An overview of Regenerative Pulp Therapy in Children

Mayur S. Bhattad[*], Sudhindra Baliga[**], Nilima Thosar[***]

A B S T R A C T

A remarkable shift in paradigm is seen with the treatment of pulp therapy procedures from conventional to regenerative ones since 10-15 years in the field of pediatric endodontics. Though conventional treatment techniques provide excellent success for many conditions, an ideal form of therapy might consist of regenerative approaches in which diseased or necrotic pulp tissues are removed and replaced with healthy pulp tissue. Regenerative pulp procedure is the creation and delivery of tissues to replace diseased, missing, and traumatized pulp. This review provides an overview on the biology of dental pulp tissue and regenerative pulp procedures like stem cells therapy, scaffolds, injectable delivery systems, pulp implantation, gene therapy, revascularization and tissue engineering including their goals and describe possible techniques that will allow this to become a reality.

Keywords: *Root Canal Revascularization, Stem Cells, Pulp Implantation, Gene Therapy*

Introduction

Pulp therapies in children are broadly classified as vital & non-vital pulp therapies.[1] Conservative pulp therapies include protective base, indirect pulp capping, direct pulp capping and pulpotomy procedures whereas radical pulp therapies include apexification and pulpectomy procedures.[1]

Although current treatment modalities offer high levels of success for many conditions, an innovative form of therapy is emerging which consist of regenerative approaches in which diseased or necrotic pulp tissues are removed and replaced with healthy pulp tissue to revitalize teeth.

Regenerative pulp procedures can be defined as biologically based procedures designed to replace damaged structures, including dentin and root structures, as well as cells of the pulp-dentin complex.[2] These regenerative pulp therapies include root-canal revascularization, postnatal (adult) stem cell

therapy, pulp implant, scaffold implant, three-dimensional cell printing, injectable scaffolds, and gene therapy.[2]

This review throws a light on regenerative procedures of pulp and describes the possible techniques that will allow these procedures to become a reality as far as dentistry is concerned. The main objectives of this therapy are regeneration of pulp-like tissue, ideally, the pulp-dentin complex, regenerate resorbed root, regenerate damaged coronal dentin and generate apical root in case of open apex.[2]

An Overview of Regenerative Pulp Therapies:

- Root canal revascularization
- Post-natal Stem cell therapy
- Pulp implantation
- Regeneration by Scaffold implantation
- Injectable scaffold delivery
- Three-dimensional Cell printing
- Gene delivery

Root canal revascularization

Revascularization is a surgical procedure for the provision of a new, additional, or augmented blood supply to a body part or organ. The term derives its name from the prefix 'Re' which in this case means restoration and vasculature, which refers to the circulatory structures of an organ.[3]

It has been proven fact that under certain specific situation revascularization can be achieved in young teeth that have been traumatically avulsed, leaving a necrotic but uninfected pulp.[4] The immature avulsed tooth has an open apex, short root, and intact but necrotic pulp tissue. Therefore, the new tissue has easy access to the root canal system and a relatively short distance for proliferation to reach the coronal pulp horns. It has been experimentally shown that the apical portion of a pulp might remain vital and proliferate coronally after reimplantation, replacing the necrotized coronal portion of the pulp.[5, 6] The speed with which the tissue completely revascularizes the pulp space is important because bacteria from the outside are continually attempting to enter the pulp space, and the presence of vital pulpal tissue greatly slows or prevents the bacterial penetration into this tissue compartment.[7] Revascularization of the pulp space in a necrotic, infected tooth with apical periodontitis was attempted by Nygaard-Ostby and Hjortdal in the 1960s but was mostly unsuccessful. However, the materials and instruments available 40–50 years ago were probably not sufficient to create an environment similar to the avulsed tooth, i.e, a canal that is free of bacteria, containing a scaffold for new tissue to grow and to be largely resistant to further bacterial penetration. With currently available technologies it could be possible to effectively disinfect an infected pulp, artificially place a scaffold, and then effectively seal the access cavity to resist subsequent infection.[8]

