

Novel approaches to dental tissue regeneration using autologous stem cells and growth factors from extracted tooth: best from biologic waste

Abstract

Stem cells are unspecialized cells that have an excellent capacity to differentiate into many different cell types when required. Mesenchymal dental stem cells including dental pulp stem cells (DPSCs) and periodontal ligament stem cells (PDLSCs) cells are promising tools for periodontal regeneration. Dental pulp stem cells (DPSCs) and periodontal ligament stem cells (PDLSCs) have been isolated with low morbidity from impacted third molar teeth which are otherwise treated as biologic waste. Endogenous regenerative technique in periodontics utilizes patient's own regenerative 'tools' i.e. growth factors and stem cells in the healing of periodontal wounds. Considering this fact, this short communication addresses the potential of autologous human impacted third molar as a source of dental stem cells and growth factors to be used as bone graft substitute in the regeneration of periodontal tissues.

Keywords: biologic waste; bone regeneration; stem cells, third molars

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Introduction

Stem cells are unspecialized cells that have an excellent capacity to differentiate into many different cell types when required. Theoretically during tissue repair, they can divide without limit to replenish any other cell type and function. Impacted third molars are routinely encountered in the dental office. Third molars are a rich source of mesenchymal stem cells, biological cues which play an integral role in wound healing. Dental pulp stem cells (DPSCs) and periodontal ligament stem cells (PDLSCs) have been isolated with low morbidity from impacted third molar teeth which are otherwise treated as biologic waste. Various periodontal regenerative techniques has shown limited success as a result of insufficient biocompatibility, resorption of bone, limited graft quantity and donor-site morbidity.¹ As a consequence, current research trends have been directed towards developing cell-based techniques for periodontal regeneration. Endogenous regenerative technique in periodontics utilizes patient's own regenerative 'tools' i.e. growth factors, stem cells in the healing of periodontal wounds.² But ex vivo culture has limitations like high cost, technique sensitivity, loss of stemness during cell passage, genetic manipulation and tumorigenic potential. Considering this fact, this short communication reveals the potential of human impacted third molar as a rich source of autologous dental stem cells and growth factors. It also describes novel approaches to dental tissue regeneration from healthy extracted tooth which is usually considered as biologic waste.

Impacted third molars: rich reservoir of mesenchymal stem cells

Dental pulp stem cells (DPSCs) and periodontal ligament stem cells (PDLSCs) have been isolated relatively non-invasively and with low morbidity from impacted third molar.^{3,4} teeth which are otherwise treated as biologic waste.

Dental pulp stem cells (DPSCs): DPSCs are mesenchymal type of stem cells inside dental pulp.⁵ DPSCs have osteogenic and chondrogenic potential *in vitro* and can differentiate into dentin, *in vivo* and also differentiate into dentin-pulp like complex.⁶ Animal studies and also human clinical trials using dental pulp stem cells assure

predictable tissue regeneration.³ DPSCs are putative candidate for dental tissue engineering due to, easy surgical access to the collection site and very low morbidity after extraction of the dental pulp. DPSCs can generate much more typical dentin tissues within a short period than non dental stem cells and it can be safely cryopreserved and recombined with many scaffolds.⁷

Periodontal ligament stem cells (PDLSCs): Periodontal ligament stem cells were first isolated from the periodontal ligament tissue of extracted human third molar teeth.⁴ These host-derived stem cells can either be subjected to isolation, ex-vivo expansion (stem cell culture) and re-implantation of these cells into periodontal wound / defect or injected directly as a suspension or delivered by biomaterial scaffolds or cell carriers.⁸ However, ex-vivo stem culture techniques are sensitive and expensive. Also on consecutive stem cell passages genetic manipulation and tumorigenic potential of the cells may be evoked (Table 1).⁹

Stem cell application in periodontal regeneration technique¹⁰⁻¹⁵ (SAI-PRT) utilizes direct application of autologous periodontal ligament stem cells derived from extracted third molar of the same host and aims at regeneration of periodontal tissues lost due to disease process. The acceleration of a patient's endogenous regenerative mechanisms by transplanting host stem/progenitor cells, for periodontal regeneration recalls mother nature at chair-side by a group of dentist, an oral and maxillofacial surgeon and a periodontist. In this procedure, the tissue adherent to third molar harboring the periodontal ligament stem cells is directly placed into the bony defect caused by periodontal diseases, by-passing the ex vivo culture. The stem cells procured are adequate to regenerate tissue specially bone in small size defects of 5 X 3mm depth. The technique derives its name from direct use of autologous stem cell application in tissue regeneration technique (SAI-TRT).

Twelve out of fifteen cases initiated under stem cell application in periodontal regeneration named as SAI-PRT resulted in successful clinical and radiographic parameters such as clinical attachment gain, decreased probing pocket depth and satisfactory defect fill of intrabony defect when evaluated for a period one year. The immediate periodic healing events were uneventful. From above authors own contribution

it can be concluded that a simple task of stem cells (PDLSCs) cementum shavings (growth factors) procurement and immediate placement using gelatin sponge scaffold are the major advantages of the current concept(SAI-PRT) has emerged as a constructive avenue in treatment of periodontal osseous defects. Studies have proved

that soft tissues on the extracted third molar root harbors PDLSCS, which have shown a clinical and radiographical improvement in the treatment of periodontal osseous defects.¹⁶⁻¹⁸ Based on this, in SAI-PRT we are attempted to transplant the autologous PDLSCs directly into the periodontal osseous defects (Table 2).

