Antibacterial agent-releasing scaffolds in dental tissue engineering

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Abstract
It seems quite challenging in tissue engineering to synthesize a base material with a range of essential activities, including biocompatibility, nontoxicity, and antimicrobial activities. Various types of materials are synthesized to solve the problem. This study aimed to provide the latest relevant information for practitioners about antibacterial scaffolds in dental tissue engineering. The PubMed search engine was used to review the relevant studies with a combination of the following terms as search queries: tissue engineering, scaffolds, antimicrobial, dentistry, dental stem cells, and oral diseases. It is noteworthy to state that only the terms related to tissue engineering in dentistry were considered. The antimicrobial scaffolds support the local tissue regeneration and prevent adverse inflammatory reactions; however, not all scaffolds have such positive characteristics. To resolve this potential defect, different antimicrobial agents are used during the synthesis process. Innovative methods in guided tissue engineering are actively working towards new ways to control oral and periodontal diseases.

Introduction
The science of tissue engineering has excellent potential for tooth regeneration. There are three essential elements in tissue engineering: scaffolds, stem cells (SCs), and growth factors (GFs). Scaffolds and growth factor carriers are important players in the regeneration of damaged tissues or teeth.

Tissue engineering requires different biomaterials with distinct properties than the ones used in engineering other tissues. Dental tissue engineering deals with regenerating damaged or lost tooth components, including enamel, dentin, and pulp. Postnatal investigation of the tooth development process is a crucial step for identifying the factors affecting the regeneration of dental tissues. As a complex tissue, a tooth consists of hard tissues, dentin, and enamel and is connected to bone through ligaments. Successive and mutual interactions between epithelial-mesenchymal cells shape teeth. While the epithelial cells have a prominent role in enamel formation, mesenchymal cells are responsible for producing differentiated cells vital for the formation of odontoblasts, pulp, and periodontal ligament.

Tissue engineering makes use of a wide range of different materials: hydroxyapatite, various composites based on bioactive glass, and synthetic/natural polymers. In addition, it is also possible to use 3D printing technology for scaffold production. None of the options mentioned above for bone and dental tissue engineering match all the characteristics of bone graft substitutes. Another instance would be electrospinning, which is a practical technique since its versatility allows for the synthesis of micro- and nano-fibers. Notably, one favorable characteristic of these nano-fibers is their optimum flexibility in the fabrication process, but low hydrophilicity and having no surface cell-recognition sites lead to sub-optimal performance of synthetic materials. Conversely, natural fibers have optimal biocompatibility performance, but mechanical performance is their Achilles heel. The final comprehensive solution to meet both mechanical and biocompatible (bioactive surface) requirements is to develop composite fibrous scaffolds, in which the synthetic polymers serve as the backbone and the natural polymers provide cellular attachment.

The main challenge after implementing scaffolds is to formulate strategies to prevent chronic infection after implantation, which is a possible complication expected in almost every surgical procedure. The culturing and implantation are the stages in which contamination is more likely. To combat contamination, antibiotics are an obvious solution, as they can effectively prevent bacterial infection after artificial bone transplantation. Typically, antibiotics should be prescribed with care since misuse leads to an adverse phenomenon: drug resistance. As a result, more effective but safer alternatives must be considered.
developed, and antibacterial scaffolds can be the ideal alternative. Standard antimicrobial scaffolds support local tissue regeneration and prevent adverse inflammatory reactions. Reportedly, in the synthesis of antibacterial scaffolds, different particles with optimal antibacterial and minimal toxic properties, such as silver nanoparticles (Ag), are used. This paper provides the latest information for practitioners about the antibacterial scaffolds in dental tissue engineering.

**Antimicrobial agents in dental tissue engineering**

The bacterial infections of the dental and periodontal defects should be managed and eradicated. Many antibiotics are embedded in polymer membranes, including but not restricted to tetracycline hydrochloride, metronidazole, and amoxicillin. Dayaghi et al compared the antimicrobial activity of Mg-Zn scaffolds containing a high tetracycline concentration and reported that this new scaffold has significant activity against Staphylococcus aureus and Escherichia coli compared to the reference scaffold mentioned above. Because of the significant antibacterial activity, if the tetracycline percentage is between 1% and 5%, it is a potential choice for bone healing applications. However, due to ever-increasing antibiotic resistance, different alternative substances have also been proposed as antimicrobial agents in dental tissue engineering, including metal and metal oxides, medicinal plants (herbal medicines), polymers, and novel drug delivery systems (nano-biomaterials).

