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Review Article

Tissue Engineering – A Dental Perspective

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Abstract

Oro-facial tissue engineering forms the basis of regenerative dentistry. A few decades ago, tissue engineering was just a coined proposition and now it is progressing rapidly towards a clinical reality. The purpose of this review was to highlight the core conceptions of tissue engineering and translate it towards its present and upcoming implications in the facial region and oral cavity. A comprehensive electronic search was carried out on PubMed, Google Scholar and Science Direct from 2000 to 2020. All articles relevant to tissue engineering in dentistry were selected including in vitro studies, comparative studies and review articles. A total of 50 articles were thoroughly appraised for the narrative review. Tissue engineering is an amalgamation of the interaction between three key components namely cells, scaffolds and signaling molecules. The focus of tissue engineering in dentistry is the regeneration of missing oral and maxillofacial tissues. Regenerative therapies can be broadly categorized as material-based and stem-cell based. In Pakistan, research on tissue engineering in dentistry is still in its preliminary phase. Attempts have successfully been made to identify and grow dental pulp stem cells from human teeth. Modified scaffolds for alveolar bone repair and regeneration have been synthesized efficiently. Tissue engineering in dentistry is an emerging field. Challenges in the growth of clinically safe and satisfactory methods for repair of oral tissues are being met effectively in spite of limited progress. Although, across the world and in Pakistan too, there is still a long way to go in this field but future of these therapies is indeed promising and with time, these procedures will become available for clinical application.
Key words: Tissue engineering, stem cells, scaffolds, growth factors, oral cavity.

Introduction

The increase in life expectancy in the recent times has led to an upsurge in the number of aged people presenting with chronic degenerative diseases and requiring "organ transplants".[1] Moreover, human transplants come with their own share of drawbacks including problems of matching and rejection, infections as well as ethical and religious issues. Such drawbacks have resulted in a search for alternatives to transplantation.[2] Tissue engineering thereby emerged, as a promising regeneration system of lost tissues which restores function and esthetics simultaneously.[3]

The terminology "tissue engineering" was first coined in the year 1987.[4] It refers to an " inter-disciplinary field which applies the principles of engineering and biological sciences toward the development of biological substitutes which restore, replace, maintain, or improve tissue function".[5] It is an amalgamation of the interaction between three key components namely cells, scaffolds and signaling molecules.[6]

Tissue engineering in dental field evolved with the inception of membranes for "guided tissue regeneration" as well as use of platelet-rich plasma for guided restoration of lost bone.[7] Today, tissue engineering in dentistry is utilizing biomodulation and incorporation of nanomaterials for reconstruction of oral and maxillofacial tissues.[2,8,9] Globally, thorough research is being carried out in basic and applied stem cells research, especially in America and European countries but in most of the under-developed countries including Pakistan, this area still remains in its preliminary phase and there is limited progress.[10] Attempts have successfully been made to identify and grow dental pulp stem cells from human teeth.[11] Modified scaffolds for alveolar bone repair and periodontal regeneration

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Authors Contribution

MN conceptualized the project. AS did the literature search. Drafting, revision and manuscript writing were done by AA. MK performed the critical review of the manuscript.

have also been successfully synthesized, although human trials are still pending.[12]

The major institutes in Pakistan the are involved in tissue engineering research in field of stem cells include, Center for Advanced Molecular Biology (CAMB), Quaid-i-Azam University, Atta ur-Rehman School of Applied Biology (ASAB), Aga Khan University Centre for Regenerative Medicine and Stem Cell Research (AKUCRM) and Bone Marrow Transplant Center (BMTC). A private organization 'Cryocell', Karachi is the first umbilical cord cells banking facility established in Pakistan.

