Journal of Dental Sciences (2021) 16, 501–507

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jds.com

Review article

Advanced technologies in periodontal tissue regeneration based on stem cells: Current status and future perspectives

Wen-Yi Zeng [a,c], Yang Ning [b,c], Xin Huang [b,c*]

[a] Hospital of Stomatology, Guanghua School of Stomatology, Sun Yat-Sen University, Guangzhou, China
[b] Department of Periodontology, Guanghua School and Hospital of Stomatology, Sun Yat-Sen University, Guangzhou, China
[c] Guangdong Provincial Key Laboratory of Stomatology, Sun Yat-Sen University, Guangzhou, China

Received 13 May 2020; Final revision received 18 July 2020
Available online 19 August 2020

KEYWORDS
Cell sheeting;
Electrospinning;
Periodontal tissue engineering;
Spheroid culture;
Stem cells;
3D printing

Abstract Periodontitis is a progressive inflammation disease, the clinical management of which remains a challenge. The traditional management may control periodontal inflammation, but failed to regenerate functional periodontium. This review summarizes the most advancing regenerative techniques regarding stem cell culture and scaffold fabrication, such as cell sheeting, spheroid culture, electrospinning and 3D printing. The applications of different techniques manifest tremendous potential of regenerating the complete and functional periodontium. Albeit promising, new technologies have met with their own drawbacks such as insufficient vascularization and precision, which necessitate deeper modification. Thus, this review also points out the potential perspectives and methods aiming at their disadvantages, illuminating the directions of future researches to successful clinical scenarios.

Introduction

Chronic periodontitis is a sort of prevalent disease characterized by the nonreversible chronic inflammatory destruction of the periodontium, which eventually leads to the premature tooth loss.[1] The morbidity of chronic periodontitis ranges from 30% to 40% in individuals over 35 years old, which makes periodontitis a global concern in oral diseases.[2] Therefore, the proper treatments remain an urgent public health issue (see Table 1).

The purpose of periodontal treatment is to suppress the inflammation and regenerate functional periodontium, including the cementum, alveolar bone, and the crucial inserted periodontal ligament.[3] Some approaches have been clinically implemented to achieve better periodontal

* Corresponding author. Department of Periodontology, Guanghua School and Hospital of Stomatology, Sun Yat-Sen University, No.56, Ling Yuan West Road, Yue Xiu District, Guangzhou, China.
E-mail address: huangx275@mail.sysu.edu.cn (X. Huang).

https://doi.org/10.1016/j.jds.2020.07.008

Table 1 The comparison of different technologies in advantages and disadvantages.

	Advantages	Disadvantages
Cell sheeting	Minimalize cell loss	Costly
	Keep cell viability	Longer culture period
	Lessen damage to function	Attach weakly to hard tissue
		Insufficient vascularization
Spheroid culture	Similar microenvironment	Spheroid size decrease
	Producing more ECM Enhanced stemness	Lack of oxygen and nutrient at the core
Electrospinning	Similarity with natural ECM	Hinder cell infiltration
	Larger surface	Difficulties in controlling the degradation rate
	Guide fiber directions	
3D printing	Custom-made scaffolds	High temperature
	Maintain great accuracy	Limited medical application
	Enable specific functions	

regeneration, such as GBR, bone grafting, and the localized delivery of growth factors.[4] These techniques have shown some improvement in periodontal regeneration in intrabony defects, but a lack of predictability in cases of advanced ones.[5] Since the isolation of PDLSC(periodontal ligament stem cell) from the extracted third molar,[6] researches based on stem cell in periodontal regeneration have surged exponentially. In light of the low efficacy in traditional tissue engineering strategies, a series of new technologies have been established. In this review, we mainly focus on advanced technologies in periodontal tissue engineering with regard to stem cell culture and scaffold fabrication and shed a light on their drawbacks and the relevant future perspectives to be put into clinical use.

Traditional strategies

Periodontal tissue engineering, by definition, incorporates culture-expanded progenitor cells into prefabricated scaffold, which is then transplanted into the defect sites to realize periodontal regeneration.[7] The traditional tissue engineering method is to inject the cells or build a composite cell-material structure in vitro, then transplanting into the defect site.[8]

Cell injection

Mesenchymal stem cells, a crucial element in tissue engineering, are capable of performing self-renewal, differentiating into various cell lines and modulating immune response by secreting anti-inflammatory factors.[9] The traditional cellular therapy utilizes single-cell suspension from the isolated cells in vitro to directly inject into the damaged site. Compared with cell-material design, cell injection is less invasive for no flaps and suitable for those with systemic diseases and not in the stage of severe periodontitis.[10] The bone marrow mesenchymal stem cell injection in a rat model exerts immunomodulatory effects at the defect sites and contributed to the periodontal repair.[11] But the drawbacks lie in the cell loss, poor engraftment, inadequate localization, spreading into surrounding tissues[12] and loss of cell fate control.[13]