Revascularization of necrotic root canal system by disinfection followed by establishing bleeding in to the canal system via over instrumentation can also be done. A blood clot can be produced to the level of the cemento-enamel junction to provide a scaffold for the in-growth of new tissue followed by a double seal of mineral trioxide aggregate in the cervical area and a bonded resin coronal restoration above it.[4] Number of groups has published preclinical research & case reports that offer a biologically based alternative to conventional endodontic treatment of complex clinical cases of this category.[9-11]

The use of the 3 mix-MP triple antibiotic paste which was developed by Hoshino and colleagues consisting of ciprofloxacin, metronidazole, and minocycline, is found to be very effective for disinfection of the infected necrotic tooth, making the conditions for subsequent revascularization.[12-14]

The most vital steps in these cases are the use of intracanal irrigants (sodium hypochlorite and chlorhexidine) with placement of antibiotics (e.g. a mixture of ciprofloxacin, metronidazole, and minocycline paste) for more than a few weeks.[10] This particular combination of antibiotics efficiently disinfects root canal system and increases revascularization of avulsed and necrotic teeth, suggesting that this is a crucial step in revascularization.[13, 15, 16]

The advantages of this are that the approach is technically easy, it can be completed using currently available instruments and medicaments and regeneration of tissue in root canal systems by the patient's own blood cells avoids the possibility of immune rejection and pathogen transmission.[2]

Postnatal stem cell therapy:

Stem cells are unspecialized cells in the human body that are capable of becoming specialized cells, each with new specialized cell functions [17] or a stem cell is commonly defined as a cell that has the ability to continuously divide and produce progeny cells that differentiate (develop) into various other types of cells or tissues.[2,18] Stem cells have the ability to continuously divide to either replicate themselves (self-replication), or produce specialized cells that can differentiate into various other types of cells or tissues (multilineage differentiation).[19] Several varieties of stem cells have been isolated and identified in vivo and in vitro. Different varieties of these cells are early embryonic stem cells; blastocyst embryonic stem cells; fetal stem cells; umbilical cord stem cells and adult stem cells.[20]

Among these varieties, adult stem cell can be used to regenerate the dental pulp tissue. This name is rather misleading, because infants and children also have stem cells. Thus the term postnatal stem cells are preferable. These stem cells found within the tissues that have already developed, directing their growth and maintenance throughout life. [20]

Post natal stem cells typically generate the cell types of the tissue in which they reside. [21] Postnatal stem cells have been found in almost all body tissues, including dental tissues. Up to date, four types of human dental stem cells have been isolated and characterized which are the Dental pulp stem cells (DPSCs), Stem cells from human exfoliated deciduous teeth (SHED); Stem cells from apical papillae (SCAP); Periodontal ligament stem cells (PDLSCs) and among them, only SHED are from deciduous teeth.[20]

SHED

SHED were first isolated by Miura et al in 2003 [22]and accordingly they were able to differentiate SHED into a variety of cell types to a much more extent than DPSCs, including neural cells, adipocytes, osteoblast-like and odontoblast-like cells[22]. The main role of these cells seems to be the formation of mineralized tissue, which can be used to enhance orofacial bone regeneration. [23, 24] The limitations of autologous postnatal stem cells with multipotentiality have made SHED an attractive alternative for dental tissue engineering. The advantages of SHED over stem cells from adult human teeth are its higher proliferation rate than stem cells from permanent teeth [22] and it can be retrieved from the tissue which is disposable and readily accessible.[25] Therefore, they are ideally suited for patients in the mixed dentition stage who have suffered pulp necrosis in immature permanent teeth due to trauma.

