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Whole Tooth Regeneration - A Dream of All the Clinicians and Researchers! An Update and Review

Neha Goel1,*Pulin Saluja2, Aparna Dave3, Manpreet Arora4 and Radhika Rai5
1.Faculty of Dental Sciences, SGT University, Gurugram, Haryana, India.
2,3,4,5.Department of Oral Pathology, Faculty of Dental Sciences, SGT University, Gurugram, Haryana, India.
***Corresponding Author:Email id:** pulin.saluja@sgtuniversity.org*

ABSTRACT
Whole tooth regeneration is the dream of every clinician and researchers. The cell-based implant for replacement of missing teeth having similar properties to that of natural tooth is a concern of every researcher. A regenerated tooth having similar physical, mechanical and functional properties is always desirable. Current treatment modalities consist of artificial replacement of teeth using removable partial denture, fixed partial denture and dental implants. Various animal-based studies have proved that it is now possible to repair and regenerate the parts of tooth like pulp-dentin complex and parts of the periodontium and not only these but also entire tooth regeneration. Researches are undergoing to assess the role of stem cells in forming a cell-based implant treatment for replacement of missing teeth.
In this review, we have assessed many articles to determine the role of dental stem cells particularly in whole tooth regeneration by scaffold and non-scaffold-based studies. Experimental Animal-based studies have concluded that whole tooth regeneration is now made possible with similar structural and functional to that of natural tooth and soon there will be an alternative to cell-based implant treatment will be available to the patient in dentistry.
Key Words: Tooth regeneration, Stem cells, cell-based implant, natural tooth

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Background
Teeth are composed of enamel, dentin, pulp and cementum. And the tooth supporting structures are known as periodontium which comprises of gingiva, periodontal ligament, alveolar bone and cementum. Enamel is the hardest structure on the tooth [1]. Before knowing about the whole tooth regeneration process the sound knowledge of tooth development is very crucial. Tooth development consists of 5 morphological stages i.e., bud, cap, early bell stage, late bell stage [2, 3]. Cap stage: As growth occurs, concavity develops on the inner side of enamel organ. During late cap stage, inner enamel epithelium develops in which the cells are cuboidal in shape which give morphology of the crown and differentiate into ameloblasts which form enamel. The outer enamel organ has outer enamel epithelium. The condensed mesenchymal cells beneath the inner enamel epithelium develop into dental papilla giving rise to dentin and pulp. And enamel organ is surrounded by fibrous capsule called dental follicle which later forms periodontal ligament. Bell stage: Formation of hard tissue occurs in this stage. Dentinogenesis (Dentin formation), amelogenesis (enamel formation), cementogenesis (cementum formation), periodontal ligament formation occurs in this stage. Thus dentin, pulp, cementum, alveolar bone, periodontal ligament is derived from neural crest cells and have shown regenerative properties which is related to the concept of dental stems cells.

INTRODUCTION
Life came to an end for many species after the complete dentition is lost [4]. In humans, teeth can be lost due to most common dental disease i.e., dental caries, trauma, periodontal disease, congenitally missing, or after a resective orofacial tumor, etc. In today's time the treatment modalities available for replacing of missing tooth are dental implants, fixed partial dentures, removal partial denture. Dental implants are becoming popular nowadays but can fail to adapt with the surrounding bone which undergoes bone remodeling throughout the life. It is an artificial material by which aseptic loosening can lead to implant failure. Whereas in regenerated tissue stimulate both bone and tooth regeneration with no artificial material being used. And treatment with Removable partial denture and Fixed partial denturehave their own disadvantages with the time. But nothing can replace the strength and properties to that of natural

tooth. With advancement in dental technology and civilization have not increased the various treatment modalities available nowadays but have also increased the health care facilities and improvement in quality of life of the patients.

This article aims to provide a review on the emerging concepts of tooth regeneration particularly highlighting the stem cell-based therapy for whole tooth regeneration. In future, replacement of missing teeth with entire tooth regeneration may become an alternative to the patients in dentistry.