The crux of this review article was to familiarize the reader with basic concepts of tissue engineering, its current uses in dentistry and its upcoming implications. It addresses the concentrated literature including a variety of oral tissue derived stem/progenitor cells, biomaterials available for scaffold fabrication, fabrication techniques, use of signaling molecules, strategies adopted for tissue engineering and applications pertinent to the facial region and oral cavity. The focus was on both material-based as well as stem-cell based therapies. As most of the growing models in regenerative dentistry have concentrated outcomes, an in-depth review combining both concepts and implications was required.

Methodology

A comprehensive electronic search was carried out on PubMed, Google Scholar and Science Direct from 2000 to 2020. The following search terms were used incorporating the Boolean operators: "Tissue engineering" or "stem cells" or "regeneration" or "scaffolds" and "dentistry" or "dental tissues" or "maxillofacial" or "oral tissues" or "periodontium". Bibliographies of all relevant articles were also searched for relevant articles.

Articles that have relevance to tissue engineering in dentistry were identified. Main selection criteria were set to include in chronological sequence: review articles, comparative studies and in-vitro studies. Only full-text articles available in English language were selected. Review articles on fundamental components of tissue engineering were selected. These mainly included articles about sources and use of stem cells, potential scaffolds administered in the maxillofacial region and function of growth factors in orofacial region. The idea was to move in a systemic manner from areas of research to potential applications in dental clinics. In vitro studies especially included studies done in Pakistan which included isolation of dental pulp stem cells and scaffolds fabricated for use in the maxillofacial area.[11-14] Finally a total of 50 articles were thoroughly appraised for the narrative review. Figure-1 depicts the entire process of identification and selection of relevant studies.

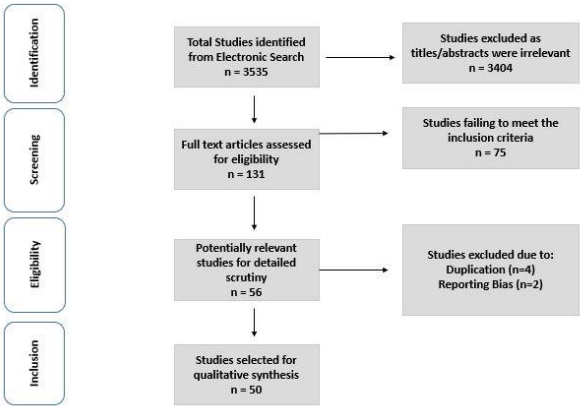

Figure 1: Protocol for selection, screening and inclusion of studies for review.

Fundamental constituents of tissue engineering

The main constituents of tissue engineering triad are given in Figure-2.

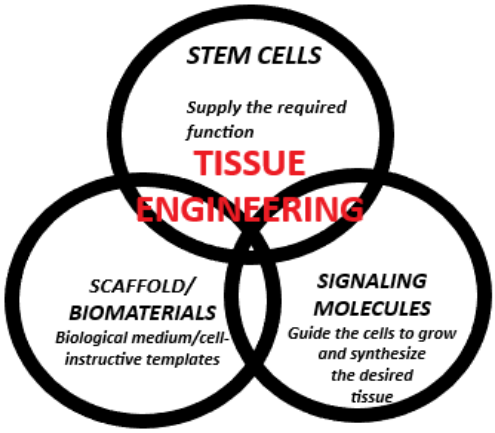

Figure 2: Fundamentals of tissue engineering.

1. Stem cells

Stem cells are immature cells which have the capability to develop into various progenies.[15] These cells are liable for normal tissue regeneration and replacement of damaged or diseased tissue after injuries as they have ability to migrate and self-replicate. Stem cells can be divided on the basis of source as *Embryonic stem cells*(ES) and *Adult stem cells*(ASC).[16]

Embryonic stem cells (ES)

These are *pluripotent* cells derived from the undifferentiated, inner mass of the blastocyst of embryo development.[15] These cells express surface proteoglycan and enzymes for alkaline phosphatase activity.[17] These cells are *allogenic* and are susceptible to immunogenic rejection, which is a potential disadvantage.[16] To overcome this problem of immunogenic incompatibility, HLA (Human leukocyte antigen) matching has been done and patient specific embryonic stem cells are being developed by nuclear transplantation from host's own cells.[15]

Adult Stem Cells (ASC)

These *multipotent* cells are also termed as *somatic* or *post-natal* stem cells. Such cells reside in the mesenchymal tissues.These cells can be isolated from the bone marrow and from the dental tissues[15]. Isolating these cells from tissues is difficult but these cells have reduced probability to cause rejection because the source of the cells is the host.[16] Types and sources of adult stem cells are elaborated in Table-1.