Table 1 Studies related to PDLSCs

Defect	Carrier	Animal model	Cell association	Duration of implant	Outcomes	References
Periodontal defect surgically created on the buccal cortex of the mandibular molar	Hydroxyapatite /b-tricalcium Phosphate particles	Immuno-deficient rats	Xenotrans-plantation	6-8 weeks	Implanted periodontal ligament stem cells demonstrated the ability to form cementum / periodontal ligament-like structures and aid periodontal tissue repair	10
Periodontal lesion of the maxilla and mandibular first molars	Hydroxyapatite and tricalcium phosphate	Miniature pig	Autologous	12 weeks	Transplanted green fluorescent protein-labeled periodontal ligament stem cells had excellent capacity to form bone, cementum and periodontal ligament when transplanted into a surgically created periodontal defect. Green fluorescent protein-labeled cells were identified in the newly formed bone, suggesting that the transplanted cells contributed to new-bone formation	11
Saddle-like through-and through defects	Hydroxyapatite/ b-tricalcium phosphate	B e a g l e dogs	Autologous	8 and 16 weeks	Transplantation of bone marrow-derived mesenchymal stem cells and periodontal ligament stem cells into peri-implant defects resulted in enhanced bone regeneration. There was no significant difference in regenerative potential between bone marrow-derived mesenchymal stem cells and periodontal ligament stem cells	12
Periodontal defect in the mesial region of the maxilla and mandibular first molars	Hydroxyapatite and tricalcium phosphate	Miniature pig	Allogeneic	12 weeks	The aim of this study was to assess the immunogenicity and immunomodulation of periodontal ligament stem cells through allogeneic transplants of periodontal ligament stem cell sheets. Allogeneic transplantation of the periodontal ligament stem cell sheets enhanced periodontal tissue repair in a manner similar to that seen in autologous transplants. Additionally, there was no evidence of rejection of the allogeneic cells	1
Cavity left after extraction of lower incisor	Root-shaped hydroxyapatite/ b-tricalcium phosphate lock and gel foam	Miniature pig	Not defined	3 months	This study implanted periodontal ligament stem cells in combination with stem cells from apical papilla in an attempt to generate a root/periodontal complex capable of supporting a porcelain crown. Together, this strategy led to the formation of bio-roots with significantly better compression strength than defects that did not receive stem cells	14

Table 2 Comparison of human studies

	Feng F et al 2010	Vandana et al 2015
Method	PDLSCs are exvivo cultured and transplanted into the infrabony defect.	Autologous soft tissue containing the PDLSCs from the extracted third molar is transplanted directly into the infrabony defect.
Duration	32-72 months	12 months
No. of cases	3	15
Disadvantage	Time consumption High cost Technique sensitive Tedious procedure Tumorigenic potential	NIL
Advantages		Less cost Less time consumption Chair side technique No chances of tumorigenic potential Efficacy of PDLSCs alone is assessed.

Impacted molars: rich source of growth factors

The chemical compositions of dentin and bone are very similar. Cementum plays a critical role in periodontal tissue regeneration and is a rich source of variety of growth factors such as IGF, FGF, BMPs, etc.¹⁹ While dentin exhibits biochemical components which are similar to bone e.g. dentin contains growth factors: insulin-like growth factor (IGF)-II, bone morphogenetic protein (BMP)-2, and transforming growth factor beta (TGF)- β . The presence of dentin promotes the formation of a calcified tissue similar to bone and accelerates healing while inhibiting inflammatory reaction.²⁰ Therefore, teeth has a very much potential to be used as a bone graft substitute.

Kim and colleagues²¹ have developed the technology of making bone graft materials using extracted teeth. However this technique requires intensive processing which involves partial demineralization and freezing-drying and thus cannot be applied directly chair-side in the treatment of osseous defects in the patients at the same time when the tooth is extracted. The extensive processing of the tooth after extraction to be used as bone graft substitute is expensive and technique sensitive. Various human and animal studies utilizing tooth as a rich source of growth factors as well as bone graft substitute have shown promising results in dental tissue regeneration.^{21,22} Considering the drawbacks tooth processing techniques, the freshly extracted tooth can be used chairside by obtaining cementum and dentin shaving which are rich in growth factors in treatment periodontal structures destroyed by disease process. As an extension of our work on direct application autologous PDLSCs- SAI-PRT, we also utilized autologous dentin and cementum shavings delivered using gelatin sponge as a scaffold. Twelve out of fifteen cases initiated have shown excellent clinical and radiographic improvements.

Conclusion

The regeneration potential of an extracted impacted molar can be tapped by utilizing the stem cells and direct application of soft tissue adherent to the extracted tooth root which harbors the PDLSCs or dental pulp which harbors the DPSCs, for autologous growth factors associated bone regeneration dentin and cementum shavings. Both autologous growth factors are of constructive potential to regenerate dental tissues especially periodontal tissues confirmed by SAI-PRT. Considering the global approach to stem cell related clinical trials, there is considerable progress in Indian scenario stem cell concepts using SAI-PRT and extended SAI-PRT which are effective chair-side, safe and in expensive. The promotion of concept depends on implementation of this technique and reach to heights of success of periodontal regeneration from one's personalized asset, instead of expensive uncertain periodontal regeneration method.

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Conflicts of interest

Authors declare that there is no conflicts of interest.

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