Tooth Regeneration

Tooth regeneration is a part of regenerative medicine to replace a tissue which is being lost to its original structure as well as function by biological means [5].

Regeneration of the teeth can include various types like [6]:

1. Regeneration of whole teeth
2. Regeneration of pulp
3. Regeneration of dentin
4. Regeneration of periodontium
5. Regeneration/ formation of enamel like structures.

Dental Stem Cells

Stem cells- these are the totipotent and unspecialized cells which are capable to form into any specialized cells/tissues. Dental Stem cells are being found in dental pulp of both deciduous and permanent teeth, dental follicle i.e., the tissue that surrounds unerupted tooth, periodontal ligament, tooth germsand from apical papilla [7, 21].

Although, different stem cell differs in aspect of rate of multiplication, marker of gene expression and differentiation property of cell which may due to variation in site of origin of tissues, function/ culture.[8]

Human third molars can be widely used as the rich source of dental stem cells because they are still in the phase of root development and often mainly discarded in practice.

Dental Pulp Stem Cells (Dpscs)

Dental pulp stem cells are mesenchymal stem cells capable of forming odontoblasts, myocytes, adipocytes, osteoblast and neural tissue [8].They express surface antigen like CD146, CD105, CD90, CD73, CD59, CD44, CD29,CD13, and STRO-1 [9]. And researchers have found that they are capable to form functional dentin-pulp complexes in *in vivo* studies in rat [10].

Stem Cells From Exfoliated Deciduous Tooth (Shed)

The pulp derived from these primary teeth has the potent stem cells. Mostly immature cell types are present in this therefore called immature DPSCs.

SHED express molecular CELL markers like CD166, CD146, CD90, CD73, CD29. They can differentiate into chondrocytes, myocytes, adipocytes, osteoblasts and even neuron like cells.[11]

Dental Follicle Progenitor Stem Cells(Dfpcs)

These are the stem cells which are present in the dental follicle around the developing tooth. These stem cells can be differentiated into odontoblasts, chondrocytes, cementoblasts and periodontal ligament cells when provided with the stimulus [12]. DFPCs express mesenchymal stem cells markers like CD90, CD59, CD29 and CD13 [13].

Stem Cells From Apical Papilla (SCAP)

The developing tooth consists of tissue connected to its top called apical papilla. They are mesenchymal stem cells showing specific cell markers like CD 146, CD90, CD44, CD24 and STRO-1 are expressed on SCAP.[14] They have the potency to differentiated into osteogenic, neurogenic and adipogenic cells in vitro.

Tooth Germ Stem Cells (TGSCS)

Tooth germ are the one giving rise to the tooth and dental tissues and is a rich source of potent stem cells. Here the differentiation capacity is very high as most of the cells are in the undifferentiated form.[15] They express surface antigen CD166, CD105, CD90, CD73 and CD29 which are the markers specific to mesenchymal stem cells [16]. These cells exhibit osteogenic, odontogenic and adipogenic cells that originate from all the 3 germ layers i.e. endoderm, ectoderm, mesoderm [17].

Periodontal Ligament Stem Cells (PDLSCS)

It is derived from neural crest cells. PDL cells act as the connection between bone and cementum. Mesenchymal stem cells in the PDL express specific markers like CD105, CD73, CD44,CD29, and CD10 [18].

The differentiating capacity of these stem cells is similar to any other dental stem cells. It has chondrogenic, osteogenic and adipogenic potency proved in the in-vitro studies by Gay et al in 2007 [19]. Also these stem cells when implanted in tooth tissues can differentiate into cementoblasts proved in in-vivo and in-vitro studies [20].

Dental Stem cells applications are not just limited to development of dental tissues but also in Parkinson's disease.[22] Stem cells from the exfoliated primary teeth i.e. SHED can be used in the therapy of systemic lupus erythematosus (SLE) also [23]. Moreover, dental pulp derived stem cells secrete insulin thus can be used in diabetic patients and in myocardial infarction by its proangiogenic properties in future after sufficient researches.