Recently, the reprogrammed or *Induced Pluripotent stem cells (iPSCs)* have been created artificially by genetic manipulation of adult stem cells.[15] Non-viral delivery methods are being considered to reduce the risk of cancer development.[16]

2. Scaffolds

Scaffolds are provisional substructures which can furnish a three-dimensional microenvironment in which cells can proliferate, differentiate and generate the required tissue.

Table 1: Classification of stem cells.

Cells	Sources	Added Advantage
1. Bone marrow derived stem cells (BMSCs)	Iliac crest (mesoderm derivative) Orofacial tissues such as maxillary and mandibular bone (neural crest cell derivate) aspirates.[15]	High replicative capacity Have a higher osteogenic potential in vivo and a lower adipogenic potential but their collectable volume is less than that of iliac crest derivatives.[15]
2. Dental-tissue derived stem cells: (a). Dental Pulp Stem cells (DPSC)	Extracted wisdom teeth, crown-fractured teeth, supernumerary teeth and inflamed pulp.[46]	Easily available.[46]
(b). Stem cells from Human Exfoliated deciduous teeth (SHED)	Exfoliated primary teeth. [15]	Inducing the development of a bone-like matrix where root resorption is followed by simultaneous bone generationaround the root.[15]
(c). Dental Follicle stem cells (DFSCs), Dental Follicular Precursor stem cells(DFPSCs) and Tooth germ progenitor cells (TGPCs)	Mesenchyme of the third molar tooth germ at the late bell stage during clinically discarded third molar extractions. [47]	The capability to differentiate into neural tissues, can be stored frozen for long periods.[47]
(d) Stem cells from the apical papilla (SCAP)	Apical parts of the roots of the developing tooth.[15]	Potential to regenerate periodontal tissues including cementum and alveolar bone.[15]
(e) Periodontal ligament derived stem cells (PDLSCs)	Extracted teeth.[15]	
3. Oral-Mucosa derived stem cells (OMSCs)	Oral epithelial progenitor stem cells which are unipotent and can only differentiate into epithelium. Human gingiva-derived mesenchymal stem cells (GMSCs) have also been isolated from the lamina propria of the gingiva. [48]	These cells possess the capability of differentiating in vitro into derivations of the three germ layers.[48]
4. Periosteum-derived stem cells (PDLSCs)	Periosteum are capable of differentiating into osteoblasts, adipocytes and chondrocytes.[15]	Cultured periosteum derived cells can be used for sinus floor augmentation.[49]
5. Salivary gland derived stem cells (SGSCs):	The cell culture of dissociated tissue. [50]	A single cell which can differentiate into all epithelial cell types within the gland has not yet been identified.[50]
6. Adipose tissue derived stem cells (ASCs):	Lipoaspirates such as from the chin or thighs.[15]	Copious source of Mesenchymal stem cells (MSCs) and these have a high osteogenic potential.[15]

Table 2: Materials used for scaffold fabrication.

