ejpmr, 2020,7(6), 290-301

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Review Article

ISSN 2394-3211

EJPMR

A CLINICAL REVIEW ON REVOLT OF TISSUE ENGINEERING IN THE MAXILLOFACIAL PRACTICE

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Article Received on 03/04/2020 **Article Revised on 24/04/2020** **Article Accepted on 14/05/2020**

ABSTRACT

Tissue engineering is an enchanting way of producing living tissues for patients who have lost an important tissue or part of it. It forms an excellent and engrossing alternative for maxillofacial reconstruction in near future. The aim of tissue engineering is to regenerate new tissues within a specific environment with help of cells, scaffold, and the signaling molecules(growth factors). Even though MSCs along with bone grafts and BMPs like growth factors forms the ideal triad of TE, various sources were available for these triads. Recombinant protein therapy, cell based therapy and gene therapy are the different approaches used in tissue engineering. A lot of options like vascularized and non vascularized flaps are available for maxillofacial reconstruction. But its shortcomings enables the tissue engineered constructs to be used as a good alternative for reconstruction with improved functional and therapeutic outcome. Today, the field of tissue engineering has expanded tremendously. New technologies like recombinant gene transfer, bioreactors, rapid prototyping etc were on the way of development in the field of tissue engineering, which may help to find out a final solution for the challenges faced during the process of tissue regeneration.

KEYWORDS: Tissue engineering; Maxillofacial reconstruction; Mesenchymal stem cells; Platelet rich plasma; Gene therapy; Bone morphogenic proteins; Bioreactors.

1. INTRODUCTION

The oral and maxillofacial region is a highly complex area with morphologically intricate skeletal elements, special sensory organs, lining and covering tissue (i.e., skin and subcutaneous fat), a rich neural and vascular network, mouth, teeth and surrounding structures, bounded superiorly by the cranial base and inferiorly by the lower jaw. The complicated interplay of embryogenic events of the craniofacial tissues increases the prevalence of developmental abnormalities and its prominent position makes it vulnerable to traumatic injuries. Regular exposure to various disease-inducing agents including UV rays, thermal insults, and carcinogens (e.g., tobacco products) paved way to various pathological lesions like malignant neoplasms. Traumatic, developmental or pathological loss of tissue integrity and continuity gave the maxillofacial surgeons a great challenge for repair and reconstruction.

Oral and maxillofacial regions are susceptible to variable environment including the mouth, sinuses, nasal passages and external environment, which may characterized by high moisture content, significant aerobic and anaerobic bacterial populations, and functional loads imposed by physiological activities like mastication. The mandible and TMJs may get exposed to compressive, shear and tensile loads depending on the type and degree of function. Tissue engineered constructs poses a great challenge to survive under these conditions, which requires the modifications to account for these conditions. The creation of composite defects in maxillofacial regions, requiring reconstruction of multiple tissue types is a special challenge to engineer composite tissues as well as to attach the various constructs to each other in their normal anatomical relationship. Facial symmetry is the other important consideration in oral and maxillofacial reconstruction. As most of the structures in the maxillofacial region including orbits, zygomas, maxilla and mandible are paired, the accurate reproduction of its form is an important aspect to preserve facial esthetics.

Within the past decade, an emerging field of "tissue engineering" or "regenerative medicine" offers an exciting alternative for maxillofacial reconstruction. Tissue engineering is a biological cell-based therapeutic approach involving regenerative cells, inducing factors and scaffolds. It is a relatively emerging therapeutic field to overcome conventional reparative treatments. The aim of this review article is to understand the history, current status, future perspectives and clinical applications of tissue engineering in the craniomaxillofacial regions.

2. Historical Perspective

The term "tissue engineering" was introduced in medicine in 1987. "Tissue engineering" was defined for the first time by the attendees of the first NSF (National Science Foundation) sponsored meeting in 1988 as "application of the principles and methods of engineering and life sciences toward fundamental understanding of structural and functional relationship in normal and pathological mammalian tissues and the development of biological substitutes for the repair or regeneration of tissue or organ function."

