How to Cite:
Mani, R., Chellapandian, K., Gayathri, K., Kumar, S. K., & Kondas, V. V. (2022). Role of nanotechnology in regeneration of pulpo-dentinal complex. *International Journal of Health Sciences*, *6*(S2), 11450–11457. https://doi.org/10.53730/ijhs.v6nS2.8068

Role of nanotechnology in regeneration of pulpo-dentinal complex

Dr Rekha Mani
Senior Lecturer, Department of Conservative Dentistry and Endodontics, SRM Kattankulathur Dental College

Dr Kingston Chellapandian
Senior Lecturer, Department of Conservative Dentistry and Endodontics, SRM Kattankulathur Dental College, Flat no S1, DAC SARA, 3rd street, old state bank colony, Tambaram West, Chennai -600045
Corresponding author email: kingstoc@srmist.edu.in

Dr Gayathri K
Senior Lecturer, Department of Periodontics, SRM Kattankulathur Dental College

Dr Shobana Krishna Kumar
Senior Lecturer**,** Department of Conservative Dentistry and Endodontics, SRM Kattankulathur Dental College

Dr Vijay Venkatesh Kondas
HOD, Department of Conservative Dentistry and Endodontics, SRM Kattankulathur Dental College, Chennai

***Abstract*---**Nanotechnology has completely revolutionized the field of Dentistry with enormous applications and opened up ample research opportunities in the field. Most research activities in Endodontics are performed in pursuit of regeneration of pulpo-dentinal complex. As in other fields, nanotechnology has ameliorated regenerative Endodontics and has brought about considerable promise to the field. Application of nanotechnology could even increase thesuccess rate of regeneration owing to biomimetic modifications in stem cells and scaffolds, which may soon be translated to clinical practice. This review highlights the important research activities in regeneration of dental pulp in collaboration with nanotechnology.

***Keywords*---**regeneration, pulpo-dentinal complex, nanotechnology, scaffolds, stem cells.

Introduction

Nanotechnology deals with synthesis of nanomaterials exhibiting improved physicochemical and biolological properties (1). Nanotechnology is now being applied to almost every field of Dentistry in toothpastes, restorative materials, bone and dental tissue regenerative procedures, implantology, drug delivery etc. leading to the genesis of a new field called Nanodentistry. Nano-hydroxyapatite tooth pastes have shown disrupt dental biofilms on the tooth surface with remineralization of incipient lesions. Nanotechnology assisted site specific drug delivery into periodontal pockets has come into practice. In Implantology, nanotechnology has led to production of implants with controlled surface architecture geometry and improved biological properties (2,3).

Regenerative Endodontic procedures endeavor to regenerate the lost cells of pulpodentinal complex(PDC) along with dentin and root structures.(4). The principal components of regeneration are stem cells, scaffolds and growth factors. Stem cells are undifferentiated cells that are capable of indefinite proliferation into various other types of cells. Dental mesenchymal stem cells include Dental pulp stem cells (DPSCs) (5),Stem cells from human exfoliated deciduous teeth(SHED), immature dental primary teeth stem cells (IDPS) (6,7), Stem cells from dental follicle(DFSCs), Periodontal ligament stem cells(PDLSC), stem cells from the apical papilla(SCAPs), stem cells derived from supernumerary teeth(SNTSC) (8-11). Among these stem cells, DPSC and SHED are capable of regenerating dental pulp(12,13). SCAP regenerated dentin and vascular pulp-like tissue *in vivo* (14,15) and SHED differentiated into endothelial cells that could contribute to the blood vessels and vascular supply of the pulp, *in vivo* ,(16).