Materials	Advantages	Disadvantages
(a) Natural/Biological Materials	Include Collagen (a fibrous protein), lyophilized bone and Coral (based on calcium carbonate). Collagen-glycosaminoglycan (GAG) can also be fabricated into scaffolds. Denatured collagen (gelatin) can also be poured into porous frameworks. [21]	High potential for pathogen transmission, immunogenic reactions and less controlled biodegradability.[21]
(b) Ceramic/Glass materials	Include inorganic materials such as calcium phosphate, Hydroxyapatite (HAP) and Tricalcium phosphate (TCP). These materials are desired by virtue of osteoconductive and osteoinductive properties. Chemical bonding with hard and soft tissue is one of the major advantages.[16]	Lengthy degradation times and devoid of inherent porosity.[21]
(c) Polymeric materials	Include PGA (polyglycolic acid), PLA (polylactic acid) and their copolymers such as PLGA (polylactic- co-glycolic acid). Materials can simply be seeded into a mesh or be extruded as fibers which can be stacked up in an anatomically designed mold. These are metabolized in vivo and the acid degradation products are eliminated from body. [16] Slower rate is suitable for controlled-release applications.[21]	Survival time is hard to manage in the body as the set end to become stifferwith degradation.[16]

Scaffolds should be biocompatible, non-toxic and non-immunogenic. These allows the transportation of necessary growth factors and permit the inflow of oxygen to maintaining metabolic activities. It should allow development of a new fractal circulatory system.[18] Scaffolds should possess sufficient mechanical resistance to withstand in vivo stresses. Linear elastic scaffolds are chosen when bone regeneration is desired and non-linear viscoelastic scaffolds are usually considered for soft tissue regeneration.[19] Sufficiently porosity to allow permeability of growth factors is desired. An optimum pore size ranging from 50-400nm is suggested.[20] Lastly, scaffolds should be biodegradable.[20] Materials used for scaffold fabrication are elaborated in Table-2.

Fabrication techniques for scaffolds

These include *Textile technologies* for example PGA and PLA are processed into fibers using textile-industrial methods. Another technique is called *Particulate leaching*.[21] In this, salt is milled into small particles and shifted into a mold of desired shape. A polymer solution is then poured into the mold. After evaporation, salts crystals are leached away, and water is used up forming pores of the scaffolds. *Phase-separation techniques* consists of lowering the temperature to induce crystallization. Polymers separate into a polymer-rich phase and a polymer-lean phase.[21]

Newer scaffolds

Nanofibrous, biomimetic constructs can be fabricated by two major techniques namely, *Electrospinning*, and *Thermally Induced Phase Separation (TIPS).*

Novel advancements have been explored in bone-tissue engineering using these scaffolds. Hybrid scaffolds comprising of natural polymers such as chitosan and synthetic polymers such as PCL(poly caprolactone) have been fabricated.[22]

Composite scaffolds

These incorporate the mechanical strength and osteoconductive properties of inorganic bone like materials such as *calcium phosphate* and *hydroxyapatite* along with processing capabilities of organic polymers.[23]

Hydrogels

Hydrogels are cross-linked hydrophilic polymers which carry large amounts of water without dissolution. *Synthetic hydrogels* include PEG (Polyethylene glycol) and polymer networks consisting of acrylic acid and acrylamide. Collagen and fibrin hydrogels are available too.[21]

Injectable hydrogels are an attractive option for clinical practice as these have the ability to pack asymmetrical defects coupled with good handling characteristics.[24] *Computer-aided Tissue Engineering (CATE)* is one of the latest areas of research where significant progress is expected for

creating innovating scaffold designs and optimization of those designs.[8]

3. Signaling molecules

Cell signaling refers to a domain of complex system of communication which controls cell activities and assembles all interactivities in a biochemical environment.[19] These are growth factors (GF) and cytokines. The fate of cell is influenced mainly by these signaling molecules.[25]

Controlled delivery of signaling molecules is desirable. They should not degrade before reaching the required site and must remain there for sufficient time to exert their effects. Combined and sequential, spatiotemporal is better than spontaneous delivery as it incorporates different release strategies, similar to that which occurs in natural microenvironment.[26] Some of the methods of delivery include

Encapsulated form: Most proteins, if administered in rawform, without any protection are highly susceptible to degradation and will be rapidly eliminated. To overcome this, localized encapsulated forms are developed.[26]