Later in 1993, Dr. Joseph Vacanti and Dr. Robert Langer defined tissue engineering as "an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain or improve tissue or organ function." In their article, they describes the early developments of this new technology and can be referenced as the beginning of this new biomedical discipline.[1,2]

James Alexander Thomson, an American developmental biologist derived the first human embryonic stem cell line in 1998 and human induced pluripotent stem cells in 2007. Human embryonic stem cells have the ability to divide unlimitedly while maintaining the potential to differentiate into different types of body cells.[3,4]

3. Principles Of Tissue Engineering

The aim of tissue engineering is to regenerate new tissues within a specific environment with help of cells, scaffold, and the signaling molecules(growth factors). It is a complex and highly orchestrated process along a uniform pathway of inflammation, proliferation, and remodeling. In this process osteogenesis is initiated by osteoblasts, which are mostly differentiated forms of stem cells provided by graft material. Embryonic stem cells, fetal cells, amniotic fluid cells, umbilical cord cells, bone marrow haematopoietic stem cells, mesenchymal stem cells, adipose tissue- derived cells and dental-derived stem cells are the different forms of stem cells that can be used in tissue engineering. Bone marrow stem cells (BMSCs) have been used more frequently.

The presence of growth factors like bone morphogenetic proteins (BMPs) helps to differentiate local or grafted stem cells. They can increase the rate of bone regeneration and helps in bone morphogenesis. Extensive variety of scaffolds were used in tissue engineering to transfer stem cells and growth factors to the defective sites. They also assist in proliferation, differentiation and biosynthesis of cells similar to the natural extracellular matrix.[5]

During this process biological signals increases the cell number and morphogenic signals which induce the tissue specific differentiation to fill the defect. Most of the biological signals can promote proliferation and induce differentiation of cells that are conveyed by polypeptide growth factors, which are supplied by local or circulating cells and blood components such as macrophages and platelets. Growth factors also originate from the extracellular matrix (ECM) where they are stored and released during tissue remodeling and repair. Extracellular matrix also serves as a three dimensional scaffold for the migration and arrangement of cells in a tissue-specific manner. When these three components are transferred to the in vitro environment of tissue-engineered constructs, the extracellular matrix is replaced by synthetic or natural scaffolds to accommodate and arrange the cells in a three-dimensional fashion. Thus the cells, signals, and scaffolds makes up the "classic triad" of tissue engineering.[2] As blood supply is essential for cell survival and development and the blood vessels acts as a reservoir for undifferentiated perivascular cells, angiogenesis and vascularization play a vital role in cellular behavior and tissue repair.

4. Components of Tissue Engineering

4a. Cells

The strategy of tissue engineering is based on a complex cell-based approaches by obtaining required quantity of cells and expanding to a volume required to form the desired amount and type of tissue. These approaches are based on variable success factors including biological quality of host tissue and scaffolding materials and handling during cultivation, to provide the biological signals and environmental factors that are necessary to revascularize the implanted constructs.

i. Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are immature undifferentiated cells obtained from bone marrow/ fat tissue/ periosteum. In vivo, these stem cells have a high proliferative potential, but not yet terminally differentiated. In contrast to embryonic stem cells, which can differentiate into a large range of different tissues, adult stem cells can differentiate into bone, cartilage, or fat cells, but are limited to tissues of the connective tissue lineage. Thus embryonic stem cells are considered to be pluripotent whereas adult stem cells are multipotent.

ESCs (Embryonic stem cells)

They are pluripotent stem cells with high proliferative potential and can differentiate into a large range of different tissues without much risk for dedifferentiation. They are isolated from the inner wall of the preimplantation blastocyst. Even though they are highly proliferative and pluripotent, their uncertainty, uncontrolled differentiation, tumourigenity, ethical issues and immune rejection make them more challenging.[5]

ASCs (Adult stem cells)

They are obtained from the fully differentiated or adult tissues such as bone marrow, periosteum, muscle, fat, brain, dental pulp, and skin. Adult stem cells derived

from bone marrow are called multipotent mesenchymal stroma cells (MSCs) and those from fat tissue are called adipose tissue-derived stem cells (ADSCs). Stem cells from bone marrow aspirates available as niches, from where stem cells are isolated as mononuclear cells by density gradient centrifugation and are separated from hematopoetic stem cells by their ability to adhere to the plastic bottom of the culture dishes. Before attaining growth, expansion should be done by passaging the cells to make it subconfluent and replating them at low density to form separate colonies of fibroblastoid cells, which is a measure for the proliferative potential of the cell population. After sufficient expansion, terminal differentiation is induced by osteogenic (ascorbic acid, dexamethasone, glycerophosphate) or chondrogenic supplements (ascorbic acid, dexamethasone, proline, pyruvate) in the culture media, depending on the tissue to be regenerated.[6,7,8]