Cell combined with materials

To limit the localization of stem cells and reduce cell loss, cells are transplanted with various materials. The most commonly used stem cells are periodontal ligament stem cells (PDLSCs),[14] and others are also available including stem cells exfoliated deciduous tissue (SHED),[15] dental pulp stem cells(DPSC)[16] and bone marrow mesenchymal stem cells(BMMSCs)[17] for periodontal regeneration. Among the most frequently used scaffold material are natural origin biomaterials like chitosan, collagen, fibrin sealants and synthetic polymers like hydroxyapatite/tricalcium phosphate(HA/TCP).[18] Lemaitre grafted adipose-derived stromal cells with collagen scaffold, and eventually the cementum regeneration took shape as well as the organization of PDL fiber in a mouse model.[19]

Supported by the safeness of the stem cells[20] and the simplicity in operation, it has potential with respect to clinical use. Yet there exists no histological evidence that functional periodontal complex can be formed with this method and it necessitates invasive surgery.

New tissue engineering technologies

As material, bioengineering, and regenerative medicine improves by leaps and bounds, a lot of new technologies in tissue engineering are emerging nowadays, giving the field of periodontal regeneration a boost. Herein, we reviewed the new technologies in cell culture and scaffold manufacturing.

New technologies in cell culture

The stem cell culture has always been a hurdle in regenerative medicine because one of the critical terms is to keep such features as stemness and differentiation capacities. Obstacles are met in integrally maintaining characteristics in vivo by mimicking microenvironment. Thus, various techniques have surfaced such as cell sheeting and spheroid culture.

Cell sheeting

As an alternative to cell suspension, cell sheeting is an advanced technology in cell culture, which equals scaffold-free cell delivery.[21] When cells are cultured, they develop cellular junction and extracellular matrix (ECM). In this context, confluent cultured stem cells can be harvested from the culture dish without any enzyme in monolayer or multilayers together with intact ECM and cellular junction as a whole(Fig. 1A).[22] Cell sheets can attach to the

adjacent one, piling in layers to fabricate thick tissues and 3D structures. Compared to the above-mentioned techniques, the complete preservation of extracellular matrix and cell-to-cell junction without enzymes such as trypsin minimalize the cell loss as well as the cell viability and lessen damage to function.[23]

A type of temperature-responsive polymer named N-isopropylacrylamide was developed to form cell sheets. Attached onto the dish surface, it is dehydrated and hydrophobic at 37 °C, which cells favor and expand on. But at temperature lower than 32 °C, it is hydrated and hydrophilic, yielding cell sheets with complete ECM.[24] In addition, other methods are also available based on magnetic force[25] and polyelectrolytes.[26]

Hu et al. transplanted human dental pulp stem cells(hDPSC) using cell injection or cell sheeting respectively, both of which significantly facilitated regenerated bone whereas hDPSC sheets group showed more bone regeneration capacity than hDPSC injection.[27] In many researches, stem cell sheets are combined with bone substitutes to stabilize stem cells within defects like tricalcium phosphate, hydroxyapatite,[15] and hydrogel.[28] Iwata et al. delivered multilayered PDL cell sheets to the nude dentin supported with woven polyglycolic acid(PGA) in the middle and osteo-inductive material porous β-tricalcium phosphate in the bone defect. This structure successfully facilitate both cementum and bone regeneration linked to well-oriented periodontal fibers.[29] Chen et al.[30] used autologous PDLSCs sheets combined with Bio-oss in a randomized clinical trial but the results showed no statistical difference, which may owe to the restricted validity of the cells from the biomaterials. Hence further researches should be implemented to better build structure between cell sheets and materials.

Regarding the drawbacks of cell sheeting, it is costly and require longer culture period as it will be fragile when not confluent enough.[24] Besides, cell sheets attach weakly on such hard tissues as the root surface. As mentioned, combining cell sheets with materials is at play but we should also assure no limitation of cell viability simultaneously. And cells may necrotize if the sheets are too thick and lacking in vascularization.[21] Of late some researchers have focused on pre-vascularization by coculturing endothelial cells in multilayer sheets.[31] Better approaches should be developed to promote the combination of cell sheets and various materials to allow supreme attachment and pre-vascularization.