Whole Tooth Regeneration

The current treatment available is dental implant. In this procedure a metallic threaded implant in placed inside the pre prepared osteotomy site for implant placement which is then loaded with crown of desired material. The prerequisite for dental implant is that some amount of bone needs to be there before its placement. Also, periodontal ligament is absent that helps as shock absorber. Whole Tooth regeneration has an advantage over it that it not only replaces the missing tooth but also cause the development of parts of periodontium, i.e., alveolar bone, pdl, cementum also. It can take and resist stress similar to that of natural tooth.

There are various conditions where bone is deficient as in the post-menopausal osteoporosis, or patient had undergone for surgical removal of bone tumors, thus bone grafts become essential for the implant placement. If the whole tooth regeneration or cell-based implant is made possible, it can avoid complex procedures and surgeries and will reduce the number of appointment/ visits of patients due to long treatment plan.

Types Of Tooth Regeneration- Scaffold And Non-Scafold Based

Tooth regeneration can be done by two ways scaffold based and non-scaffold based.[25] Various scaffold used for tooth regeneration are discussed below briefly. [26]It can be a biological polymer scaffold or ceramic scaffold.It consists of biological polymer scaffold that can consists of collagen, chitosan, silk protein, alginate, hyaluronic acid and derivatives, peptides.

Artificial polymer scaffolds have proved to have better mechanical properties and machinable in comparison to biological ones. They are biodegradable and used extensively in dental regeneration.They are moderately short lasting polymers e.g. such as polyglycolic acid (PGA), polyglycolic acid-poly-L-lactic acid (PGA-PLLA), polylactic acid (PLA) and polylactic polyglycolic acid (PLGA). Whearas ceramic scaffold have long lasting properties and biological polymer scaffold are of intermediate type.

It has been seen in studies that scaffold based whole tooth regeneration resulted in tooth with smaller morphology and different properties as that of natural tooth in some cases. In non scaffold based tooth regeneration do not require any of the mentioned scaffold for tooth regeneration. Nowdays no only parts of tooth but Whole tooth regeneration is possible according to the in vitro and in vivo studies in experimental animal [27, 28].

Young et al in the year 2002 carried the study in immunodeficient rat for 30weeks by using pig's third molars tooth buds as a source of stem cells and use PGLA (Poly(lactide-co-glycolide)) as the scaffold. The result revealed appreciable tooth structures formed that contained dentin, odontoblasts and well-defined pulp chamber [28].

Duailibi et al in the year 2004 also carried the research on adult rats for 12 weeks by using rat post-natal tooth bud cells as the stem cells source and PGA (3-phosphoglycerate**)** and PLGA (Poly(lactide-co-glycolide)) as the scaffold and implanted in the omentum of adult rats. The study concluded that bioengineered tooth structures were formed [29].

Ohazama et al in the year 2004 conducted a study by using rat oral embryonic epithelium (ED 10), non-dental cell population from ES Cell, neural crest cells as the stem cell source. This is a scaffold free technique. He transplanted the recombined tissue in its kidney for 2 weeks and resulted in tooth structured formation and bone and soft tissue. And also implanted ED 14.5 molar rudiments in diastema region of adult mice and resulted in tooth structure formation of normal size and demonstrated normal histology [30].

Honda et al in the year 2005 did a study on rats by using pig's third molar tooth buds as source of stem cells and using PGA mesh as the scaffold and implanted into the omentum of rats for 2-25 weeks. The result showed regeneration process natural to odontogenesis. But the tooth size, shape, and root formation timing were different [31].

In the same year 2005, young et al also conducted the scaffold-based research i.e. PGA and PLGA were used and implanted in rats for one month and also the same is implanted in rat omentum, grown in bioreactor for 10 days and concluded that formation of hybrid tooth bone which is similar to bone and periodontal tissues [32].

In the year 2007 Nakao et al conducted a scaffold free based study. The source of stem cell was murine tooth bud embroyonic stem cells.It was seen that multiple similar tooth in morphology was formed in vitro and vivo after transplantation in jaw [33].