**Metals and metal oxides**

The macromolecular construction suggests a tool to improve antimicrobial activity as numerous antimicrobial moieties, such as metals and metal oxides, can be conjugated to the scaffold. New studies and tests on gram-positive and gram-negative bacteria, particularly multidrug-resistant strains. Xing et al compared the antimicrobial activity of metallic silver particle-loaded poly-(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) scaffolds and poly-(3-hydroxybutyrate-co-3-hydroxyvalerate) and reported the significant antibacterial activity of Ag-loaded scaffold against S. aureus and Klebsiella pneumonia compared to the free PHBV scaffold. Numerous studies have incorporated Ag into scaffolds for sustained release and antibacterial activity. Tests on the antimicrobial activity of Ag scaffolds against S. aureus and E. coli showed that the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values for each strain were 32.0 and 32.0 μg/mL for S. aureus, and 64.0 and 85.3 μg/mL for E. coli. The control scaffold (without Ag) did not exhibit any antimicrobial effects. In another study, TiO$_2$-loaded scaffolds exhibited significant antibacterial activity. TiO$_2$ might cause oxidative and mechanical damage to bacteria through contact activities and the production of reactive oxygen species (ROS). ROS exerts oxidative stress on bacteria while the concentration is higher than the bacterial antioxidant defense system's inhibitory potential, thereby destroying the organization and action of bacteria. In addition, the contact between the bacterial cell wall and TiO$_2$ leads to mechanical stresses and distortion of the bacterial cell membrane.

**Medicinal plants (herbal medicines)**

**Aloe vera**

According to reports, the aloe vera plant, its bioactive components, and glucomannan (known as acemannan) have antiviral and bactericidal effects. It has been reported that these plants exhibit potent anti-inflammatory, antioxidant, and antibacterial activities due to their anthraquinones (e.g., barbaloin, emodin, and anthranol) and phenolic compounds. In addition to the antimicrobial activity of aloe vera, positive interactions with dental cells make it an appropriate candidate for periodontal therapy. It has been reported that aloe vera has antimicrobial activity against the primary bacteria associated with periodontitis. The gel of aloe vera has been shown to prevent the growth of both gram-negative and gram-positive bacteria compared to conventional antibiotics, such as vancomycin, methicillin, bacitracin, and erythromycin.

**Manuka honey**

Manuka honey (MH) contains a unique Manuka factor, providing a supplementary antibacterial agent. The effect of MH-containing scaffold (hydrogel type) was investigated on bacterial elimination, adhesion, and cellular adhesion. The results showed higher antimicrobial activity for MH-containing scaffolds compared to conventional scaffolds.

**Berberine**

Berberine acts as a natural antimicrobial agent and is active against many different bacteria, without toxic effects on mammalian cells. Huang et al prepared berberine-loaded scaffolds that showed more than 150 hours of berberine release, with potent antibacterial activity against S. aureus.

**Curcumin**

Numerous reports demonstrated the antibacterial, antiviral, antifungal, and antimalarial activities of curcumin. Based on recent studies, curcumin inhibits the growth and proliferation of E. coli at a concentration of 8 mg/mL. Besides, curcumin can prevent the growth...
of different methicillin-resistant *S. aureus* strains at concentrations of 125-250 mg/mL. Previous studies have also shown that local use of curcumin-containing scaffolds decreases gingival inflammation. Moreover, curcumin can efficiently inhibit the activation of inflammatory mediators and positively impact periodontal diseases.

**Polymers**

*Chitosan*

Chitosan is a well-known scaffold in different tissue engineering fields. It has non-toxic degradation products with little effect due to its low hydrophilicity and low cell compatibility. It also has excellent antimicrobial activity against different bacteria. Besides, chitosan monomers support the regeneration of dental pulp wounds and are useful scaffolds for dental pulp cells.