Carrier systems: Most growth factors are susceptible to harsh proproteolytic environments. Various morphological carrier systems such as micelles, vesicles or tubes using biodegradable synthetic polymers are available. Various methods include solvent casting, soaking in solutions or injection molding. These carrier systems serve as artificial ECM (extracellular matrix) and minimize the release of signaling molecules to non-target sites.[25]

Platelet rich plasma (PRP): Use of PRP as a reservoir of growth factors and matrix elements is a relatively new option.[26]

Hydrogels: This is one of the most convenient methods for delivery.[19]

Important Signaling molecules are illustrated in Figure-3.

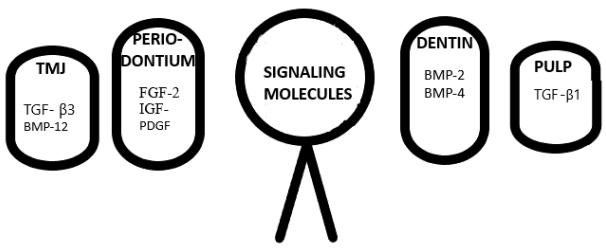

Figure 3: Signaling molecules.

In orofacial reconstruction, the most frequently used growth factors include BMP(Bone morphogenic protein), specifically BMP-2, BMP-4 and BMP-7, and Transforming growth factor specially TGF-β1.[7] Others includes FGF (fibroblast growth factor), IGF (Insulin-like growth factor) and PDGF (Platelet-derived growth factor).[27]

Strategies for tissue engineering

Various strategies have been advocated for developing new tissues. This includes seeding of cells, in which undifferentiated cells (usually not from the host) are inserted directly into the area of injury.[16] Similarly cell induction may be carried out. Growth and differentiation factors are injected within the selected site. Nearby cells are thereby induced to differentiate. Thirdly, preformed cells in a scaffold may be seeded with cells from a patient. Growing cells on multi-dimensional substructures for eventual implantation afterwards is demanding.[16,28]

Current applications of tissue engineering in dentistry

Tissue loss due to trauma, disease or congenital malformation presents a crucial health issue.[9] The focus of tissue engineering in dentistry is the regeneration of missing oral and maxillofacial tissues. Regenerative therapies can be broadly categorized as *material-based* and *stem-cell based*.[29]

Material-based therapies utilize scaffolds and growth factors. Many treatments based on these protocols is being practiced in dentistry. *Stem-cell based therapies,* on the other hand, incorporate progenitor cells and cell sheets. These are rationally difficult to enact in general dental practice due to technique sensitivity, high cost and labor.[29,30]

Periodontal ligament fabrication using principles of tissue engineering

Chronic periodontitis is a quotidian condition and it causes destruction of tooth supporting connective tissues (PDL, root cementum and alveolar bone). *GTR/GBR (Guided Tissue Regeneration and Guided Bone Regeneration)* are the routinely practiced procedures.[9] This approach entails positioning of a membrane beneath the mucosa and overlying the remaining bone. It is commonly used in periodontal treatment.[31] GTR/GBR periodontal membranes are now being developed by combining natural and synthetic polymers using methods such as film casting, dynamic filtration and electrospinning.[32]

Resorbable membranes include Collagen membranes and PLGA (poly lactic co-glycolic acid).Non-resorbable membranes include expanded

Polytetrafluoroethylene (e-PTFE) and titanium membranes. Usually such barrier membranes are bioinert. Osteoconductive bioactive materials such as Calcium phosphate (CaP) are usually added to induce bone formation and facilitate direct attachment with bone. These materials are osteoconductive but not osteoinductive as these do not stimulate the development of *de novo*bone in non-bonyareas.[29] Another approach is *Endogenous Restorative Technology (*ERT) which depends on endogenous resources, such as cells and growth factors for tissue regeneration.[29] Two strategies are currently being employed in this regard. The first strategy garners actual PDL cells from the host, which are cultured in lab. These are then placed as a monolayer sheet and adapted in situ on the tooth surface to repair periodontal defect. In the second option, the cells are injected onto a multi-dimensional framework which is grown in vitro and eventually inserted into the periodontal defect.[16]