The simplest method to administer the post natal stem cells of appropriate regenerative potential is to inject these cells into the disinfected root canal systems after the apex is opened. The post natal stem cells can be derived from multiple tissues including skin, buccal mucosa, fat and bone.[26]

One possible approach would be to use dental pulp stem cells derived from autologous (patient's own) cells that have been taken from a buccal mucosal biopsy, or umbilical cord stem cells that have been cryogenically stored after birth; an allogenic purified pulp stem cell line that is disease and pathogen-free; xenogenic (animal) pulp stem cells that have been grown in the laboratory.[2]

The advantages of postnatal stem cells are firstly, the autogenously stem cells are relatively easy to harvest, can be delivered by a syringe and the cells have the potential to induce new pulp regeneration. Secondly, this approach is already used in regenerative medical applications, including bone marrow replacement, and a recent review has described several potential endodontic applications.[27] On the other hand, there are several disadvantages to a delivery method of injecting cells. Primarily the cells may have low survival rates and subsequently the cells might migrate to different locations within the body, possibly leading to aberrant patterns of mineralization.[27]

Pulp Implantation

In pulp implantation, the cultured pulp tissue is transplanted into cleaned and shaped root canal systems. The pulp tissue is grown in sheets in vitro on biodegradable polymer

nanofibers or on sheets of extracellular matrix proteins such as collagen I or fibronectin.[28, 29]

The potential problems associated with the implantation of sheets of cultured pulp tissue is that these specialized procedures may be required to ensure that the cells properly adhere to root canal walls. Growing dental pulp cells on collagens I and III has still not proved to be successful.[30] The advantage of having the cells aggregated together is that, it localizes the postnatal stem cells in the root canal system. The disadvantage of this technique is that implantation of sheets of cells may be technically difficult. The sheets are very thin and fragile, so more clinical research is required to develop reliable implantation techniques.

Regeneration by Scaffold Implantation

The scaffold is a physicochemical and biological three dimensional microenvironment for cell growth and differentiation, promoting cell adhesion, and migration. A great number of current tissue engineering strategies are based on the development of a cell scaffold construction to play a role in repairing and regenerating tissue defects.[31] The strategies for successful regeneration include the use of biologic or biocompatible materials to build a bridge across the injured area. Nowadays, the scaffold lies at the heart of all the new tissue engineering approaches.

Ideal requirements of a scaffold [32-34]

It should be porous to allow placement of cells and growth factors, should allow effective transport of nutrients, oxygen, and waste, should be biodegradable leaving no toxic byproducts, should be replaced by regenerative tissue while retaining the shape and form of the final tissue structure, should be biocompatible and finally should have adequate physical and mechanical strength.

Types of scaffold

a) Biological/natural scaffolds[35, 36]

These consist of natural polymers such as collagen and glycosaminoglycan, which offer good biocompatibility and bioactivity.

b) Artificial scaffolds

These are synthetic polymers with controlled physicochemical features such as degradation rate, microstructure, and mechanical strength.[37] For example: Polylactic acid (PLA), polyglycolic acid (PGA), and their copolymers, poly lactic-co-glycolic acid (PLGA), Synthetic hydrogels include polyethylene glycol (PEG) based polymers. Scaffolds modified with cell surface adhesion peptides, such as arginine, glycine, and aspartic acids (RGD) improve cell adhesion and matrix synthesis within the three-dimensional network.[38] Scaffolds containing inorganic compounds such as hydroxyapatite (HA), tricalcium phosphate (TCP) and calcium polyphosphate (CPP), are used to enhance bone conductivity, and have proved to be very effective for tissue engineering of DPSCs.[39, 40] Micro-cavity-filled scaffold also enhances cell adhesion.[41-43]

Injectable Scaffold Delivery

To regenerate bone and bony parts, it is preferable to have rigid tissue engineered scaffold structures as they provide excellent physical support to the cell. However, in the root canal system it is preferable to have a soft three dimensional scaffold matrix such as polymer hydrogel which can be non invasive and can be easily delivered by injecting into the root canal systems.[2] The hydrogel may promote pulp regeneration by providing a substrate for cell proliferation and differentiation into an organized tissue structure.[44,45]