Fat tissue for stem cell isolation are derived by liposuction. The aspirate undergoes washing and subsequent digestion using collagenase treatment. After centrifugation and resuspension of the resulting pellets, the cells are plated at low density of 100 cells/cm2. Epithelial stem cells are more difficult to isolate and expand.

ii. Induced Pluripotent stem cells (iPSC's)

They are mature skin cells which get transformed into pluripotent cells by inserting 4 genes. (Oct3/4, Klf4, Sox2, and c-Myc) into the cell nucleus. Even though they are still under trial, it will offer a great promise for craniofacial regeneration.[9,10,11]

iii. Recombinant cells

Recombinant technology is an emerging idea in the field of regenerative medicine to improve the functionality of the implanted devices and the therapeutic outcome. It allows to use gene transfer to the seeded cells which allows overexpression of the required growth factors making them less dependent on host tissue. Recombinant growth factors are similar to human growth factors from the view of amino acid sequences and 3D structure. They are produced by eukaryotic cells or E coli bacteria by transfecting with the respective gene.

iv. Differentiated Osteoblasts

These cells are committed mesenchymal cells directing towards the osteogenic lineage. As they are already differentiated, they can achieve the repair of the defect rapidly. They can also drive the process of repair locally before the recruitment of host cells. The main limitation of this type cells is the limited proliferative capacity.

4b. SCAFFOLD

The ultimate goal of tissue engineering is to create a three-dimensional(3D) biocompatible support which can be inserted into a tissue to repair a defect by allowing cell adhesion, proliferation and differentiation without triggering any inflammatory responses or rejection from the body. This can be achieved by selecting a suitable scaffold. Scaffolds act as a biological platform to facilitate repair and restoration of physiological/ histological features of defective tissues during the healing process. Its architecture will define the ultimate shape of the new bone and cartilage.

Characteristics of an ideal bone scaffold

- Should be osteogenic, osteoconductive and osteoinductive.
- Should be three dimensional.
- Sufficiently porous with an interconnected pore network for cell growth and to transport nutrients and metabolic waste.
- Mechanical stability to resist external forces, cell contraction forces in healing process
- Should have sufficient compressive strength not exceeding that of the surrounding bone, that may cause bone resorption.
- Mechanical properties should match the tissues at implantation sites.
- Biocompatible and bioresorbable with controllable resorption rate.
- Rate of degradation should match rate of regeneration.
- Suitable surface chemistry with adequate porosity for cell attachment, proliferation, and differentiation.
- Reproducible
- Sterilizable

Ideally the “living”bone can be considered as the only real osteogenic scaffold due to the unique characteristic of osteoblast to generate bone tissue. Even though several scaffold materials like hydroxyapatite (HA), poly hydroxyl esters and natural polymers such as collagen and chitin are under investigations, commonly used materials includes bone grafts, matrices, ceramics, polymeric materials, composite scaffolds and recently advanced nanoscaffolds.

i. Bone grafts

Autologous bone graft is considered as the gold standard among all bone grafts. In autologous bone grafting, the donor’s own bone is taken from a healthy part of the donor’s body minimizing rejection issues.

A cortical bone graft provides good structural support and reduced resorption. Due to its high density, revascularization of the newly formed tissue is slow and difficult resulting in engraftment delay. In contrast, a cancellous bone graft ensures early revascularization, resulting in faster engraftment, lesser risk of infection, and a shorter time for implant placement.

The allografts are derived from donors of the same species (usually from bone banks) but lose many of their characteristics during the long and expensive process of sterilization and decellularization. Although these processes are necessary to minimize rejection or disease

transmission, they deprive the bone of its osteogenic properties, making it a simple, empty scaffold. These grafts can be further classified as freeze-dried bone allograft (FDBA) and demineralized freeze-dried bone allograft (DFDBA). Due to their high manufacturing costs and increased rate of resorption their use is limited to small and medium defects.