**Novel drug delivery systems (nanobiomaterials)**

Extensive data have shown that antibiotic pastes and chemical irritants might influence dental stem cells’ viability and function. In this case, a biocompatible intracanal drug delivery device based on nano-fibers is suggested to establish a bacteria-free atmosphere conducive to tissue restoration. In summary, a polymer-loaded solution must be formulated with the selected antibiotic(s) at the desired concentration. After this, antibiotic eluting nano-fibers are developed by modifying the electrospinning variables (e.g., flow rate, power of the field, etc.). These therapeutic nano-fibers could be conveniently inserted into the necrotic dental root canal system as a three-dimensional (3D) tubular structure, with excellent clinical potential since it will ensure that the antibiotics are distributed on microbial biofilms. The contaminated dentin subjected to triple-antibiotic-eluting nano-fibers showed remarkable bacterial mortality based on data from confocal laser scanning microscopy.

**The use of antibacterial agent-releasing scaffolds in dental tissue engineering**

*Endodontics and pediatric dentistry*

The clinical treatment of premature (open apex) teeth with diseased pulp induced by trauma or bacterial infection is a concern for endodontists and pediatric dentists. The favorable treatment has, over the years, been compatible with the concepts of apexification, i.e., calcium hydroxide disinfection accompanied by root canal obturation using gutta-percha. However, fresh dental pulp recovery opportunities due to the evoked bleeding (EB) were raised during the past decade. However, the patient-dependent reconstruction process’s outcome is still unattainable and somewhat uncertain, despite the cases described in the clinical and histological analyses. Several factors were assumed to account for the unsuccess, including but not restricted to using rather cytotoxic antibiotic pastes. Several studies have reported the application and transmission prospects of 3D nano-fibers appropriate for antibiotic removal as a local technique for interior drug delivery that combined with injectable scaffolds, enriched or not with stem cells and growth factors (GFs), can increase the likelihood of the restoration of the human dental pulp.

**Periodontitis**

Periodontitis is one of the most aggressive recurrent oral inflammatory disorders and damages soft and hard tissue consistency, resulting in tooth loss in severe tissue destruction cases. To restore the periodontal system's architecture and function, in principle, targeted procedures of tissue reconstruction are used. In summary, an occlusive biocompatible polymer-based membrane is effectively used as a barrier to prevent the movement of epithelial and connective tissue cells to the regenerating site. Thus, smaller migrating ancestral cells in the residual periodontal ligament (PDL) can increase the root region’s porosity such that they can be differentiated into new periodontal tissues. The last decade has witnessed substantial improvements in the production of membranes with antimicrobial benefits with varying clinical success rates using this strategy. The works reported in the literature have included antimicrobials and inorganic particles (e.g., calcium phosphates) and biomolecules (e.g., growth factors) in the fabrication of membranes with therapeutic functions. More recently, combining known materials and biomolecules with advanced technologies has enabled the management of significant periodontal disorders. 3D printing has also been used for the first patient-specific growth factor adjusted scaffold (rhPDGF-BB).

In recent years, novel treatments for dental pulp restoration, including the EB procedure, have been promising to improve treatment outcomes. During EB, after thorough root canal disinfection, periapical tissue laceration is purposely undertaken to induce bleeding and establish a fibrin-based scaffold to interfere with innate stem cells and growth factors. To disinfect, the EB procedure has ideally used a triple (ciprofloxacin/CIP, metronidazole/MET, and minocycline/MINO) or double (minocycline-free) antimicrobial ingredient made of very concentrated antibiotic pastes. Nevertheless, no therapeutic dosage was found to improve antibiotic mixture-specific antimicrobial activity, decreasing the host tissue and cell toxicity. Regardless of EB's promising results in treating immature permanent teeth with necrotic pulps, one case study showed pulp-like tissue development. Evidently, most histological observations suggest that periapical tissue comprising bone-like hard tissue and cement-like content has been invaginated, contributing to the thickening of root canal walls. While the EB approach was suggested for treating immature teeth, a new survey showed that undifferentiated MSCs had reached the pulp area of mature teeth with apical defects from the apical area.
**Conclusion**

New methods in dental tissue engineering have been employed towards new ways of managing oral and periodontal diseases. Antimicrobial agent-containing scaffolds in dental tissue engineering not only can support the local tissue regeneration but also can prevent adverse local inflammatory processes. Despite the currently available studies, more in vitro and in vivo studies are necessary in this field.

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**Authors’ contributions**

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**References**

1. Prautsch KM, Schmidt A, Paradiso V, Schaefer DJ, Guzman R, Kalbemmannen DF, et al. Modulation of human adipose stem cells’ neurotrophic capacity using a variety of growth factors for neural tissue engineering applications: axonal growth, transcriptional, and phosphoproteomic analyses in vitro. Cells. 2020;9(9):1939. doi: 10.3390/cells9091939.

