REVIEW ARTICLE

# Stem cell therapy in oral and maxillofacial region: An overview

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**ABSTRACT**

Cells with unique capacity for self-renewal and potency are called stem cells. With appropriate biochemical signals stem cells can be transformed into desirable cells. The idea behind this article is to shortly review the obtained literature on stem cell with respect to their properties, types and advantages of dental stem cells. Emphasis has been given to the possibilities of stem cell therapy in the oral and maxillofacial region including regeneration of tooth and craniofacial defects.

***Key words:*** Oral and maxillofacial region, stem cells from exfoliated deciduous teeth, stem cell marker, tooth regeneration

## INTRODUCTION

Stem cells are unique type of cells that have specialized capacity for self-renewal and potency, can give rise to one and sometimes many different cell types. "They are found in almost many of the multi cellular organisms and are characterized by the ability to renew through mitotic cell division while maintaining the undifferentiated state."[1] Stem cell therapy involves manipulation of the cells *in vitro* and using for therapeutic purposes. The possible applications of stem cells are replacement and repair of tissues and organs. Replacement of oromaxillofacial structure is difficult, because functions such as facial expression, articulation, chewing, and swallowing are delicate and made of a complex anatomical structure formed from soft and hard tissues.[2] Stem cells, biomimmetic materials, and growth factors are essential to form these three-dimensional structures. Regeneration of oral and maxillofacial structures can be carried out using stem cell therapy that has gained momentum in the recent days.

## STEM CELL PROPERTIES

A classic stem cell should possess two properties namely self-renewal and potency.

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- Self-renewal is the capacity of the cell to undergo numerous cycles of cell division maintaining the undifferentiated state.[3] An ideal stem cell should have the capacity of self renewal beyond the "Hayflicks" limit (the ability of the cell to proliferate to about 40-60 population doublings before it achieves senescence).[4]
- Potency means the differentiation capacity of the stem cell.[5]

## STEM CELL TYPES

Stem cells can be broadly divided into

1. Embryonic stem cell
2. Adult stem cell
   - Hematopoietic stem cell
   - Mesenchymal stem cell
3. Induced pluripotent stem cell

### Embryonic stem cell

Embryonic stem cells are capable of multipotential differentiation but clinical feasibility is limited due to ethical issues. The inner cell mass (the part that would form fetus) of the embryo is used to form embryonic cell lines.[6] Embryonic stem cells has a potential to differentiate into germ layers namely ectoderm, endoderm and mesoderm.[7] Tumorigenesis and immune rejection is common with embryonic stem cells.[8]

### Adult stem cell

Adult stem cells are multipotent stem cells.[1] They have been harvested from different kind of tissues like bone marrow, umbilical cord, amniotic fluid, brain tissue, liver, pancreas,

cornea, dental pulp, and adipose tissue. Adult stem cells are comparatively easier to isolate and do not have any ethical issues. Immune rejection and teratoma formation is also rare with adult stem cells. Adult stem cells are commonly used in current day practice.

### Induced pluripotent stem cell

Induced pluripotent stem cells(IPS) is an evolving concept in which 3–4 genes found in the stem cells are transfected into the donor cells using appropriate vectors. The stem cells thus derived by culturing will have properties almost like embryonic stem cells.[9] This path breaking discovery may have a major role in future stem cell therapy.

## SOURCES OF STEM CELLS

The oral and maxillofacial region can be treated with stem cells from the following sources

1. Bone marrow
2. Adipose tissue
3. Stem cells from oral and maxillofacial region

### Bone marrow

Bone marrow stem cells (BMSCs) can be harvested from sternum or iliac crest. It is composed of both hematopoietic stem cells and mesenchymal stem cells (MSCs). The majority of oro-maxillofacial oral structures are formed from mesenchymal cells. The advantage of bone marrow is that it has a larger volume of stem cells and can be differentiated in to wide variety of cells. Isolation of BMSCs can be carried out only under general anesthesia with possible post operative pain.

### Adipose tissue

They can be harvested from the lipectomy or liposuction aspirate. Adipose derived stem cells (ADSCs) contain a group of pluripotent mesenchymal stem cells that exhibit multilineage differentiation.[10] Advantage of adipose tissue is that it is easily accessible and abundant in many individuals.