Spheroid culture technique

Apart from the aforementioned cell sheeting technology, multiple cell culture techniques have been developed. Different from 2D culture of monolayer, 3D culture reconstitute in vivo environment, including cell—cell and cell-ECM interactive networks.[32] Spheroid culture, an emerging and promising method, works by cell aggregates and self-assembly.[33] Compared to cell suspension or cell sheeting,

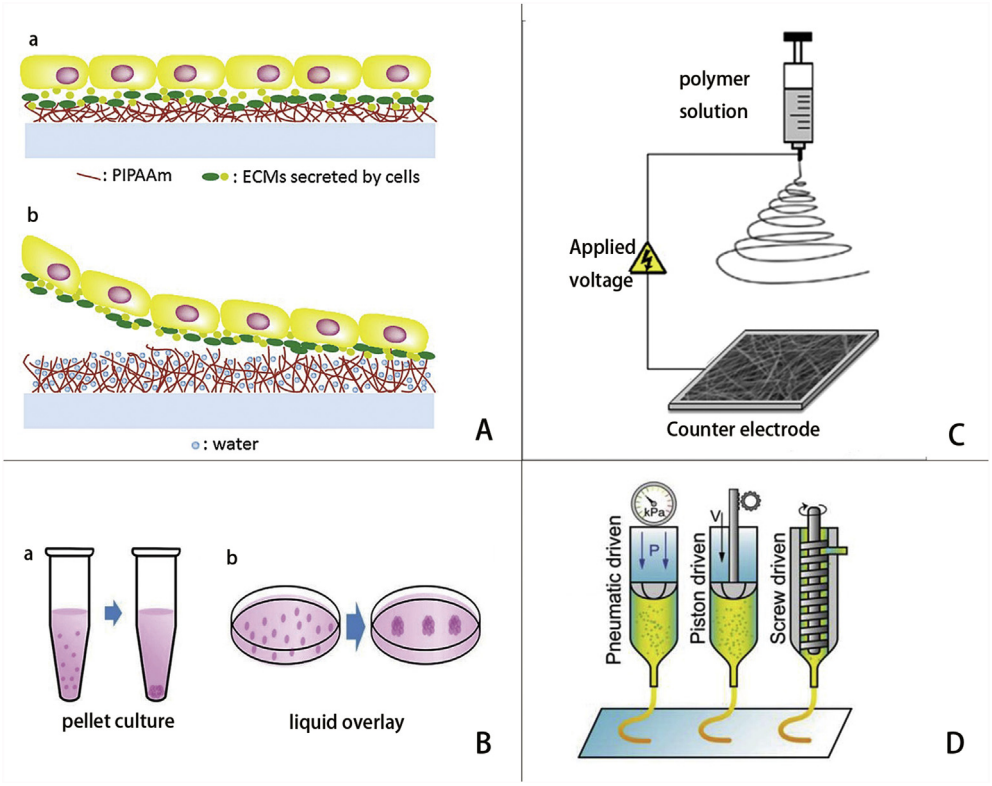

Figure 1 The principle of cell sheeting and electrospinning. (A) Cells attach to the surface at 37 °C, and detach from it at temperature lower than 32 °C. (B) Under electrical field, polymer deposits into electrospinning fibers. (C)The scheme of pellet culture and liquid overlay in spheroid culture. (D) Extrusion-based techniques in 3D printing. Adapted from References[24,32,41,50] with permission.

spheroid cells are capable of producing ECM and other growth factors at a higher level.[34]

The principles of spheroids depend on the culture microenvironment wherein cell—cell adhesion outweigh cell-material adhesion.[35] In periodontal tissue engineering, various spheroid forming methods have been established such as hanging drop, pellet culture, membrane-based aggregation[36] and microwell chips approach(Fig. 1B).[37] Pellet culture utilizes centrifugal force, and hanging drop utilizes surface tension and gravitational forces. Microwell chips can better control spheroid size by changing the size of microwell scale.[37] Spheroids of hPDLMSCs (human periodontal ligament mesenchymal stem cells) were formed using microwell chips, showing greater stemness and enhanced osteogenic potential, which may be beneficial for hard tissue repair.[38] Iwasaki et al. utilized pellet culture in polypropylene tubes to form PDLSC spheroids, which upregulated the anti-inflammation and angiogenesis expression, promoting periodontal regeneration theoretically. Yet it showed a failure of periodontal regeneration probably owing to the dead cells contained in the central necrotic zone.[39]

To conclude, spheroid culture has various advantages associated with the similarity in vivo microenvironment, such as the enhanced stemness and osteogenic potential as well as the immunomodulatory effects, making it promising conditioned medium for periodontal regeneration. However, the spheroid size shows a time-dependent decrease probably due to the smaller cell size in spheroids, the reduced proliferating cells and the increased death cells. Lack of oxygen and nutrients at the core of spheroids accounts for the limitations of spheroid culture.[39] Hence, further researches should be established to illustrate the optimization of cell size and number.