2. Chen R, Mozaffarian M, Habibyar N. The cell growth behavior of starch-based electrospun nanofibers on human osteoblast-like osteosarcoma SaOs-2 cell line. J Adv Chem Pharm Mater. 2019;2(1):109-10.

3. Sharifi S, Samiei M, Dalir Abdolahinia E, Khalilov R, Shahi S, Maleki Dizaj S. Gelatin-hydroxyapatite nanofibers as promising scaffolds for guided tissue regeneration (GTR): preparation, assessment of the physicochemical properties and the effect on mesenchymal stem cells. J Adv Periodontol Implant Dent. 2020;12(1):25-30. doi: 10.1016/j.japid.2020.001.

4. Nakashima M, Reddi AH. The application of bone morphogenetic proteins to dental tissue engineering. Nat Biotechnol. 2003;21(9):1025-32. doi: 10.1038/nb864.

5. Alipour M, Aghazadeh M, Akhbarzadeh A, Vafajoo Z, Aghazadeh Z, Raiesdasteh Hokmabad V. Towards osteogenic differentiation of human dental pulp stem cells on PCL-P(EG)-PCL/zeolite nanofibrous scaffolds. Artif Cells Nanomed Biotechnol. 2019;47(1):3431-7. doi: 10.1080/21691401.2019.1652627.

6. Ghavimi MA, Bani Shahabadi A, Jaromlasad S, Memar MY, Maleki Dizaj S, Sharifi S. Nanofibrous asymmetric collagen/curcumin membrane containing aspirin-loaded PLGA nanoparticles for guided bone regeneration. Sci Rep. 2020;10(1):18200. doi: 10.1038/s41598-020-75454-2.

7. Huang GT, Songyama W, Liu Y, Liu H, Wang S, Shi S. The hidden treasure in apical papilla: the potential role in pulp/dentin regeneration and bioroot engineering. J Endod. 2008;34(6):645-51. doi: 10.1016/j.joen.2008.03.001.

8. Kelly CN, Miller AT, Holllister SJ, Guldberg RE, Gall K. Design and structure-function characterization of 3D printed synthetic porous biomaterials for tissue engineering. Adv Healthc Mater. 2018;7(7):e1701095. doi: 10.1002/adhm.201701095.

9. O’Keefe RJ, Mao J. Bone tissue engineering and regeneration: from discovery to the clinic—an overview. Tissue Eng Part B Rev. 2011;17(6):389-92. doi: 10.1089/teng.2011.0475.

10. Agarwal S, Wendorf JH, Greiner A. Progress in the field of electropinning for tissue engineering applications. Adv Mater. 2009;21(32-33):3343-51. doi: 10.1002/adma.200803092.

11. Chen W, Liu Y, Courtney HS, Bettenga M, Agramwal CM, Bumgardner JD, et al. In vitro anti-bacterial and biological properties of magnetron co-sputtered silver-containing hydroxyapatite coating. Biomaterials. 2006;27(32):5512-7. doi: 10.1016/j.biomaterials.2006.07.003.

12. Nogueira F, Granadeiro L, Mouro C, Gouveia IC. Antimicrobial and antioxidant surface modification toward a new silk-fibroin (SF)-L-Cysteine material for skin disease management. Appl Surf Sci. 2016;364:552-9. doi: 10.1016/j.apsusc.2015.12.174.

13. Shahverdi AR, Fakhimi A, Shahverdi HR, Minaiaa S. Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against Staphylococcus aureus and Escherichia coli. Nanomedicine. 2007;3(2):168-71. doi: 10.1016/j.nano.2007.02.001.

14. Halaffaee AD, Socransky SS. Microbial etiological agents of destructive periodontal diseases. Periodontol 2000. 1994;5:78-111. doi: 10.1111/j.1600-0757.1994.tb00020.x.

15. Zamani M, Morshed M, Varshosaz J, Janessari M. Controlled release of metronidazole benzene from poly epsilon-caprolactone electrospun nanofibers for periodontal diseases. Eur J Pharm Biopharm. 2010;75(2):179-85. doi: 10.1016/j.ejpb.2010.02.002.