A scaffold acts as a 3 dimensional temporary framework for seeding and proliferation of stem cells until replacement by newly formed array of host cells (17,18). Scaffolds play a pivotal role by serving as a biological platform for seeding of stem cells, regulating internal communications and cellular activities. Collagen, with excellent biocompatibility is the most extensively studied natural scaffold material for tissue engineering in dentistry. Collagen has demonstrated in vitro differentiation of SHED to odontoblast and in vivo dental pulp regeneration to the full length of the root (19).Synthetic materials such as polylactic acid (PLA), polyglycolic acid (PGA), and their copolymer poly lactic-glycolic acid (PLGA), poly-D,L-lactide/glycolide (PLG), have all shown desirable outcomes in the delivery of stem cells to the target site, all the three being FDA approved. While PGA was found to be more conducive for proliferation DPSCs (20), PLG scaffolds with SCAP could miraculously regenerate pulp-like tissue in empty root canal spaces accompanied by dentin formation along the walls of the canal, deposited by newly formed odontoblast-like cells (21).The pore size of the scaffold is crucial and must be of minimum 100 μm for tissue regeneration (22). Platelet-rich fibrin (PRF) and Platelet-rich plasma (PRP) derived from blood have also demonstrated highly favourable proliferation and dentinogenic differentiation of DPSCs owing to ability to release angiogenic growth factors like vascular endothelial growth factor (VEGF), basic fibroblast growth factor(bFGF), platelet-derived growth factor (PDGF) etc (23,24).

There are two methods of regenerating pulpo dentinal complex, one is cell transplantation and the other one is cell homing. Cell transplantation consist isolating the stem cells, cultivating them invitro, increase them in number and then insert them into scaffolds with or without adding growth factors. The entire assembly is then transported to the sterile endodontic space. In cell homing, residual stem cells are stimulated using hydrogels and dental pulp regeneration is brought about. In this technique, in vitro isolation and manipulation of stem cells is not needed (26-27).

Growth factors, in the local environment, play a key role in determining the fate of stem cells. Multitude of growth factors with definite implications in pulp tissue regeneration have been identified (28). Addition of bone morphogenetic proteins(BMPs) resulted in synthesis of extracellular matrix, osteoblast-like cell proliferation and rapid tertiary dentin formation(29). There are two major strategies implied in the regeneration of pulpodentinal complex, implanting stem cells with or without scaffolds or implanting prefabricated tissue constructs into target location. These tissue constructs consist of engineered stem cells and scaffolds (3). The comprehensive research data pertaining to the role of nanotechnology in dental pulp regeneration seems to be limited so the aim of the review is to provide a comprehensive update of current research activities in the field.

Materials and Methods

An electronic search was conducted in Pubmed and Google scholar search engines until september 2020 using the keywords were used 'Nanotechnology','dental Pulp',‘regeneration','Nanodentistry' , ‘pulpo-dentinal complex’,'Nanoparticles','Endodontics'. Relevant article published so far in English language were retrieved.

Applications Of Nanotechnology In Pulp Regeneration

Stem Cells And Nanotechnology

a. Magnetic nanoparticles - After stem cells have been transplanted, it is important to track the fate of these cells, its survival and migration in vivo. This tracking can be done by labelling with magnetic nanoparticles like super paramagnetic iron oxide (SPIO) which can be visualized using MRI (30-32). These particles are in the size range of 60-150nm. These particles contain aqueous soluble iron coated with dextran or carboxy dextran to prevent aggregation (33,34). These particles are biodegradable and can be recycled by cells. SPIO particles can be attached to either stem cell surface or internalized by phagocytosis. The process of phagocytosis does not seem to affect viability or differentiation of stem cells (35).
b. Quantum dots (Qdots)- consist of light-emitting nanoparticles for labelling of stem cells. These nanocrystals with 2-10nm diameter can be detected using simple optical imaging rather than complex MRI (36,37).Qdots are usually internalized by endocytosis after which they reach the perinuclear region of the cells (38-40).When Qdots are degraded, it leads to

mitochondrial dysfunction and ultimately cell death (41). There are conflicting results regarding the cytotoxicity of Qdots.

c. Intracellular delivery of genes and proteins – when nanomaterials are used for intracellular gene delivery, the stem cells can be directed to specific sites and can be made to differentiate into the required cell types (42, 43). Gene delivery can be done using nanoparticles, carbon nanotubes or nanowires made of silicon (44,45). Polymeric nanoparticles which are biodegradable can be used for delivery of proteins or other chemicals into stem cells. These particles are in size range of 100-300nm and they reach the perinuclear region of the cells after internalization. The particles do not affect the viability of stem cells but have a negative impact on their differentiation (30).