Three dimensional cell printing

It is a rapid prototyping computer-aided 3D technology which is based on using layer by layer deposition of cell and/or cell aggregates into a 3D gel with sequential maturation of the printed construct into perfused and vascularized living tissue or organ.[46] In theory, an ink-jet like device is used to dispense layers of cells suspended in a hydrogel to recreate the structure of the tooth pulp tissue.[47] Three dimensional cell printing technique can be used to precisely position cells and this method has the potential to create tissue constructs that mimic the natural tooth pulp tissue structure.[48] The ideal positioning of cells in a tissue engineering construct would include placing odontoblastoid cells around the periphery to maintain and repair dentin, with fibroblasts in the pulp core supporting a network of vascular and nerve cells.[49]

Gene therapy:

Gene therapy has been recently used as a means of delivering genes for growth factors, morphogens, transcription factors and extracellular matrix molecules locally to the somatic cells of individuals, with resulting therapeutic effect. The gene can stimulate or induce a natural biological process by expressing the molecules which are involved in the regenerative response for the tissue of interest. [(49)] An in-vivo and ex-vivo, both approach can be used for gene therapy.[49,50]

Gene therapy is a relatively a new field in dentistry and evidence is lacking to demonstrate that this therapy has the potential to rescue the necrotic pulp.[43,50]

Conclusion

Regenerative pulp therapy in pediatric dentistry today is seen with an eye of speculation because each one of the regenerative techniques has certain advantages and disadvantages and some of the techniques are even hypothetical, or at an early stage of development. The future development will require a comprehensive research program which is directed at each of these components and their application to patients. With fast growing literature towards these modalities, it would make us believe that the day is not so far when regenerative pulp therapy will be

performed on day to day basis in clinical pediatric dental practice.

References

1. Shobha Tandon. Textbook of Pedodontics: 2nd Ed. Paras Medical Publisher, 2008
2. Murray PE, Garcia-Godoy F and Hargreaves KM. Regenerative endodontics - a review of current status and a call for action. J Endod 2007;33(4):377-90.
3. Merriam-Webster Online Dictionary 2008: http://Merriam-webster.com/dictionary/ revascularization
4. Trope M. Regenerative potential of dental pulp. J Endod 2008;34(7): S13- S17.
5. Rule DC, Winter GB. Root growth and apical repair subsequent to pulpal necrosis in children. Br Dent J 1966;120:586 –90.
6. Iwaya SI, Ikawa M, Kubota M. Revascularization of an immature permanent tooth with apical periodontitis and sinus tract. Dent Traumatol 2001;17:185–87
7. Love RM. Bacterial penetration of the root canal of intact incisor teeth after a simulated traumatic injury. Endod Dent Traumatol 1996;12**:**289-93.
8. Nygaard-Ostby B, Hjortdal O. Tissue formation in the root canal following pulp removal. Scand J Dent Res 1971;79:333–48.
9. Hargreaves KM, Geisler T. Regeneration potential of the young permanent tooth: what does the future hold? J Endod 2008;34(7S): S51- S56.
10. Johnson WT, Goodrich JL, James GA. Replantation of avulsed teeth with immature root development. Oral Surg Oral Med Oral Pathol 1985;60:420–27.
11. Laureys W, Beele H, Cornelissen R, Dermaut L. Revascularization after

[ABOUT THE AUTHORS:

*Dr Mayur Bhattad is a senior lecturer in the Department of Pedodontics & Preventive Dentistry, HSRSM Dental College & hospital, Hingoli, Maharashtra, India. Email address: mayur_b99@yahoo.co.in

**Dr Sudhindra Baliga is professor & Head in the Department of Pedodontics & Preventive Dentistry, Sharad Pawar Dental College, Sawangi(M),Wardha