16. Dayaghi E, Bakhsheshi-Rad HR, Hamzah E, Akhavan-Farid A, Ismail AF, Aziz M, et al. Magnesium-zinc scaffold loaded with tetracycline for tissue engineering application: in vitro cell biology and antibacterial activity assessment. Mater Sci Eng C Mater Biol Appl. 2019;102:53-65. doi: 10.1016/j.msec.2019.04.010.

17. Samberg ME, Ondorff PE, Monteiro-Riviere NA. Antibacterial efficacy of silver nanoparticles of different sizes, surface conditions and synthesis methods. Nanotoxicology. 2011;5(2):244-53. doi: 10.1080/17435390.2010.525669.

18. Xing ZC, Zhe WA, Baek YJ, Choi MJ, Jung Y, Kang K. In vitro assessment of antibacterial activity and cytocompatibility of silver-containing PHBV nanofibrous scaffolds for tissue engineering. Biomacromolecules. 2010;11(5):1248-53. doi: 10.1021/bm100372i.

19. Samberg ME, Mente P, He T, King MW, Monteiro-Riviere NA. In vitro biocompatibility and antibacterial efficacy of a degradable poly-L-lactide-co-episolon-caprolactone) copolymer incorporated with silver nanoparticles. Ann Biomed Eng. 2014;42(7):1482-93. doi: 10.1007/s10439-013-0929-9.

20. Shuai C, Shuai C, Feng P, Gao C, Peng S, Yang Y. Antibacterial capability, physicochemical properties, and cytocompatibility of nTiO2 incorporated polymeric scaffolds. Polymers (Basel). 2018;10(3):328. doi: 10.3390/polym10030328.

21. Sharma P, Jha AB, Dubey RS, Pessarakli M. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. J Bot. 2012;2012:217037. doi: 10.1155/2012/217037.

22. Persat A, Nadell CD, Kim MK, Ingreameau F, Siriyaporn A,
Drescher K, et al. The mechanical world of bacteria. Cell. 2015;161(5):988-97. doi: 10.1016/j.cell.2015.05.005.

23. Bozzi A, Perrin C, Austin S, Arce Vera F. Quality and authenticity of commercial Aloe vera gel powders. Food Chem. 2007;103(1):22-30. doi: 10.1016/j.foodchem.2006.05.061.

24. Banu A, Sathyarayana B, Chattannavar G. Efficacy of fresh Aloe vera gel against multi-drug resistant bacteria in infected leg ulcers. Australas Med J. 2012;5(6):305-9. doi: 10.4066/amj.2012.1301.

25. Bashir A, Saeed B, Mujahid TY, Jehan N. Comparative study of antimicrobial activities of Aloe vera extracts and antibiotics against isolates from skin infections. Afr J Biotechnol. 2011;10(19):3835-40. doi: 10.5897/ajb07.572.

26. Hixon KR, Bogner SJ, Ronning-Arnesen G, Janowiak BE, Sell SA. Investigating manuka honey antibacterial properties when incorporated into cryogel, hydrogel, and electrospray tissue engineering scaffolds. Gels. 2019;5(2):21. doi: 10.3390/gels5020021.

27. Huang D, Zhu Y, Zou Q, Zhang L, Li J, Cheng L, et al. Antibacterial chitosan coating on nano-hydroxyapatite/polyamide66 porous bone scaffold for drug delivery. J Biomater Sci Polym Ed. 2011;22(7):931-44. doi: 10.1080/0920506010496576.

28. LaColla P, Tramontano E, Musui C, Marongiu ME, Novellino E, Greco G. Curcumin-like derivatives with potent activity against HIV-1 integrase: synthesis, biological evaluation and molecular modeling. Antiviral Res. 1998;37(3):57.

29. Anand P, Kunnunakkara AB, Newman RA, Apparwal BB. Bioavailability of curcumin: problems and promises. Mol Pharm. 2007;4(6):807-18. doi: 10.1021/mp700113r.

30. Bellio P, Brisidelli F, Perilli M, Sabatini A, Bottoni C, Segatore B, et al. Curcumin inhibits the SOS response induced by levofloxacin in Escherichia coli. Phytomedicine. 2014;21(4):430-4. doi: 10.1016/j.phymed.2013.10.011.

31. Farjana HN, Chandrasekaran SC, Gita B. Effect of oral curcuma gel in gingivitis management-a pilot study. J Clin Diagn Res. 2014;8(12):ZC08-10. doi: 10.7860/jcdr/2014/8784.5235.