Scaffolds And Nanotechnology

a. Nano-fibrous scaffolds –Scaffolds, being templates for 3D tissue growth, should emulate the extracellular matrix(ECM). The native ECM is nano-fibous in nature. Nanotechnology can support synthesis of biomimetic nano-fibrous scaffolds which not only support endodontic regeneration but also act as reservoirs of growth factors, anti-inflammatory and antibacterial molecules. Nano-fibrous scaffolds have superior surface area to volume ratio and promising microstructural properties, exhibiting highly interconnected porous networks (46). There are three techniques by which nano-fibrous scaffolds can be fabricated namely electrospinning, molecular self-assembly and thermally induced phase separation (TIPS) (47). In Electrospinning, a polymer solution is drawn from an orifice to a collector using an electric field to produce polymer fibers having diameter in the nanoscale and microscale levels (48). Molecular self-assembly is a process by which structurally organized arrangement is stabilized by non-covalent interactions like hydrogen bond, Van der Waals forces etc. Most common example of such assembly in nature is collagen. Inspired by nature, researchers have designed peptide molecules which, under favourable conditions, can self-assemble into a nano-fibrous structure (48). The third technique, TIPS, consist of controlling the temperature of the polymer solution to induce phase separation. This results in formation of two phases, one being polymer-rich and the other being polymer-lean. The solvent is then removed by evaporation or extraction and the residual polymer-rich phase undergoes solidification to form polymer foam, the microstructure of which can be varied based on the type of the polymer and solvent (48). Recently, electrospun scaffoldings have found new application as drug delivery vehicle. Antibiotic scaffolds fabricated by electrospinning can disinfect root canals more effectually owing to the slow but effective release of antibiotics and also continue serve as matrix for seeding of stem cells from apical papilla after induced bleeding. Nanocomposite polydioxanone II scaffolds that contain metronidazole or ciprofloxacin have shown powerful antimicrobial activity against Enterococcus faecalis and help pulpal regeneration (49). Electrospunnanohydroxy apatite containing gelatin scaffolds promote differentiation of DPSC into odontoblast like cells whereas nano-fibrous scaffolds functionalized by dexamethasone and BMP - 7 promote regeneration of dentin (50,51).

b. Designing of artificial stem cell niches using nanotechnology – stem cells can be directed towards a definite site and function by creating artificial microenvironments using nanotechnology. The cell adhesion sites created by artificial nanostructures vary in its size, shape, spacing, concentration and surface chemistry. The artificial nanostructures can be nanotubes or nanolines and the shapes they form can be of pores, pits, grooves or ridges (52-54). Studies have shown that surface irregularities improve the adhesion and osteogenic differentiation of human mesenchymal stem cells (53).

Conclusion

Nanotechnology has promising applications in the regeneration of pulpodentinal complex. It is now possible to precisely deliver, track and induce highly specific differentiation of stem cells with an appropriate biological seat for seeding them. However, one should accept the fact that these reseach activities, may take quite some time to be translated to our dental clinics.