Xenografts are cheaper alternative to allografts but undergo similar sterilization procedures. They are animal derived grafts, mainly obtained from cattle, pigs and horses. They provide support and survival similar to those of autologous bone grafts without the osteogenic properties. The deproteinized bovine bone in sterilized granules or blocks is commonly used xenograft in bone regeneration. Several alloplastic grafts, such as β-tricalciumphosphate (β-TCP), bioceramics, and hydroxyapatite (HA), have also been used for bone regeneration.[12]

ii. Synthetic ceramic scaffolds

Synthetic ceramic scaffolds including hydroxyapatite (HA), tricalcium phosphate (TCP), HA-TCP, beta-tricalcium phosphate / bioactive glass (β-TCP/BG), β-TCP with Mg (β-TCMP), Calcium phosphate cement (CPC) have been used in cranial, calvarial, or subcutaneous defects. They are available in the size range of 5mm to 12cm. As per the sudies the defect closure with synthetic ceramic scaffolds was achieved in one to 10 weeks after the surgery.[13]

iii. Natural ceramic scaffolds

The studies showed that natural ceramic scaffolds has been used in defects of size ranging from 1.5 to 3 cm and the results can be attained by 1-4 month after surgery. Human demineralized cancellous bone, human autoclaved cancellous bone, demineralized bone matrix are commonly used natural ceramic scaffolds.

iv. Non-ceramic and polymeric scaffolds

Scaffolds including polycaprolactone (PCL), PCL/collagen (PCL/Col), polyurethane, phosphoester-poly (ethylene glycol) (PhosPEG), poly (lactic-co-glycolic) acid (PLGA) and open-cell poly-L-lactic acid (OPLA) have been used in cranial, calvarial, femoral, or subcutaneous defects of size ranging from 4mm - 1.2cm. The defect closure was observed 12 days to 12 weeks after the surgery.[13]

v. Composite scaffolds (polymer+ceramic)

They are used in cranial, femoral, or subcutaneous defects of size 2.5mm to 5mm. PCL/TCP, fibrin-alginate-HA, PLGA-CaP, PLGA-bioactive glass are few examples of composite scaffolds. The defect closure was observed 4 days to 6 months after the surgery.

vi. Metal-based scaffolds

It includes titanium and titanium alloys (Ti6 AL4 V and Ti6 AL4 V with a CaP coating), titania-silica coated Ti fibers and silver. The results found to be attained in 1-12 weeks after surgery. They are applicable in defects of 1.5-4 mm.[13]

vii. Nano-scaffolds

Nano-scaffolds are recently developed biological platform to attain 3D support in tissue engineering. They have been used in recent studies for the closure of 8-25mm defects Nano-sintered, nanocrystalline, phase-pure HA and silica-CaP nanocomposite are few examples which can attain results within 4 to 12 weeks post-surgery.

4c. Growth Factors

Growth factors play an important role in regenerative medicine by promoting the bone regeneration. Growth factors are proteins produced by cells that act as signaling molecules on an appropriate cell to carry out a desired function. In craniofacial reconstruction the primary focus of growth factors was directed towards the repair of mesenchymal tissues and interaction between mesenchymal tissue and epithelium during mucosa repair. GF binds its receptor to initiate intracellular signaling, which regulates the synthesis of proteins and receptors and thereby activating the cellular proliferation, matrix deposition, migration and differentiation of tissues. These molecules are fairly important for tissue formation during embryonic development, by taking part in limb patterning and organ morphogenesis. In postnatal life their role is restricted to tissue regeneration and repair. Therefore any defects in the genes that code for these GF produce various craniofacial skeletal dysostoses like apert syndrome, crouzon syndrome and achondroplasia.

Bone morphogenetic protein is one of the best studied growth factor in regenerative medicine. In 1965, the observations of bone formation from demineralized bone matrix made by Urist paved the way for the development of BMP. BMPs are under the superfamily of TGF-b proteins.[14] They promotes bone regeneration by recruiting mesenchymal stem cells to the healing site and differentiate them into the osteogenic lineage for bone deposition. Although there are many BMPs described, BMP-2, -4, -6 and -7 are the best studied in craniofacial biology with great potential for regeneration.[15]

The major limitation with growth factors is the shortage of naturally derived factors isolated from biological tissue, that has been addressed by developing biologically active proteins using recombinant engineering techniques. Researches and investigation with recombinant human BMP-2 (rhBMP-2) as growth factors for tissue regeneration would be attained popularity in near future. Herford etal reviewed several cases of mandibular reconstruction using BMPs, highlighting its versatility.[16] Jung et al. studied the effect of rhBMP-2 with a xenogeneic bone substitute, which improves membrane-guided bone regeneration therapy of osseous defects in dental implant placement.[17]

The major consideration with recombinant factors is that it should be administered in excess of naturally occurring concentrations which may potentially evoke harmful biological effects, such as malignant transformation of cells. It paves the way in search of an alternative with stable biological effects via development of PRP and gene therapy.