New technologies in creating multiphasic scaffold

The regeneration of the periodontal complex is in need of coordinated process among each individual component. Inspired from the anatomical structure of the periodontium, multiphasic scaffolds are comprised of hierarchical structures to facilitate regeneration by managing a series of complicated spatiotemporal interactions occurring in periodontal remodeling procedure.[40]

Electrospinning

Electrospinning mimics natural ECM to provide suitable environment for cell development, standing out as a universal micro to nanofiber manufacturing technique.[41] A typical machine consists of a syringe that carries polymer solution or the polymer melt, a pump to pulse the polymer, a source of electrical field and a collector plate.[42] Under the electrical difference between the syringe and the collector, polymer deposits in the form of micro to nanofibers(Fig. 1C).[43] Solution electrospinning fibers are inorganized nanofibers with smaller pores, while melt electrospinning fibers are organized microfibers with larger pores.[40]

Due to the similarity with natural ECM, electrospinning scaffolds favors cell adhesion, proliferation, migration and differentiation.[44] Electrospinning, a resourceful and cost-effective way to fabricate nanofiber, has the advantages of larger surfaces that can improve protein interaction and offer binding sites.[45] For tissue engineering, the electrospinning fibers, whether in random or in alignment, are capable of guiding the growth direction of fibers or cells topographically, benefiting the specifically oriented tissues such as the hierarchical periodontal fibers.[46]

Promising as electrospinning is, there exist a few limitations standing in the way in the further progress in periodontal regeneration. For solution electrospinning fibers, the smaller fiber diameter is, the bigger superficial area to volume ratio will be, which supplies more protein and molecule binding sites but may exert detrimental effects on the cell infiltration due to the reduced pore sizes.[45] Hence, it is of great significance to control the different pore sizes in accordance with various structures. The drawbacks also lies in the difficulties in controlling the degradation rate synchronizing with the periodontal tissue remodeling.[47] If the degradation begins ahead of tissue maturation, the scaffold may collapse and 3D network vanish, hindering the growth and remodeling of the fiber.

3D printing

3D printing, also acknowledged as rapid prototyping or additive manufacturing, emerges as a pioneering technique in tissue engineering.[48]

In the context, scaffolds are produced in a layer-by-layer manner compared with subtractive manufacturing.[49] Bioprinting takes advantage of cell-laden bioinks to yield functional tissue and organ, wherein cells are embedded in matrix.[48] Nowadays the bioprinting technologies involve stereolithography, inkjet, laser-induced forward transfer(-LIFT) and extrusion.[50] Among these techniques, extrusion bioprinting is a common way for fabricating scaffolds in periodontal regeneration. In most cases, controlled extrusion is driven by the piston, screw or pneumatic system in the form of filament or struct from the printer head to the collector(Fig. 1D).[51] As a common example of this technique, fused deposition modeling utilizes thermoplastic polymer melted at the nozzle at elevated temperature, empowering the deposition of semi-liquid struct above the collector.[50] The frequently-used materials for this technique include polycarbonate (PC), acrylonitrile butadiene styrene (ABS), polycaprolactone (PCL), and polylactic acid(PLA) owing to the relatively low melting points.[52]

3D bioprinting, relying on data from computed tomography in digital imaging system,[53] makes it easy to fabricate custom-made scaffolds, maintain great accuracy in details and enables specific functions.[54] Regarding FDM, it has the advantages of low-cost, high speed printing due to the simultaneous printing from multi-nozzle. Yet the high temperature eliminates FDM as an alternative for biomedical applications.

Application of multiphasic scaffolds

Multiphasic scaffolds are fabricated in many manners to show optimized spatiotemporally control over the periodontal regeneration process.[55]

Biphasic scaffolds with cell sheets. Some studies combined the cell sheeting with biphasic scaffolds. Vaquette et al. fabricated a biphasic scaffold, composed of a 3D printed PCL scaffold for bone area via fused deposition modeling with osteoblast culture and a solution

electrospinning membrane for the PDL compartment integrated with multiple PDL cell sheets.[56] Applied on the dentin block, scaffolds gained better attachment of cell sheets to the dentin surface, owing to the thin mineralized tissue. But the periodontal fibers remained mainly parallel to the dentin surface. One reason may be that the solution electrospinning membrane had small pores partially hindering the further insertion of new periodontal ligament fibers into the bone. Another reason may be lack of fiber guiding structure.

