplot, I.D. Rupenthal. Dendrimers for gene delivery–a potential approach for ocular therapy? *J. Pharm. Pharmacol.,* 66 (4) (2014), pp. 542–556

[126]S. Huang, M. Kamihira. Development of hybrid viral vectors for gene therapy. *Biotechnol. Adv.,* 31 (2) (2013), pp. 208–223

[127]O. Anusaksathien, W.V. Giannobile. Growth factor delivery to re-engineer periodontal tissues. *Curr. Pharm. Biotechnol.,* 3 (2) (2002), pp. 129–139

[128]A. Voutetakis, I. Bossis, M.R. Kok, W. Zhang, J. Wang, A.P. Cotrim, *et al.* Salivary glands as a potential gene transfer target for gene therapeutics of some monogenetic endocrine disorders. *J. Endocrinol.,* 185 (3) (2005), pp. 363–372

[129]D. Sheyn, O. Mizrahi, S. Benjamin, Z. Gazit, G. Pelled, D. Gazit. Genetically modified cells in regenerative medicine and tissue engineering. *Adv. Drug Deliv. Rev.,* 62 (7) (2010), pp. 683–698

[130]Y. Chen, P.K. Chen, L. Jeng, C. Huang, L. Yang, H. Chung, *et al.* Periodontal regeneration using ex vivo autologous stem cells engineered to express the BMP-2 gene: an alternative to alveolaplasty. *Gene Ther.,* 15 (22) (2008), pp. 1469–1477

[131]T. Yokoi, M. Saito, T. Kiyono, S. Iseki, K. Kosaka, E. Nishida, *et al.* Establishment of immortalized dental follicle cells for generating periodontal ligament in vivo. *Cell Tissue Res.,* 327 (2) (2007), pp. 301–311

[132]S.V. Murphy, A. Atala. 3D bioprinting of tissues and organs. *Nat. Biotechnol.,* 32 (8) (2014), pp. 773–785

[133]F. Obregon, C. Vaquette, S. Ivanovski, D. Hutmacher, L. Bertassoni. Three-dimensional bioprinting for regenerative dentistry and craniofacial tissue engineering. *J. Dent. Res.,* 94 (9) (2015), pp. 143S–152S

[134]N.E. Fedorovich, J. Alblas, W.E. Hennink, F.C. Öner, W.J. Dhert. Organ printing: the future of bone regeneration? *Trends Biotechnol.,* 29 (12) (2011), pp. 601–606

[135]N. Annabi, A. Tamayol, J.A. Uquillas, M. Akbari, L.E. Bertassoni, C. Cha, *et al.* 25th anniversary article: rational design and applications of hydrogels in regenerative medicine. *Adv. Mater.,* 26 (1) (2014), pp. 85–124

[136]D.W. Hutmacher, M. Sittinger, M.V. Risbud. Scaffold-based tissue engineering: rationale for computer-aided design and solid free-form fabrication systems. *Trends Biotechnol.,* 22 (7) (2004), pp. 354–362

[137]S. Michna, W. Wu, J.A. Lewis. Concentrated hydroxyapatite inks for direct-write assembly of 3-D periodic scaffolds. *Biomaterials,* 26 (28) (2005), pp. 5632–5639

[138]S.E. Bakarich, R. Gorkin III, M. in het Panhuis, G.M. Spinks. Three-dimensional printing fiber reinforced hydrogel composites. *ACS Appl. Mater. Interfaces,* 6 (18) (2014), pp. 15998–16006

[139]J.C. Reichert, A. Cipitria, D.R. Epari, S. Saifzadeh, P. Krishnakanth, A. Berner, *et al.* A tissue engineering solution for segmental defect regeneration in load-bearing long bones. *Sci. Transl. Med.,* 4 (141) (2012), p. 141ra93

[140]P.F. Costa, C. Vaquette, Q. Zhang, R.L. Reis, S. Ivanovski, D.W. Hutmacher. Advanced tissue engineering scaffold design for regeneration of the complex hierarchical periodontal structure. *J. Clin. Periodontol.,* 41 (3) (2014), pp. 283–294