### Stem cells from the oro-maxillofacial region

Stem cells from oral and maxillofacial region predominantly contain mesenchymal stem cells. In oral and maxillofacial area different types of dental stem cells were isolated and characterized. They include

- Dental pulp stem cells (DPSCs)[11]
- Stem cells from exfoliated deciduous teeth (SHED)[12]
- Periodontal ligament stem cells (PDLSCs)[13]
- Stem cells from apical papilla (SCAP)[14]
- Dental follicle progenitor cells (DFPCs)[15]

These dental stem cells have MSC like qualities, such as self-renewal and differentiation potential.[16]

1. DPSCs were successfully isolated by Gronthos *et al*., in 2000. They were able to demonstrate odontoblast like cells from DPSCs producing ectopic dentin in the immunocompromized mice.[11]
2. SHED were identified to be cells of higher proliferation rate, with increased population doublings, immature multipotent clonogenic cells isolated from deciduous teeth that can differentiate into several cell types.[12]
3. PDLSCs were isolated from periodontal ligament of 25 human third molars by Seo BM *et al*. They demonstrated cementoid cells, adipocytes when transplanted into immunocompromized rodents.[13]
4. Sonoyama *et al*., isolated mesenchymal stem cells from apical papilla otherwise called SCAP which is capable of forming odontoblast like cells *in vivo*.[14]
5. Morsczeck C *et al*.[15] obtained stem cells from dental follicle called dental follicle precursor cells(DFPCs) that can form cementoblasts, PDL cells and osteoblasts.

## DENTAL STEM CELL ADVANTAGES

The advantages of stem cells from oral and maxillofacial region is that

1. Have high plasticity.
2. It can be cryopreserved for longer period (Ideal for stem cell banking).
3. It showed good interaction with scaffold and growth factors.
4. Stem cells transplantations can cause pathogen transmission and also need immunosuppression, so autologous stem cell source is the best option. Dental pulp stem cells will be better fitting tool due to easy surgical access, the very low morbidity of the anatomical site after the collection of the pulp.[17]

## STEM CELLS STORAGE AND TRANSPORT

Tissue samples containing stem cells were placed in a screw top vial containing an appropriate media, which nourishes it during transport. The sample should reach the processing storage facility before 40 hours. In the laboratory the samples were trypsinized and passaged to yield colonies of stem cells. The required cell type can be manipulated by utilizing right inductive signals and appropriate growth factors to the stem cells.

## STEM CELL MARKERS AND SCAFFOLD

Cultured stem cells should be passed through stem cell markers like Oct4, Nanog, SSEA4, TRA-1-60 and TRA-1-81 before it is administered to patients to know the lineage of the cell. Compulsory endotoxin test should be subjected to the cultured stem cells to rule out any microbial contamination. Stem cells are loaded in an appropriate carrier called "scaffold" to close the defects or replace the organ. Scaffold can be of different shapes, pattern and biomaterials. Depending

upon the necessity it can be made up of natural or artificial materials and can be biodegradable or non biodegradable. Materials such as poly lactic acid, polyglycolic acid (PGA), polyethylene terepthalate, polypropylene fumarate, hydroxyapatite/tricalcium phosphate, fibrin, alginates, and collagen are used.[18]

## CLINICAL APPLICATION OF STEM CELL THERAPY IN THE ORO-MAXILLOFACIAL REGION

The structures of interest in oral and maxillofacial region include the enamel, dentin, dental pulp, cementum, periodontal ligament, craniofacial bones, the temporo mandibular joint, ligaments, skeletal muscles, tendons, skin, subcutaneous soft tissue, and salivary glands.

### Regeneration of dentin, pulp

Dental pulp tissue has the regenerative potential to form dentin in response to any injury.[19] Tubular dentin formation was observed when human pulp stem cells with scaffold (hydroxyapatite/tricalcium phosphate) were implanted in immunocompromised mice.[20] Reparative dentin formation on amputed pulp was found when stem cells were combined with recombinant human bone morphogenetic protein 2 (BMP 2) in experimental studies on animal models.[21]

Regeneration of the pulp inside the damaged tooth can be the basic clinical application of stem therapy in dentistry. Root canal treatment in a young permanent molar will stop the tooth's continuous maturation process there by leaving thin egg shell like weak tooth that is susceptible to fracture. Regeneration of pulp with stem cell therapy will be a better option. Stem cells harvested from the pulp of unwanted teeth like third molar can be utilized to regenerate the pulp of severely injured tooth there by preventing the need for endodontic treatment in adults.