Hence, following on this study, Costa et al. applied a calcium phosphate layer in the bone area to increase the osteo-conductivity and a melt electrospinning membrane with larger pore sizes to allow the attachment of functionally oriented periodontal ligament.[57]

Biphasic scaffolds. Park et al. developed compartmentalized biphasic scaffolds with fiber-guiding properties via computer-aided manufacturing and 3D wax printing. They fabricated polycaprolactone (PCL) bone region and polyglycolic acid (PGA) PDL region respectively with human dentin slices before subcutaneous implantation. After the cell-seeding process into the ectopic mice model, this biphasic scaffold successfully demonstrated bone and periodontal ligament regeneration along with the formation of parallel and obliquely oriented fiber.[53]

However, the periodontal model was geometrically highly standardized.[58] To enhance adaptability, Park et al. designed scaffolds according to micro-computed tomographic imaging of the defects. This time, biphasic scaffolds were formed with perpendicularly oriented microchannels by casting a PCL solution in the mold. Finally, the microchannels enhanced the controlled orientation of fiber.[59] Based on the image-based and fiber-guidance system, they advocated the manufacture of customized scaffolds by 3D printing.[60]

Triphasic scaffolds. As a further evolution of biphasic scaffolds, a triphasic scaffold surfaced via fused deposition modeling. Different sizes of microchannels are fabricated for each region. Phase A, the cementum–dentin interface, had 100-μm channels; phase B, the PDL compartment, had 600-μm channels; phase C, the bone region, had 300-μm channels. By combining spatially released bioactive cues, oblique fibers were yielded and inserted into the newly formed bone and cementum. Albeit promising, one of the limitations may be the stiffness of PCL scaffolds, making it difficult to adapt into the complex anatomical structures. And it's also unclear how the cementum component can attach onto the root surface regarding clinical applications.[61]

Clinical trials. Clinically, Rasperini et al. reported the PCL scaffold via selective laser sintering (SLS) to treat periodontal defect. A customized scaffold was fabricated by a computed tomography scan of the periodontal defect. In the internal region, there are ports for delivering growth factors and pegs perpendicular to the root surfaces, while the external region would resorb over time. However, the constructs became exposed 13 months post-implantation, leading to a failure from a clinical perspective. The exposure may result from the slow-degrading and stiff bulky polymer that created misfit in the soft tissue.[62] In another study, Vaquette et al. designed PCL melt electrospinning scaffolds for the bone region with PCL solution electrospun membrane for PDL region.[63] The melt electrospinning scaffold made it possible for a construct of macropore size with aligned microfibers and lower stiffness than the previous study, which ultimately regenerated newly formed obliquely periodontal ligament fibers and successful insertion. Thus, it is presumed that the changes in porosity and stiffness contribute to the integrity of soft tissue and reduces the risk of exposure.

Prospects and conclusions. In the aforementioned researches, the fiber guiding features lacked consistency and regularity. Of late, studies related to micropattern and microgroove of scaffolds have mounted up.[64] To enhance control over fiber orientation, Pilipchuck et al. designed PCL periodontal ligament scaffolds where organized grooved pillars in various sizes can be seen in a 3D patterned PCL thin film.[65] Park et al. utilized high resolution printers to produce micro-grooved molds via digital slicing process with parallel, oblique and perpendicular orientations. The assessment in vitro presented high efficacy in aligning human PDL cells.[66]

In brief, there still exist insufficient accuracy in terms of the multiphasic scaffolds. Even though micro-to nano-structures can be manufactured, the whole size of scaffolds reaches up to millimeter scale, marking a striking gap between the scaffold and the natural periodontium. Additionally, further endeavors should be made in designing biomimetic angulations and precise microstructures in the scaffolds to mimic the natural periodontal ligament anatomical structure.

Conclusion

Undoubtedly, tissue engineering based on stem cells have made evident progress in both cell culture and scaffold fabrication. But it's still not sufficient to put into clinical scenarios. Of necessity is the optimization of cell culture methods and related standard protocol as a whole. Multiphasic scaffolds should be improved in accuracy and adaptation regarding the angulation and three-dimension formation of micro-structures. The limitation also lies in the scarce source of stem cells as well as ethic and safe issues when using allogenic stem cells. Currently, the traditional clinical managements are still the options of most cases. It's concluded that stem-cell based tissue engineering warrants further researches and investigations in an effort for clinical use.