[141]B.T. Goh, L.Y. Teh, D.B.P. Tan, Z. Zhang, S.H. Teoh. Novel 3D polycaprolactone scaffold for ridge preservation–a pilot randomised controlled clinical trial. *Clin. Oral Implants Res.,* 26 (3) (2015), pp. 271–277

[142]R. Schek, J. Taboas, S.J. Hollister, P. Krebsbach. Tissue engineering osteochondral implants for temporomandibular joint repair. *Orthod. Craniofacial Res.,* 8 (4) (2005), pp. 313–319

[143]K. Kim, C. Lee, B. Kim, J. Mao. Anatomically shaped tooth and periodontal regeneration by cell homing. *J. Dent. Res.,* 89 (8) (2010), pp. 842–847

[144]E. Ikeda, R. Morita, K. Nakao, K. Ishida, T. Nakamura, T. Takano-Yamamoto, *et al.* Fully functional bioengineered tooth replacement as an organ replacement therapy. *Proc. Natl. Acad. Sci.,* 106 (32) (2009), pp. 13475–13480

[145]W. Zhang, I.P. Ahluwalia, P.C. Yelick. Three dimensional dental epithelial-mesenchymal constructs of predetermined size and shape for tooth regeneration. *Biomaterials,* 31 (31) (2010), pp. 7995–8003

[146]G.M. Whitesides, E. Ostuni, S. Takayama, X. Jiang, D.E. Ingber. Soft lithography in biology and biochemistry. *Annu. Rev. Biomed. Eng.,* 3 (1) (2001), pp. 335–373

[147]A. Rosenthal, A. Macdonald, J. Voldman. Cell patterning chip for controlling the stem cell microenvironment. *Biomaterials,* 28 (21) (2007), pp. 3208–3216

[148]C.J. Flaim, D. Teng, S. Chien, S.N. Bhatia. Combinatorial signaling microenvironments for studying stem cell fate. *Stem Cells Dev.,* 17 (1) (2008), pp. 29–40

[149]A.B. Ennett, D. Kaigler, D.J. Mooney. Temporally regulated delivery of VEGF in vitro and in vivo. *J. Biomed. Mater. Res. A,* 79 (1) (2006), pp. 176–184

[150]R.S. Kane, S. Takayama, E. Ostuni, D.E. Ingber, G.M. Whitesides. Patterning proteins and cells using soft lithography. *Biomaterials,* 20 (23) (1999), pp. 2363–2376

[151]D.I. Rozkiewicz, Y. Kraan, M.W. Werten, F.A. de Wolf, V. Subramaniam, B.J. Ravoo, *et al.* Covalent microcontact printing of proteins for cell patterning. *Chem. Eur. J.,* 12 (24) (2006), pp. 6290–6297

[152]J.-Y. Zhang, B.A. Doll, E.J. Beckman, J.O. Hollinger. Three-dimensional biocompatible ascorbic acid-containing scaffold for bone tissue engineering. *Tissue Eng.,* 9 (6) (2003), pp. 1143–1157

[153]P. Kim, H.E. Jeong, A. Khademhosseini, K.Y. Suh. Fabrication of non-biofouling polyethylene glycol micro-and nanochannels by ultraviolet-assisted irreversible sealing. *Lab Chip,* 6 (11) (2006), pp. 1432–1437

[154]Y. Ling, J. Rubin, Y. Deng, C. Huang, U. Demirci, J.M. Karp, *et al.* A cell-laden microfluidic hydrogel. *Lab Chip,* 7 (6) (2007), pp. 756–762

[155]N.W. Choi, M. Cabodi, B. Held, J.P. Gleghorn, L.J. Bonassar, A.D. Stroock. Microfluidic scaffolds for tissue engineering. *Nat. Mater.,* 6 (11) (2007), pp. 908–915