Huang *et al*.[22] in his review article summarized new protocol for endontically involved immature permanent teeth in which minimal instrumentation was done in it followed by disinfection with triple antibiotic paste. Treated tooth is coated with mineral trioxide aggregate (MTA) and filled with glass ionomer cement. Periodical observation was done to ascertain root maturation.

## STEM CELLS IN PERIODONTAL REGENERATION

Stem cells will be a promising tool for regenerating the periodontal structures such as periodontal ligament and other supporting elements. BMSCs have been used by Kawaguchi *et al*. for their capability to regenerate periodontal tissue and repair periodontal defects. BMSCs have the ability to produce alveolar bone, periodontal ligament, and *in vivo* cementum after implantation into the periodontal defects. Thus, it was proved BMSCs provides an alternative source for the treatment of periodontal diseases.[23] Autologous mesenchymal stem cells from iliac crest in combination with platlet rich plasma from peripheral blood was used for periodontal regeneration. Significant closure of bone defect and improvement of attachment level was observed after one year follow up. It also showed good healing and regeneration of interdental papilla.[24]

Nagatomo *et al*. in their experimental studies found that PDL cells having stem cell properties can regenerate periodontium.[25] Transplantation of PDL derived cells into animal models were shown to regenerate periodontal tissue.[26]

Iwata *et al*. harvested and expanded primary canine PDL cells *in vitro* and also made into transplantable constructs containing PGA Scaffold and PDL cell sheets. The transplantable constructs in combination with porous bTCP(b –tricalcium phosphate) induced regeneration of periodontal structures, including alveolar bone, cementum, and periodontal fibers.[27] Liu *et al*. regenerated periodontal tissue in miniature swine using scaffolds seeded with periodontal ligament derived stem cells.[28] PDLSCs can differentiate into cells that can colonize on biocompatible scaffold, suggesting an easy and efficient autologous source of stem cells for regeneration of dental tissues.[29] Marie MK *et al*. in their experimental on goat was able to regenerate periodontal tissues around titanium implant using autologous bone marrow stem cells with scaffold.[30]

### Regeneration of craniofacial defects

Stem cells can be useful in the regeneration of bone and to correct large craniofacial defects due to cyst enucleation, tumor resection, and trauma. The closure of a bone defect is commonly carried out with the transfer of tissue, which have disadvantages like, not able to restore the unique function of the lost part, donor site morbidity, accompanied by scarring, infection and loss of function.[31] Adipose derived stem cells was used to treat the calvarial defect (120 $cm^2$) of a 7-year-old girl who had severe head injury. Autologous adipose stem cells were extracted from gluteal region along with iliac crest bone graft. Autologous fibrin glue that holds the cells in place was prepared by cryoprecipitation. This successful technique has given new rays of hope that ADSCs can be used for difficult reconstructive procedures.[32]

Soft tissue reconstruction in the oromaxillofacial region is of paramount importance when there is significant loss of soft tissues during surgery or trauma. Various methods including graft and flap transfer has been tried that produced donor site morbidity. Alhadlaq *et al*. in their experimental studies found human MSCs can turn into adipose cells when they exposed to adipogenic inducing medium. Adipose cells with appropriate shaped scaffold can be used for reconstruction of soft tissues.[33]

Stem cells isolated from dental pulp has a potential to differentiate into osteoblasts and are a good source for bone formation.[33] Stem cells from oral and maxillofacial region can be combined with bone marrow stem cells to correct larger defects. Oromaxillofacial bone tissue repair with stem cells was done using collagen sponge scaffold and dental pulp stem cells harvested from third molars of the same patient.[31] Lagenbach *et al*. in their *in vitro* studies used microspheres (scaffold free tissue construct) to close the critical size bone defects. They found osteogenically differentiated microspheres with outgrowing cells can be used to fill up bone defects. This new procedure has added advantage of permitting the transplantation of more cells and better integrity compared with cell suspensions or gels.[34] Stem cells isolated from SHED has significantly promoted wound healing in nude mice, proving deciduous teeth can be utilized for the treatment of chronic wounds. This application can be extended into oromaxillofacial region to enhance wound healing.[35]