Declaration of competing interest

The authors have no conflicts of interest related to this article.

Acknowledgement

This study was supported by grants from Undergraduate Teaching Quality Engineering Project of Sun Yat-Sen

University (No. 87000-31911131), Guangdong Financial Fund for High-Caliber Hospital Construction (No. 174-2018-XMZC-0001-03-0125/C-01) and National Natural Science Foundation of China (No. 81500838).

References

1. Kinane DF, Stathopoulou PG, Papapanou PN. Periodontal diseases. *Nat Rev Dis Primers* 2017;3:17038.
2. Righolt AJ, Jevdjevic M, Marcenes W, Listl S. Global-, regional-, and country-level economic impacts of dental diseases in 2015. *J Dent Res* 2018;97:501—7.
3. Sculean A, Chapple IL, Giannobile WV. Wound models for periodontal and bone regeneration: the role of biologic research. *Periodontol* 2000;68:7—20. 2015.
4. Chen FM, Shelton RM, Jin Y, Chapple IL. Localized delivery of growth factors for periodontal tissue regeneration: role, strategies, and perspectives. *Med Res Rev* 2009;29:472—513.
5. Hernandez-Monjaraz B, Santiago-Osorio E, Monroy-Garcia A, Ledesma-Martinez E, Mendoza-Nunez VM. Mesenchymal stem cells of dental origin for inducing tissue regeneration in periodontitis: a mini-review. *Int J Mol Sci* 2018;19:944.
6. Seo BM, Miura M, Gronthos S, et al. Investigation of multipotent postnatal stem cells from human periodontal ligament. *Lancet* 2004;364:149—55.
7. Bartold PM, Gronthos S, Ivanovski S, Fisher A, Hutmacher DW. Tissue engineered periodontal products. *J Periodontal Res* 2016;51:1—15.
8. Iwata T, Yamato M, Ishikawa I, Ando T, Okano T. Tissue engineering in periodontal tissue. *Anat Rec* 2014;297:16—25.
9. Huang GT, Gronthos S, Shi S. Mesenchymal stem cells derived from dental tissues vs. Those from other sources: their biology and role in regenerative medicine. *J Dent Res* 2009;88:792—806.
10. Li G, Han N, Zhang X, et al. Local injection of allogeneic stem cells from apical papilla enhanced periodontal tissue regeneration in minipig model of periodontitis. *BioMed Res Int* 2018; 2018:3960798.
11. Du J, Shan Z, Ma P, Wang S, Fan Z. Allogeneic bone marrow mesenchymal stem cell transplantation for periodontal regeneration. *J Dent Res* 2014;93:183—8.
12. Lu L, Liu Y, Zhang X, Lin J. The therapeutic role of bone marrow stem cell local injection in rat experimental periodontitis. *J Oral Rehabil* 2019:1—10. 00.
13. Abou Neel EA, Chrzanowski W, Salih VM, Kim HW, Knowles JC. Tissue engineering in dentistry. *J Dent* 2014;42:915—28.
14. Han J, Menicanin D, Gronthos S, Bartold PM. Stem cells, tissue engineering and periodontal regeneration. *Aust Dent J* 2014; 59(Suppl 1):117—30.
15. Fu X, Jin L, Ma P, Fan Z, Wang S. Allogeneic stem cells from deciduous teeth in treatment for periodontitis in miniature swine. *J Periodontol* 2014;85:845—51.
16. Ferrarotti F, Romano F, Gamba MN, et al. Human intrabony defect regeneration with micrografts containing dental pulp stem cells: a randomized controlled clinical trial. *J Clin Periodontol* 2018;45:841—50.
17. Zhang Y, Xiong Y, Chen X, Chen C, Zhu Z, Li L. Therapeutic effect of bone marrow mesenchymal stem cells pretreated with acetylsalicylic acid on experimental periodontitis in rats. *Int Immunopharm* 2018;54:320—8.
18. Chen FM, Sun HH, Lu H, Yu Q. Stem cell-delivery therapeutics for periodontal tissue regeneration. *Biomaterials* 2012;33:6320—44.
19. Lemaitre M, Monsarrat P, Blasco-Baque V, et al. Periodontal tissue regeneration using syngeneic adipose-derived stromal cells in a mouse model. *Stem Cells Transl Med* 2017;6:656—65.
20. Chen FM, Liu X. Advancing biomaterials of human origin for tissue engineering. *Prog Polym Sci* 2016;53:86—168.