32. Guimaraes-Stabili MR, de Aquino SG, de Almeida Curylofo F, Tasso CO, Rocha FRC, de Medeiros MC, et al. Systemic administration of curcumin or piperine enhances the tissue regeneration. Alpha Omegan. 1992;85(4):19-24.

33. Malekzadeh M, Kia SJ, Mashaei L, Moosavi MS. Oral nanocurcumin on gingival inflammation in patients with gingivitis and mild periodontitis. Clin Exp Dent Res. 2021;7(1):78-84. doi: 10.1002/cre.2.330.

34. Yang X, Han G, Pang X, Fan M. Chitosan/collagen scaffold containing bone morphogenetic protein-7 DNA supports dental pulp stem cell differentiation in vitro and in vivo. J Biomed Mater Res A. 2020;108(12):2519-26. doi: 10.1002/jbm.a.34064.

35. Matsunaga T, Yanagiiguchi K, Yamada S, Ohara N, Ikeda T, Hayashi Y. Chitosan monomer promotes tissue regeneration on dental pulp wounds. J Biomed Mater Res A. 2006;76(4):711-20. doi: 10.1002/jbm.a.30588.

36. Diogenes AR, Ruparel NB, Teixeira FB, Hargreaves KM. Translational science in disinfection for regenerative endodontics. J Endod. 2014;40(4 Suppl):S52-7. doi: 10.1016/j.joen.2014.01.015.

37. Pankajakshan D, Albuquerque MT, Evans JD, Kamocka MM, Gregory RL, Bottino MC. Triple antibiotic polymer nanofibers for intracanal drug delivery: effects on dual species biofilm and cell function. J Endod. 2016;42(10):1490-5. doi: 10.1016/j.joen.2016.07.019.

38. Bottino MC, Kamocki K, Yassen GH, Platt JA, Vail MM, Ehrlich Y, et al. Bioactive nanofibrous scaffolds for regenerative endodontics. J Dent Res. 2013;92(11):963-9. doi: 10.1177/0022034513505770.

39. Albuquerque MT, Valera MC, Nakashima M, Nör JE, Bottino MC. Tissue-engineering-based strategies for regenerative endodontics. J Dent Res. 2014;93(12):1222-31. doi: 10.1177/0022034514549089.

40. Diogenes A, Henry MA, Teixeira FB, Hargreaves KM. An update on clinical regenerative endodontics. Endod Topics. 2013;28(1):2-23. doi: 10.1111/etp.12040.

41. Larsson L, Decker AM, Nibali L, Pilipchuk SP, Berglundh T, Giannobile WV. Regenerative medicine for periodontal and peri-implant diseases. J Dent Res. 2016;95(3):255-66. doi: 10.1177/0022034515561887.

42. Carter SD, Costa PF, Vaquette C, Ivanovski S, Hutmacher DW, Malda J. Additive biomanchurating: an advanced approach for periodontal tissue regeneration. Ann Biomed Eng. 2017;45(1):12-22. doi: 10.1007/s10439-016-1687-2.

43. Karrer P, Pilipchuk SP, Flanagan CL, Park CH, Pagani G, Hollister SJ, et al. 3D-printed bioresorbable scaffold for periodontal repair. J Dent Res. 2015;94(9 Suppl):1535-7S. doi: 10.1177/0022034515558830.

44. Seabra AB, Justo GZ, Haddad PS. State of the art, challenges and perspectives in the design of nitric oxide-releasing polymeric nanomaterials for biomedical applications. Biotechnol Adv. 2015;33(6): Pt 3:1370-9. doi: 10.1016/j.biotechadv.2015.01.005.

45. Shimizu E, Jong G, Partridge N, Rosenberg PA, Lin LM. Histologic observation of a human immature permanent premolar with chronic apical abscess after revascularization/revitalization. J Endod. 2012;38(9):1293-7. doi: 10.1016/j.joen.2012.06.017.

46. Becerra P, Ricucci D, Loghin S, Gibbs JL, Lin LM. Histologic study of a human immature permanent premolar with chronic apical abscess after revascularization/revitalization. J Endod. 2014;40(1):133-9. doi: 10.1016/j.joen.2013.07.017.

47. Chrepa V, Henry MA, Daniel BJ, Diogenes A. Delivery of apical mesenchymal stem cells into root canals of mature teeth. J Dent Res. 2015;94(12):1653-9. doi: 10.1177/0022034515596527.