***Dr Nilima Thosar is a professor in the Department of Pedodontics & Preventive Dentistry, Sharad Pawar Dental College, Sawangi(M),Wardha

cryopreservation and auto-transplantation of immature and mature apicoectomized teeth. Am J Orthod Dentofacial Orthop 2001;119:346-52

12. Hoshino E, Kurihara-Ando N, Sato I et al. In vitro antibacterial susceptibility of bacteria taken from infected root dentine to a mixture of ciprofloxacin, metronidazole, and minocycline. Int Endod J 1996;29:125-30
13. Sato T, Hoshino E, Uematsu H, Noda T. In vitro antimicrobial susceptibility to combinations of drugs on bacteria from carious and endodontic lesions of human deciduous teeth. Oral Microbiol Immunol 1993;8:172-76.
14. Sato I, Ando-Kurihara N, Kota K, Iwaku M, Hoshino E. Sterilization of infected root canal dentine by topical application of a mixture of ciprofloxacin, metronidazole, and minocycline in situ. Int Endod J 1996;29:118-124.
15. Ritter AL, Ritter AV, Murrah V, Sigurdsson A. Trope M. Pulp revascularization of replanted immature dog teeth after treatment with minocycline and doxycycline assessed by laser doppler flowmetry, radiography, and histology. Dent Traumatol 2004;20:75-84.
16. Yanpiset K, Trope M. Pulp revascularization of replanted immature dog teeth after different treatment methods. Endod Dent Traumatol 2000;16:211-17.
17. Bongso A. Stem cells - From bench to bedside; world scientific publishing co. pte. Ltd Jul 2005: Page No: 1-13.
18. Morrison SJ, Shah NM and Anderson DJ. Regulatory mechanisms in stem cell biology – review. Cell 1997;88:287-98.
19. Rao MS. Stem sense: a proposal for the classification of stem cells. Stem Cells Dev 2004;13:452-55.
20. Shehab EM. Tissue engineering in endodontics. J of Oral Science 2009;51(4):495-07.
21. Menasche P. The potential of embryonic stem cells to treat heart disease. Curr Opin Mol Ther 2005;7:293-99.
22. Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, Shi S. SHED: stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci USA 2003;100:5807-12
23. Tonomura A, Sumita Y, Ando Y, Iejima D, Kagami H, Honda MJ, Ueda M. Differential inducibility of human and porcine dental pulp derived cells into odontoblasts. Connect tissue Res 2005;48:229-38.
24. Papaccio G, Graziano A, D'Aquino R, Graziano MF, Pirozzi G, Menditti D, De Rosa A, Carinci F, Laino G. Long-term cryopreservation of dental pulp stem cells (SBP-DPSCs) and their differentiated osteoblasts: a cell source for tissue repair. J Cell Physiol 2006;208:319-25.