21. Chen M, Xu Y, Zhang T, et al. Mesenchymal stem cell sheets: a new cell-based strategy for bone repair and regeneration. *Biotechnol Lett* 2019;41:305—18.
22. Kirby GTS, Michelmore A, Smith LE, Whittle JD, Short RD. Cell sheets in cell therapies. *Cytotherapy* 2018;20:169—80.
23. Onizuka S, Iwata T. Application of periodontal ligament-derived multipotent mesenchymal stromal cell sheets for periodontal regeneration. *Int J Mol Sci* 2019;20:2796.
24. Iwata T, Washio K, Yoshida T, et al. Cell sheet engineering and its application for periodontal regeneration. *J Tissue Eng Regen Med* 2015;9:343—56.
25. Cheng K, Wang T, Yu M, et al. Effects of rgd immobilization on light-induced cell sheet detachment from tio2 nanodots films. *Mater Sci Eng C Mater Biol Appl* 2016;63:240—6.
26. Zahn R, Thomasson E, Guillaume-Gentil O, Vörös J, Zambelli T. Ion-induced cell sheet detachment from standard cell culture surfaces coated with polyelectrolytes. *Biomaterials* 2012;33: 3421—7.
27. Hu J, Cao Y, Xie Y, et al. Periodontal regeneration in swine after cell injection and cell sheet transplantation of human dental pulp stem cells following good manufacturing practice. *Stem Cell Res Ther* 2016;7:130.
28. Fawzy El-Sayed KM, Mekhemar MK, Beck-Broichsitter BE, et al. Periodontal regeneration employing gingival margin-derived stem/progenitor cells in conjunction with il-1ra-hydrogel synthetic extracellular matrix. *J Clin Periodontol* 2015;42: 448—57.
29. Iwata T, Yamato M, Tsuchioka H, et al. Periodontal regeneration with multi-layered periodontal ligament-derived cell sheets in a canine model. *Biomaterials* 2009;30:2716—23.
30. Chen FM, Gao LN, Tian BM, et al. Treatment of periodontal intrabony defects using autologous periodontal ligament stem cells: a randomized clinical trial. *Stem Cell Res Ther* 2016;7: 33.
31. Laschke MW, Menger MD. Prevascularization in tissue engineering: current concepts and future directions. *Biotechnol Adv* 2016;34:112—21.
32. Ryu NE, Lee SH, Park H. Spheroid culture system methods and applications for mesenchymal stem cells. *Cells* 2019;8.
33. Petrenko Y, Sykova E, Kubinova S. The therapeutic potential of three-dimensional multipotent mesenchymal stromal cell spheroids. *Stem Cell Res Ther* 2017;8:94.
34. Kelm JM, Fussenegger M. Scaffold-free cell delivery for use in regenerative medicine. *Adv Drug Deliv Rev* 2010;62:753—64.
35. Langenbach F, Naujoks C, Smeets R, et al. Scaffold-free microtissues: differences from monolayer cultures and their potential in bone tissue engineering. *Clin Oral Invest* 2013;17: 9—17.
36. Cheng NC, Wang S, Young TH. The influence of spheroid formation of human adipose-derived stem cells on chitosan films on stemness and differentiation capabilities. *Biomaterials* 2012;33:1748—58.
37. Sakai Y, Yoshida S, Yoshiura Y, et al. Effect of microwell chip structure on cell microsphere production of various animal cells. *J Biosci Bioeng* 2010;110:223—9.
38. Moritani Y, Usui M, Sano K, et al. Spheroid culture enhances osteogenic potential of periodontal ligament mesenchymal stem cells. *J Periodontal Res* 2018;53:870—82.
39. Iwasaki K, Nagata M, Akazawa K, Watabe T, Morita I. Changes in characteristics of periodontal ligament stem cells in spheroid culture. *J Periodontal Res* 2019;54:364—73.
40. Carter SD, Costa PF, Vaquette C, Ivanovski S, Hutmacher DW, Malda J. Additive biomanufacturing: an advanced approach for periodontal tissue regeneration. *Ann Biomed Eng* 2017;45: 12—22.
41. Braghirolli DI, Steffens D, Pranke P. Electrospinning for regenerative medicine: a review of the main topics. *Drug Discov Today* 2014;19:743—53.