## Future tissues

Future tissues like tissue engineered bone grafts, engineered joints and cranial sutures can be developed with stem cell therapy. A team of professionals including stem cell biologists, molecular biologists, geneticists, polymer and materials scientists, mechanical engineers and clinicians with knowledge of oral and maxillofacial disorders is needed to develop the field of craniofacial tissue engineering.[36] The ability to design anatomically viable and functional bone would have great potential for oromaxillofacial reconstructions of congenital defects, cancer resections, and trauma. The anatomically shaped viable bone grafts like articular condyles can be engineered by using adult mesenchymal stem cells and biomimetic scaffold bioreactor.[37,38]

Tissue engineered temporo mandibular joint was created by having natural bone building process as an inspiration. Condyle shaped scaffolds were made using decellularized bone with help of digitized clinical images. Stem cells were seeded into the scaffold and placed in a bioreactor chamber containing culture medium.[39] In future this technique can be applied to regenerate other bones in oromaxillofacial region.

## Tooth regeneration

The regeneration of adult teeth will be possible in future with the newer advancement in stem cell therapy and tissue engineering.[40] Regenerative procedures would be better fitting and alternative tool in place of dental implants. Experimental studies with animal models have shown that the tooth crown structure can be regenerated using tissue engineering techniques that combine stem cells and biodegradable scaffolds.[41] Epithelial mesenchymal interactions are mandatory in tooth development. "These interactions are characterized by the reciprocal exchange of signals between these two naïve germ layer tissues and result in the emergence of unique terminal phenotypes with their supporting cells".[42]

Tooth regeneration involves three key elements which include

- Inductive morphogenes
- Stem cells
- Scaffold

Steps involved in regeneration of tooth are

1. Harvesting and expansion of adult stem cells.
2. Seeding the stem cells into scaffold which provides optimized environment.
3. Cells are instructed with targeted soluble molecular signals spatially.
4. Confirming the gene expression profile of the cells for next stage in odontogenesis.[43]

Duailibi *et al*., in their experimental studies were able to form tooth structures from single cell suspensions of cultured rat tooth bud cells. They demonstrated bioengineered rat teeth developed in 12 weeks with PGA and PLGA scaffold.[44] Honda *et al*. developed tissue engineered teeth, when implanted into omentum of rat using porcine tooth bud cells and PGA fiber mesh scaffold that resembles the model of odontogenesis.[45] Young *et al*., using porcine tooth bud cells, PGA and PLGA scaffolds generated a hybrid tooth bone for the treatment of tooth loss along with alveolar bone resorption.[46]

## CONCLUSION

The future dentistry will be more of regenerative based, where patients own cells can be used to treat diseases. Stem cell therapy has got a paramount role as a future treatment modality in dentistry. Regenerative dentistry will have to go in pace with regenerative medicine. On the other hand, stem cells should be differentiated to the appropriate cell types before they can be used clinically, otherwise it might lead to deleterious effects. Determining the role of local conditions such as the type of scaffold and the presence of the microorganisms should be very carefully analyzed. Longer patient follow up is needed to study the life time of regenerated tissue.

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Announcement

## Training –Cum – Awareness Program for Indian Biomedical Editors organized by JOMFP

Unique one day training cum awareness program for Indian Biomedical editors for IndMed and MedInd Indexing, the Government of India's official Indexing agency/portal was conducted under the auspices of Journal of Oral & Maxillofacial Pathology on 21.1.2012. The course was conducted by Faculty of National Informatics Centre, New Delhi, Dr. Rekha Gupta and Dr. Sukhdev Singh.

JOMFP Editor, Dr. Elizabeth Joshua had organized this unique program with the active support of Ragas Dental College and Hospital, Chennai.

This was first of its kind to be hosted by a Dental Speciality Journal for the benefit of the other medical journals in this part of India. Editors and editorial members of various dental journals including Several Oral and Maxillofacial Pathologists, from various parts of India attended the function. President Dr. Alka Kale, President Elect Dr. GS Kumar, Vice President Dr. N. Malathy and Hon. Secretary of IAOMP Dr. K Ranganathan graced the occasion. The program was a very interactive one with all the delegates, benefiting much from this resource program.