Growth factors used in maxillofacial reconstruction

In most of the studies, six growth factors are commonly used for bone regeneration in animal models. They are also proved the success in bone regeneration while used in maxillofacial reconstruction. It includes Platelet-derived growth factor (PDGF), Basic fibroblast growth factor (bFGF), Insulin-like growth factor (IGF), Transforming growth factor beta (TGFb), Vascular endothelial growth factor (VEGF) and Bone morphogenetic proteins (BMPs).

4d. Platelet Rich Plasma (PRP)

PRP is one of the recently evolved autonomous alternative which works on the same principle of tissue engineering. It promotes wound healing and tissue regeneration due to the increased concentration of growth factors like TGF β, VEGF, PDGF, FGF in the form of platelet concentrates. It was first introduced in maxillofacial practices by Whitman etal in 1997 and makes it popular by Marx et al in 1998.[18,19] According to Marx, the autogenous bone with PRP in mandibular continuity defects results in faster radiographic maturation and histologically denser bone. It also improves soft tissue healing by increasing collagen content, thereby promoting angiogenesis and wound strength.

PRP acts by the degranulation of the alpha granules in platelets which releases the pre packaged growth factors within 10 minutes of clotting process, so that it should be developed in an anticoagulated state and used clinically within 10 minutes of clot initiation. The growth factors bind to transmembrane receptors of the cell membrane of cells in the grafts or flaps. The platelets synthesize and secrete additional growth factors for 7 more days of their life span after the initial release of growth factors by degranulation. Once the platelets gets exhausted and dies off, macrophages get recruited and secretes some more growth factors to regulate wound healing. Therefore the number of platelets in the blood clot within the graft, wound, or adherent to a flap sets the rate of wound healing.[20]

The PRP can be activated by calcium alone or a mixture of 10% CaCl2 and bovine thrombin or autologous whole blood and some autogenous cancellous bone, both containing thrombin.[21,22] PRP can also available in gelatinous form known as PRP gel by activating PRP, which mimics final stages of clotting process.

5. Approaches Of Tissue Regeneration

First, recombinant protein therapy consists of delivering the appropriate growth factor on a scaffold in order to stimulate certain cells in the target site, which reduce or eliminate the need for autogenous bone grafts. But it require higher concentrations of bone morphogenic proteins to achieve the results due to slower osteogenic potential and less availability or response of osteoprogenitor cells.[23,24]

The second approach is cell-based therapy which involves direct contribution of cells to tissue regeneration and differentiation into various tissue types. An interesting feature of cells is “Plasticity”, which refers to the ability of adult stem cells to differentiate into various tissue types according to the gene expressed and the local environment.[25]

The third approach is the gene therapy which is relatively a new therapeutic pattern in medicine where specific genetic information is delivered to the cells directing them to secrete a certain protein product.[26,27] The major considerations of this approach are selection of appropriate gene encoding for the stimulant protein (where the plasmids are usually used as gene delivery vehicles) and gene delivery (either adenoviral vector or a non-viral vector using chemical supplements such as calcium phosphate complexes). Alternatively, lipofection has also been employed in which liposomes that encase the plasmid vectors are used to pass through the cell membrane.

6. Phases Of Tissue Engineering[28,29]

- Fabrication of bioresorbable scaffold
- Seeding of the osteoblasts/chondrocytes populations into the polymeric scaffold in a static culture (petri dish)
- Growth of premature tissue in a dynamic environment (spinner flask)
- Growth of mature tissue in a physiologic environment (bioreactor)
- Surgical transplantation
- Tissue-engineered transplant assimilation /remodeling

7. Current Methods of Oral and Maxillofacial Reconstruction

Various techniques were used for maxillofacial reconstructions since decades ago. Most of the reconstructive techniques used nowadays includes.

1) Soft tissue pedicled flaps
2) Non-vascularized soft and hard tissue grafts
3) Vascularized soft and hard tissue grafts
4) Alloplastic reconstructions with prosthetic appliances

Non vascularized graft establishes a vascular network in a delayed fashion, depending on tissue diffusion, whereas vascularized graft perfused immediately via an existing AV system. Occasionally composite technique was used for reconstruction, where soft and hard tissues

are added in different stages with a time interval in between.