References

1. Kelsall RW, Hamley IW, Geoghegan M, editors. Nanoscale science and technology. Chichester: Wiley; 2005 Apr 15.
2. Valiev RZ, Zehetbauer MJ, Estrin Y, Höppel HW, Ivanisenko Y, Hahn H, Wilde G, Roven HJ, Sauvage X, Langdon TG. The innovation potential of bulk nanostructured materials. Advanced Engineering Materials. 2007 Jul;9(7):527-33.
3. N. Malhotra and K. Mala, “Regenerative endodontics as a tissue engineering approach: past, current and future,” Australian Endodontic Journal, vol. 38, no. 3, pp. 137–148, 2012.
4. Hargreaves K, Cohen S. COHEN'S pathways of the pulp. 10th ed. St Louis: Mosby Elsevier; 2011.
5. Gronthos, S.; Mankani, M.; Brahim, J.; Robey, P.G.; Shi, S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc. Natl. Acad. Sci. 2000, 97, 13625–13630.
6. Kerkis, I.; Kerkis, A.; Dozortsev, D.; Stukart-Parsons, G.C.; Gomes Massironi, S.M.; Pereira, L.V.;Caplan, A.I.; Cerruti, H.I. Isolation and characterization of a population of immature dental pulp stem cells expressing OCT-4 and other embryonic stem cell markers. Cells Tissues Organs. 2006, 184, 105–116.
7. Miura, M.; Gronthos, S.; Zhao, M.; Lu, B.; Fisher, L.W.; Robey, P.G.; Shi, S. SHED: Stem cells from human exfoliated deciduous teeth. Proc. Natl. Acad. Sci. 2003, 100, 5807–5812.
8. Seo, B.M.; Miura, M.; Gronthos, S.; Bartold, P.M.; Batouli, S.; Brahim, J.; Young, M.; Robey, P.G.;Wang, C.Y.; Shi, S. Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet 2004, 364,149–155.
9. Sonoyama W, Liu Y, Fang D, Yamaza T, Seo BM, Zhang C, Liu H, Gronthos S, Wang CY, Shi S, Wang S. Mesenchymal stem cell-mediated functional tooth regeneration in swine. PloS one. 2006 Dec 20;1(1):e79
10. Sonoyama,W.; Liu, Y.; Yamaza, T.; Tuan, R.S.;Wang, S.; Shi, S.; Huang, G.T.-J. Characterization of the apical papilla and its residing stem cells from

human immature permanent teeth: A pilot study. J. Endod. 2008, 34, 166–171.
11. Morsczeck, C.; Gotz, W.; Schierholz, J.; Zeilhofer, F.; Kuhn, U.; Mohl, C.; Sipple, C.; Hoffmann, K.H.Isolation of precursor cells (PCs) from human dental follicle of wisdom teeth. Matrix Biol. 2005, 24, 155–165.
12. Casagrande, L.; Cordeiro, M.M.; Nör, S.A.; Nör, J.E. Dental pulp stem cells in regenerative dentistry.Odontology2011, 99, 1–7.
13. Iohara, K.; Nakashima, M.; Ito, M.; Ishikawa, M.; Nakasima, A.; Akamine, A. Dentin regeneration by dental pulp stem cell therapy with recombinant human bone morphogenetic protein 2. J. Dent. Res. 2004, 83,590–595.
14. Huang, G.T.; 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.
15. Huang, G.T.; Yamaza, T.; Shea, L.D.; Djouad, F.; Kuhn, N.Z.; Tuan, R.S.; Shi, S. Stem/progenitorcell-mediated de novo regeneration of dental pulp with newly deposited continuous layer of dentin in an in vivo model. Tissue Eng. Part A 2010, 16, 605–615.
16. Sakai, V.T.; Zhang, Z.; Dong, Z.; Neiva, K.G.; Machado, M.A.A.M.; Shi, S.; Santos, C.F.; Nör, J.E. SHED differentiate into functional odontoblasts and endothelium. J. Dent. Res. 2010, 89, 791–796.
17. K. M. Galler, R. N. D'Souza, J. D. Hartgerink, and G. Schmalz, "Scaffolds for dental pulp tissue engineering," Advances in Dental Research, vol. 23, no. 3, pp. 333–339, 2011.
18. P. E. Murray, F. Garcia-Godoy, and K. M. Hargreaves, "Regenerative endodontics: a review of current status and a call for action," Journal of Endodontics, vol. 33, no. 4, pp. 377–390, 2007.
19. V. Rosa, Z. Zhang, R. H. M. Grande, and J. E. Nor, "Dental pulp ¨ tissue engineering in full-length human root canals," Journal of Dental Research, vol. 92, no. 11, pp. 970–975, 2013.
20. K. S. Bohl, J. Shon, B. Rutherford, and D. J. Mooney, "Role of synthetic extracellular matrix in development of engineered dental pulp," *Journal of Biomaterials Science, Polymer Edition*, vol. 9, no. 7, pp. 749–764, 1998.
21. G. T.-J. Huang, T. Yamaza, L. D. Shea et al., "Stem/Progenitor cell-mediated de novo regeneration of dental pulp with newly deposited continuous layer of dentin in an in vivomodel," *TissueEngineering Part A*, vol. 16, no. 2, pp. 605–615, 2010.
22. Karageorgiou, V.; Kaplan, D. Porosity of 3D biomaterial scaffolds and osteogenesis. Biomaterials 2005, 26, 5474–5491.
23. X. Li, J. Hou, B. Wu, T. Chen, and A. Luo, "Effects of platelet-rich plasma and cell coculture on angiogenesis in human dental pulp stem cells and endothelial progenitor cells," *Journal of Endodontics*, vol. 40, no. 11, pp. 1810–1814, 2014.
24. R. E. Marx, "Platelet-rich plasma: evidence to support its use," *Journal of Oral andMaxillofacial Surgery*, vol. 62,no. 4, pp. 489–496, 2004.
25. Kim, J.Y.; Xin, X.; Moioli, E.K.; Chung, J.; Lee, C.H.; Chen, M.; Fu, S.Y.; Koch, P.D.; Mao, J.J. Regeneration of dental-pulp-like tissue by chemotaxis-induced cell homing. Tissue Eng. Part A 2010, 16, 3023–3031.
26. Suzuki, T.; Lee, C.H.; Chen, M.; Zhao, W.; Fu, S.Y.; Qi, J.J.; Chotkowski, G.; Eisig, S.B.; Wong, A.; Mao, J.J. Induced migration of dental pulp stem cells for in vivo pulp regeneration. J. Dent. Res. 2011, 90, 1013–1018.