In a randomized controlled study of twenty patients, injecting autogenic fibroblasts was reported to be successful in replacing the damaged interdental papillae.[33] The use of platelet rich plasma (PRP) is another method to achieve periodontal regeneration. According to reports, PRP was effective in the treating periodontal infra-bony defects but this cannot be compared to GTR. An appropriate amount of PRP is essential around implanted or re-implanted teeth.[9] A commercially available EMD (Enamel matrix derivate) is also being used for periodontal regeneration. EMD comprises mainly of amelogenin. Various studies have proposed that EMD facilitates growth of periodontal fibroblasts and impedes epithelial cell proliferation.[29]

Alveolar bone augmentation

Vertical and horizontal bone loss results in the reduced height of alveolar ridge. This limits the prosthodontic implications. Material-based strategies for alveolar bone regeneration include graft materials with recombinant growth factors. Autologous cancellous bone grafts are usually considered for large defects. The clinical consequences of material-based strategies indicate that partial infra-bony or furcation defects can be managed conveniently utilizing traditional methods. However, it is clinically observable that bone augmentation of acutely atrophic alveolar ridges especially in the case of sinus lifting procedures, cannot be achieved, using the traditional methods alone.[34] Bone graft materials are not osteo-inductive. As a result of immune response by the activated osteoclasts, unavoidable bone resorption occurs. In these cases, stem-cell based therapies need to be considered. There are two approaches to these therapies.

The first one employs the use of BMSCs aspirated from iliac crest which are later expanded in vitro. The second approach utilizes patient-derived cellular grafts developed at chair-side or use commercially fabricated allografts which contain BMSCs derived from cadavers. A report of stem-cell based alveolar bone renewal was cited in literature regarding a twenty-year old patient. The patient reported with a missing maxillary central incisor due to a road-traffic accident. BMSCs from iliac crest, in a HA (Hydroxy apatite) scaffold were injected in the defect side.[35]

Tissue engineering and Implant Dentistry

Osteogenic stem cells in implant osteotomy sites can yield the essential environment for better bone formation, thereby increasing the long term success of implants.[29] An interesting method to increase the osteointegration of titanium implants has been proposed. The endosseous implants are coated with extra-cellular matrix components such as collagen, BMP and chondroitin sulphate.[19]

Use of tissue-engineered oral mucosa

It includes collagen products and silk fibroins. It follows the same protocol as that of tissue-engineered skin, which includes development of epithelial oral sheets. This is done by seeding keratinocytes on decellularized dermis of human cadaver.[9]

Craniofacial bone tissue engineering, including temporomandibular joint (TMJ)

Two major methods for these are *Distraction Osteogenesis* (which facilitates bone formation through the eventual separation of osteogenic fronts) and *Cell-based therapies*.[36] It is important to know that cartilage has limited ability to regenerate invivo. Chondrocytes from numerous locations in the body can be obtained, cultured in vitro and implanted.[37]

Trauma or arthritis can cause damage to the articular disc or condyle, which causes severe pain and discomfort to the patient. Human-shaped mandibular condyle has been successfully engineered in a goat model using rat BMSCs encapsulated in a matrix.[38]

Research on tissue engineering in dentistry in Pakistan

Research focusing on tissue engineering in the domain of dentistry in Pakistan is in its initial stages and rather slow. However, attempts have been made to restore orofacial function and

esthetics using bioengineered products. Major work done in this regard is hereby quoted.