In the year 2008 duailibi et al conducted a study in rat using rat tooth bud cells as stem cell source. It used PGA/ PLLA and PLGA as scaffold ans stems stems cells seeded into scaffold and implanted into fresh extraction socket for 12 weeks. The study conducted revealed that the small tooth crown with enamel,

dentin. Pulp and periodontal tissues were formed. But they were less organized as compared to when grown in omentum [34].
In 2009, Ikeda et al conducted the scaffold free based study in rat. Stem cells used were rat ED14.5 molar tooth stem cells. They implanted bioengineered tooth germ in the jaw. The tooth formed was fully erupted, well in occlusion with tooth in opposite arch. Structure and hardness was similar to natural tooth. Moreover, the noxious stimuli was present [35].
In the year 2011, Oshima et al conducted the study on rat. It was a scaffold free based study. It used ED 14.5 molar tooth stem cells. In this technique they develop mature tooth in sub-renal capsule and finally implantation into the missing tooth site. In this, tooth structure developed similar to natural tooth with periodontal tissue. After it is implanted in the jaw, alveolar bone regeneration was seen. Tooth also responded to mechanical and noxious stimuli [36].
In the year 2013, Cai et al carried a scaffold free study in rat, using integrated free human derived iPSCs(ifhU-iPSCs) and rat ED 14.5 molar mesenchyme. Differentiation of ifhU-iPSCs to epithelial sheets and recombination with rat molar mesenchyme. And other technique consists of 3 weeks culture of recombinant in sub renal culture.
The study concluded that in the recombinant, similar tooth like structure is formed. Not only this but also molecular markers proved differentiation of ifhU-iPSCs into enamel forming cells called ameloblast and raman spectroscopy and nano indentation revealed similar properties of enamel.[37]

Challenges

Whole tooth regeneration is a full challenge [25, 7, 27]. Although we have come so far from regeneration of parts to tooth to entire tooth regeneration, there are still challenges ahead. Mostly the researches have been done on experimental animals, no such stem cell therapy have been done on humans. The major challenge is to identification of the specific type of odontogenic stem cells, its isolation and purification in research centers to yield the desired results. This may takes years of ongoing studies for isolation of specific types of cells.
Also stromal progenitor cells are less potent than embroyonic stem cells. And embroyonic stem cells may raise an ethical issue. Also the development of tooth formation in slower than the mice [27].
Immunological reaction is the one that may pose the major problem in the patients. Although autologous stem cells if being used do not have this disadvantage. There is a big question whether teeth like structures formed can replaced the natural teeth.
These stem cells may deviate from their odontogenic potency after in vitro expansion and may not yield the desired effects of the tooth regeneration [38].
Further researches in future on tooth regeneration may give the desired results and overcoming all the challenges we are facing today.

CONCLUSION

As to regenerate the entire tooth have been the dream of every dentist, researchers and clinicians. According to the reviewed articles, it has been seen that we have come a long way in development of entire tooth regeneration. The most common experimental animal is rat.
Various in vivo researches in rat have proved that not only pulp-dentin complex but in factentire tooth regeneration is now possible. It has been seen along with the regenerated tooth; development of periodontium is also seen.Removable partial denture, fixed partial denture, implants are artificial replacements of natural tooth in which various structures like enamel, dentin, pulp, PDL,etc. Are absent increasing the chances of failures. This regenerated tooth will have all the properties similar to natural tooth including development of periodontium including PDL along with the regenerated tooth.
Thus, whole tooth regeneration can be proved as a boon in the field of dentistry which not increase the treatment modalities available to the patient for replacement of missing teeth but also helps in avoiding complex surgical procedures or surgeries for multiple teeth replacement.
Whole tooth regeneration is challenging. As of now there is no such research which have fully formed the regenerated tooth with desirable physical, mechanical, structural and functional to that of a natural tooth. With collaboration of different sectors like molecular biology, embryology, bio-materials, tissue engineering are required for the goal of whole tooth regeneration are needed. Researches are ongoing and thus soon there will be another treatment modality for whole tooth regeneration will be available in future.

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