27. Yang, J.W.; Zhang, Y.F.; Wan, C.Y.; Sun, Z.Y.; Nie, S.; Jian, S.J.; Zhang, L.; Song, G.T.; Chen, Z. Autophagy in SDF-1α-mediated DPSC migration and pulp regeneration. Biomaterials 2015, 44, 11–23.
28. K. Bessho, N. Tanaka, J. Matsumoto, T. Tagawa, and M. Murata, "Human dentin-matrix-derived bone morphogenetic protein," Journal of Dental Research, vol. 70, no. 3, pp. 171–175, 1991.
29. Kikuchi N, Kitamura C, Morotomi T, et al. Formation of dentin-like particles in dentin defects above exposed pulp by controlled release of fibroblast growth factor 2 from gelatin hydrogels. J Endod2007;33:1198–202.
30. Ferreira L, Karp JM, Nobre L, Langer R (2008) New opportunities: the use of nanotechnologies to manipulate and track stem cells, *Cell Stem Cell*, 3(2), 136-146.
31. Thu MS, Bryant LH, Coppola T, Jordan EK, Budde MD, Lewis BK, Chaudhry A, Ren J, Varma NR, Arbab AS, Frank JA (2012) Self-assembling nanocomplexes by combining ferumoxytol, heparin and protamine for cell tracking by magnetic resonance imaging, *Nat Med*, 18(3), 463-467.
32. Lewin M, Carlesso N, Tung CH, Tang XW, Cory D, Scadden DT, Weissleder R (2000) Tat peptide-derivatized magnetic nanoparticles allow in vivo tracking and recovery of progenitor cells, *Nat Biotechnol*, 18(4), 410-414.
33. Reimer P,Balzer T (2003) Ferucarbotran (Resovist): a new clinically approved RES-specific contrast agent for contrastenhanced MRI of the liver: properties, clinical development, and applications, *EurRadiol*, 13(6), 1266-1276.
34. Wang YX, Hussain SM, Krestin GP (2001) Superparamagnetic iron oxide contrast agents: physicochemical characteristics and applications in MR imaging, *EurRadiol*, 11(11), 2319-2331.
35. Song YS,Ku JH (2007) Monitoring transplanted human mesenchymal stem cells in rat and rabbit bladders using molecular magnetic resonance imaging, *NeurourolUrodyn*, 26(4), 584-593.
36. Bruchez MP (2011) Quantum dots find their stride in single molecule tracking, *CurrOpin Chem Biol*, 15(6), 775-780.
37. Lin S, Xie X, Patel MR, Yang YH, Li Z, Cao F, Gheysens O, Zhang Y, Gambhir SS, Rao JH, Wu JC (2007) Quantum dot imaging for embryonic stem cells, *BMC Biotechnol*, 7, 67.
38. Chakraborty SK, Fitzpatrick JA, Phillippi JA, Andreko S, Waggoner AS, Bruchez MP, Ballou B (2007) Cholera toxin B conjugated quantum dots for live cell labeling, *Nano Lett*, 7(9), 2618-2626.
39. Slotkin JR, Chakrabarti L, Dai HN, Carney RS, Hirata T, Bregman BS, Gallicano GI, Corbin JG, Haydar TF (2007) In vivo quantum dot labeling of mammalian stem and progenitor cells, *Dev Dyn*, 236(12), 3393-3401.
40. Hsieh SC, Wang FF, Lin CS, Chen YJ, Hung SC, Wang YJ (2006) The inhibition of osteogenesis with human bone marrow mesenchymal stem cells by CdSe/ZnS quantum dot labels, *Biomaterials*, 27(8), 1656-1664.
41. Maysinger D, Lovric J, Eisenberg A, Savic R (2007) Fate of micelles and quantum dots in cells, *Eur J Pharm Biopharm*, 65(3), 270-281.
42. Derfus AM, Chen AA, Min DH, Ruoslahti E, Bhatia SN (2007) Targeted quantum dot conjugates for siRNA delivery, *Bioconjug Chem*, 18(5), 1391-1396.
43. Harris TJ, Green JJ, Fung PW, Langer R, Anderson DG, BhatiaSN (2010) Tissue-specific gene delivery via nanoparticle coating, *Biomaterials*, 31(5), 998-1006.