Successful rehabilitation of oral function using dental implants necessitates the presence of optimum alveolar bone volume. With the loss of teeth, alveolar bone starts resorbing and may require repair and augmentation. Zeeshan et al. successfully synthesized "Hydroxy-propyl-methyl cellulose (HPMC) crosslinked chitosan (CH) based scaffolds containing bioactive glass (BG) and zinc oxide (ZnO)" which may be used for repair of residual alveolar ridge.[12]

One of the major diseases plaguing the developing countries and increasing the disease burden of edentulism is periodontitis. To counter the loss of periodontium, Shah et al.[13] have synthesized "tri-layered functionally graded membrane" that can be implanted in vivo and aid in regeneration of periodontium. However, clinical reports are scarce.[39,40]

If conventional ways are used, drug administered to patients may not be optimally effective owing to decreases blood supply to hard tissues. To optimize the amount of drug reaching the affected site, "implanted drug delivery systems based on biomaterials" have also been generated.[14]

Recently, researchers at Aga Khan University, Karachi attempted to culture dental stem cells. They collected teeth, both deciduous and permanent, from 13 human subjects, extirpated their dental pulps and cultured them successfully via "explant method in a stem cell defined media".[11]

Future Prospects of Tissue Engineering in Dentistry

Restorative Dentistry and Regenerative Endodontics

Enamel remineralization or regeneration has already been explored by administration of inorganic polymers and amorphous proteins in nanostructured crystalline forms. [28,41] DPSCs and SHED can be used to develop tissues similar to dentin-pulp complexin an empty human root canal accompanied by the addition of mineralized tissue onwalls of canal.[40] These cells have also been transplanted immersed in scaffolds in immunocompromised mice and after a period of six weeks, a collagenous matrix was deposited.[19] In the future, hydrogel scaffolds may be potentially poured into the canal and self-polymerize eventually.[24]

Salivary Gland and Tongue Regeneration

In conditions like Sjogren Syndrome and radiotherapy for head and neck tumors, the loss of salivary gland parenchyma and the inability to produce saliva ,markedly affects the quality of life of the individual.[42] Currently the modality is only limited to the use of salivary substitutes and sialagogues.[9] Regenerative strategies includes creating an artificial salivary gland or the application of stem cells to damaged salivary gland tissues. Surgical resection may result in loss of tongue tissue. Stem cell based tongue tissue reconstruction has been cited in a rat model.[29]

Tooth regeneration

Tooth regeneration is an adjunct of the current developments in tissue engineering.[43] The ultimate goal is to develop fully functional bioengineered teeth.[44] Initial experiments in rats have led to the development of teeth which are about 2mm wide. A porous scaffold mimicking the appearance of human tooth was fabricated and was injected with individual cells from a tooth bud. This assembly was inserted in the omentum of a mouse.[16] Post-natal dental stem cells are currently being used in animal models but research suggests use of iPSCs for achieving this goal effectively.[45] Regeneration of entire individual teeth remains a challenging goal.[9]

Currently stem cell transplantation in Pakistan has been established for treatment diseases such thalassemia and aplastic anemia. Furthermore, nowadays, stem cell therapy is being used by orthopedic surgeons to inject stem cells in affected joints of osteoarthritis patients.[10] National Bioethics Committee which was established at Pakistan Health Research Council (PHRC) allocates protocols for stem cell research and regulation in Pakistan.

However, application of stem cells and scaffolds in dentistry remains an emerging field. There is a still a long path while translating it from laboratory to the dental clinic. In-vitro approaches pertaining to most oral tissues have been attempted with promising outcomes. Current research is mainly focused on harnessing stem cell technologies to develop regionally relevant induced pluripotent stem cell lines for use in orofacial region. This can ultimately lead to development of economically viable therapies and contribute towards improved oral health. Challenges in the growth of clinically safe and satisfactory methods for repair of oral tissues are being met effectively despite limited progress. Key challenges in this field include complexity of oral tissues, lack of remodeling ability in enamel and dentin, ethical concerns and cost-effectiveness of procedures.

Conflict of interest: None declared.

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