25. Cordeiro M, Dong Z, Kaneko T, Zhang Z, Miyazawa M, Shi S, Smith J Nör J. Dental pulp tissue engineering with stem cells from exfoliated deciduous teeth. J Endod 2008;34:962-69.
26. Kindler V. Postnatal stem cell survival: does the niche, a rare harbor where to resist the ebb tide of differentiation, also provide lineage-specific instructions? J Leukoc Biol 2005;78:836–44.
27. Nakashima M, Akamine A. The application of tissue engineering to regeneration of pulp and dentin in endodontics. J Endod 2005;31:711-18.
28. Venugopal J, Ramakrishna S. Applications of polymer nanofibers in biomedicine and biotechnology. Appl Biochem Biotechnol 2005;125:147-58.
29. Fukuda J, Khademhosseini A, Yeh J, Engl G, Cheng J, Farokhzad OC, Langer R. Micropatterned cell co-cultures using layer-by-layer deposition of extracellular matrix components. Biomaterials 2006;27:1479-86.
30. Huang GT, Sonoyama W, Chen J, Park SH. Various isolation methods and culturing environments. Cell Tissue Res 2006;324:225-36.
31. Martina M, Hutmacher DW. Biodegradable polymers applied in tissue engineering research: a review. Polym Int 2007;56:145-57.
32. Xiaoming Li. Recent patents on polymeric scaffolds for tissue engineering. Rec Patents on Biomed Engi 2009;2:65-72.
33. Vacatello M, D'Auria G, Falcigno L, Dettin M, Gambaretto R, Di Bello C, Paolillo L Conformational analysis of heparin binding peptides. Biomaterials 2005;26:3207-14.
34. Prescott R, Alsanea R, Fayad M, Johnson B, Wenckus C, Hao J, John AS, George A. Invivo generation of dental pulp-like tissue by using dental pulp stem cells, a collagen scaffold and dentin matrix protein 1 after subcutaneous transplantation in mice. J Endod 2008;34:421-26.
35. Feng Z, Yamato M, Akutsu T, Nakamura T, OkanoT, Umezu M. Investigation on the mechanical properties of contracted collagen gels as a scaffold for tissue engineering. Artif Organs 2003;27:84-91.
36. Thibodeau B, Teixeira F, Yamauchi M, Caplan DJ, Trope M. Pulp revascularization of immature dog teeth with apical periodontitis. J Endod 2007;33:680-89.
37. Sharma B, Elisseeff JH. Engineering structurally organized cartilage and bone tissues. Ann Biomed Eng 2004;32:148-59.
38. Burdick JA, Anseth KS. Photoencapsulation of osteoblasts in injectable RGD-modified PEG hydrogels for bone tissue engineering. Biomaterials2002;23:4315-23.
39. Jadlowiec JA, Celil AB, Hollinger JO. Bone tissue engineering: recent advances

and promising therapeutic agents. Expert Opin Biol Ther 2003;3:409-23.

40. Wang FM, Qiu K, Hu T, Wan CX, Zhou XD, Gutmann JL. Biodegradable porous calcium polyphosphate scaffolds for the three-dimensional culture of dental pulp cells. Int Endod J 2006;39:477-83.
41. Graziano A, d'Aquino R, Cusella-De Angelis MG, Laino G, Piattelli A, Pacifici M, De Rosa A, Papaccio G. Concave pit-containing scaffold surfaces improve stem cell-derived osteoblast performance and lead to significant bone tissue formation. PLoS One 2007;2:e496.
42. Graziano A, d'Aquino R, Cusella-De Angelis MG, De Francesco F, Giordano A, Laino G, Piattelli A, Traini T, De Rosa A, Papaccio G. Scaffold's surface geometry significantly affects human stem cell bone tissue engineering. J Cell Physiol 2008;214:166-72.
43. Misako Nakashima. Tissue engineering in endodontics. Aust Endod J 2005;31:111-13.
44. Alhadlaq A, Mao JJ. Tissue-engineered osteochondral constructs in the shape of an articular condyle. J Bone Joint Surg Am 2005;87:936–44.
45. Desgrandchamps F. Biomaterials in functional reconstruction. Curr Opin Urol 2000; 10: 201– 206.
46. Mironov V, Boland T, Trusk T, Forgacs G and Markwald RR. Organ printing: computer aided jet-based 3D tissue engineering. Trends in Biotech 2003;21(4):157-61.
47. Sanjana NE, Fuller SB. A fast flexible ink-jet printing method for patterning dissociated neurons in culture. J Neurosci Methods 2004;136:151-63.
48. Barron JA, Krizman DB, Ringeisen BR. Laser printing of single cells: statistical analysis, cell viability, and stress. Ann Biomed Engl 2005;33:121-30.
49. Anilkumar K, Geetha A**.** Tissue Engineering future concepts in endodontics – a short overview. J Clin and Diag Res 2010;4:3282-86.
50. Jullig M, Zhang WV, Stott NS. Gene therapy in orthopaedic surgery - the current status. ANZ J Surg 2004;74:46-54.