44. Bianco A, Kostarelos K, Prato M (2011) Making carbon nanotubes biocompatible and biodegradable, *Chem Commun (Camb)*, 47(37), 10182-10188.
45. Kostarelos K (2010) Carbon nanotubes: Fibrillar pharmacology, *Nat Mater*, 9(10), 793-795.
46. M. Nakashima and A. Akamine, "The application of tissueengineering to regeneration of pulp and dentin in endodontics,"Journal of Endodontics, vol. 31, no. 10, pp. 711–718, 2005.
47. Bottino MC, Thomas V, Schmidt G, Vohra YK, Chu TM, Kowolik MJ, et al. Recent advances in the development of GTR/GBR membranes for periodontal regeneration—a materials perspective. Dent Mater 2012;28(7):703–21
48. Smith LA, Liu X, Ma PX. Tissue engineering with nano-fibrous scaffolds. Soft Matter. 2008;4(11):2144-9.
49. Kamocki K, Nör JE, Bottino MC. Effects of ciprofloxacin-containing antimicrobial scaffolds on dental pulp stem cell viability—In vitro studies. Archives of oral biology. 2015 Aug 1;60(8):1131-7.
50. Wang J, Liu X, Jin X, Ma H, Hu J, Ni L, Ma PX. The odontogenic differentiation of human dental pulp stem cells on nanofibrous poly (L-lactic acid) scaffolds in vitro and in vivo. Acta biomaterialia. 2010 Oct 1;6(10):3856-63.
51. Wang J, Ma H, Jin X, Hu J, Liu X, Ni L, Ma PX.The effect of scaffold architecture on odontogenic differentiation of human dental pulp stem cells. Biomaterials. 2011 Nov 1;32(31):7822-30.
52. Dalby MJ, Andar A, Nag A, Affrossman S, Tare R, McFarlane S,Oreffo RO (2008) Genomic expression of mesenchymal stem cellsto altered nanoscale topographies, *J R Soc Interface*, 5(26), 1055-1065.
53. Dickinson LE, Kusuma S, Gerecht S (2011) Reconstructing thedifferentiation niche of embryonic stem cells using biomaterials,*MacromolBiosci*, 11(1), 36-49.
54. Lipski AM, Pino CJ, Haselton FR, Chen IW, Shastri VP (2008)The effect of silica nanoparticle-modified surfaces on cellmorphology, cytoskeletal organization and function, *Biomaterials*,29(28), 3836-3846.