42. Soares RMD, Siqueira NM, Prabhakaram MP, Ramakrishna S. Electrospinning and electrospray of bio-based and natural polymers for biomaterials development. *Mater Sci Eng C Mater Biol Appl* 2018;92:969—82.
43. Zhuang Y, Lin K, Yu H. Advance of nano-composite electrospun fibers in periodontal regeneration. *Front Chem* 2019;7:495.
44. Wang X, Ding B, Li B. Biomimetic electrospun nanofibrous structures for tissue engineering. *Mater Today* 2013;16:229—41.
45. Zafar M, Najeeb S, Khurshid Z, et al. Potential of electrospun nanofibers for biomedical and dental applications. *Materials* 2016;9:73.
46. van Manen EH, Zhang W, Walboomers XF, et al. The influence of electrospun fibre scaffold orientation and nano-hydroxyapatite content on the development of tooth bud stem cells in vitro. *Odontology* 2014;102:14—21.
47. Yang XT, Yang B, Tian WD. Development of new technology in periodontal tissue engineering. *Zhonghua Kou Qiang Yi Xue Za Zhi* 2018;53:490—4.
48. Vijayavenkataraman S, Yan WC, Lu WF, Wang CH, Fuh JYH. 3d bioprinting of tissues and organs for regenerative medicine. *Adv Drug Deliv Rev* 2018;132:296—332.
49. Kolesky DB, Homan KA, Skylar-Scott MA, Lewis JA. Three-dimensional bioprinting of thick vascularized tissues. *Proc Natl Acad Sci U S A* 2016;113:3179—84.
50. Tao O, Kort-Mascort J, Lin Y, et al. The applications of 3d printing for craniofacial tissue engineering. *Micromachines* 2019;10:480.
51. Placone JK, Engler AJ. Recent advances in extrusion-based 3d printing for biomedical applications. *Adv Healthc Mater* 2018;7:e1701161.
52. Wang X, Jiang M, Zhou ZW, Gou JH, Hui D. 3d printing of polymer matrix composites: a review and prospective. *Compos B Eng* 2017;110:442—58.
53. Park CH, Rios HF, Jin Q, et al. Biomimetic hybrid scaffolds for engineering human tooth-ligament interfaces. *Biomaterials* 2010;31:5945—52.
54. Ma Y, Xie L, Yang B, Tian W. Three-dimensional printing biotechnology for the regeneration of the tooth and tooth-supporting tissues. *Biotechnol Bioeng* 2019;116:452—68.
55. Ivanovski S, Vaquette C, Gronthos S, Hutmacher DW, Bartold PM. Multiphasic scaffolds for periodontal tissue engineering. *J Dent Res* 2014;93:1212—21.
56. Vaquette C, Fan W, Xiao Y, Hamlet S, Hutmacher DW, Ivanovski S. A biphasic scaffold design combined with cell sheet technology for simultaneous regeneration of alveolar bone/periodontal ligament complex. *Biomaterials* 2012;33:5560—73.
57. Costa PF, Vaquette C, Zhang Q, Reis RL, Ivanovski S, Hutmacher DW. Advanced tissue engineering scaffold design for regeneration of the complex hierarchical periodontal structure. *J Clin Periodontol* 2014;41:283—94.
58. Staples RJ, Ivanovski S, Vaquette C. Fibre guiding scaffolds for periodontal tissue engineering. *J Periodontal Res* 2020;55:331—41.
59. Park CH, Rios HF, Jin Q, et al. Tissue engineering bone-ligament complexes using fiber-guiding scaffolds. *Biomaterials* 2012;33:137—45.
60. Park CH, Rios HF, Taut AD, et al. Image-based, fiber guiding scaffolds: a platform for regenerating tissue interfaces. *Tissue Eng C Methods* 2014;20:533—42.
61. Lee CH, Hajibandeh J, Suzuki T, Fan A, Shang P, Mao JJ. Three-dimensional printed multiphase scaffolds for regeneration of periodontium complex. *Tissue Eng* 2014;20:1342—51.
62. Rasperini G, Pilipchuk SP, Flanagan CL, et al. 3d-printed bioresorbable scaffold for periodontal repair. *J Dent Res* 2015;94:153S—7S.
63. Vaquette C, Saifzadeh S, Farag A, Hutmacher DW, Ivanovski S. Periodontal tissue engineering with a multiphasic construct and cell sheets. *J Dent Res* 2019;98:673—81.
64. Park CH. Biomaterial-based approaches for regeneration of periodontal ligament and cementum using 3d platforms. *Int J Mol Sci* 2019;20:4364.
65. Pilipchuk SP, Monje A, Jiao Y, et al. Integration of 3d printed and micropatterned polycaprolactone scaffolds for guidance of oriented collagenous tissue formation in vivo. *Adv Healthc Mater* 2016;5:676—87.
66. Park CH, Kim KH, Lee YM, Giannobile WV, Seol YJ. 3d printed, microgroove pattern-driven generation of oriented ligamentous architectures. *Int J Mol Sci* 2017;18:1927.