8. Strategies of Tissue Engineering In Oral & Maxillofacial Surgery

There are various strategies of tissue engineering existing in the maxillofacial practices. TE primarily depends upon mechanical and biological properties of scaffolds and its interactions with cells. Even though a lot of options are available for maxillofacial reconstruction, its shortcomings enables the tissue engineered constructs to be used as a good alternative for reconstruction with improved therapeutic outcome. Currently TE is in an evolving stage focusing on regeneration or reconstruction of bony skeleton, lining epithelium, temporomandibular joint, cartilages, auricle, and nose, and the teeth and surrounding periodontal tissue.

Bone applications

A tissue engineered bone construct should combine the biocompatibility and osteoinductive potential of autologous bone, with the availability and structural characteristics of allogeneic bone. Scaffolds should have sufficient mechanical integrity and surface area for cell attachment. Delivery of osteogenic factors or cells can be achieved via in situ crosslinking or poly- merization via chemical reactions initiated by mixing chemicals immediately before injection, transcutaneous photopolymerization or thermogelation.[30,31] Growth factors act as mediators of cellular growth and differentiation during tissue regeneration, which may be present at the wound site or added to the scaffold as an exogenous factor at the time of fabrication.

Attempts to address the shortcomings of exogenous growth factors like high cost and potential for harmful stimulation or disease transmission have spurred the researches and investigations into the use of PRP and gene delivery for tissue engineering, which results in higher and more constant levels of protein production.

Cartilage applications

Cartilage engineering in the craniofacial complex remains a significant challenge due to the inherent lack of remodeling in cartilage due to decreased cellularity and vascularity of cartilages and presence of various cartilaginous tissue types in the craniofacial complex like articulating cartilage in mandibular condyle, TMJ disk, nasal and auricular cartilages.

The structural cartilage in the nose and ears supports the skin to create openings critical for the senses. As per the studies and its reviews regarding the scaffolding materials for cartilage engineering, PGA is an acceptable scaffold material that underwent rapid degradation, leaving constructs with limited mechanical integrity in few weeks. PLA non-woven mesh as a scaffolding material gives a promising results with sufficient tensile and compressive integrity.[32]

Wang et al., in his famous review on cell sources for TMJ engineering suggested that phenotypically different chondrocytes from articular cartilage and TMJ disk require expansion which may results in dedifferentiation and loss of this phenotype where as, stem cells can proliferate and induce into a chondrocyte-like cell with help of growth factors.[33] In a study by Bailey et al. condylar chondrocytes are compared with human umbilical cord cells and demonstrated that umbilical cord cells on PGA scaffolds can proliferate extensively and produce more extracellular matrix with components similar to that of native articulating cartilage.[34] They also reported that costal and ankle chondrocytes can synthesis more collagen and glycosaminoglycans compared to chondrocytes from the TMJ disk. The studies and reviews comparing various growth factors suggested that lower concentrations favor biosynthesis and higher concentrations favor proliferation. It also reported that the most beneficial growth factors appear to be IGF-I and bFGF, both of which produce significant increase in collagen synthesis and cell proliferation.

Metabolic supplements also have impact on cell proliferation and matrix synthesis. Ascorbic acid, L-glutamine, sodium pyruvate and insulin increased cell proliferation, while L-proline at high concentrations decreased matrix production.[35] Initial cell seeding plays a pivot role in tissue-engineering due to cell-to-cell interactions and signaling. PGA scaffolds seeded at saturation increased cellularity and extracellular matrix (ECM) content relative to scaffolds seeded below saturation.[36] As the native TMJ disk undergoes significant compression, tension, and shear, mechanical stimuli may be required to produce an optimal tissue-engineered TMJ construct while cells proliferate and produce ECM in static culture.

Oral mucosa applications

Various scaffold materials (collagen, fibrin, gelatin, poly (lactic-co-glycolic acid (PLGA), and poly- caprolactone), cell sources like keratinocytes and fibroblasts and growth factors like epidermal growth factor have been investigated for oral mucosal engineering in a famous study by Moharamzadehe. Oral mucosal regeneration aims to have a full thickness mucosa and structural components including a basement membrane and extracellular matrix. But cultured epithelial sheets yields inadequate results due to lack of underlying supporting tissue, fragility and contractility. Composite grafts for muco-cutaneous junctions such as the lips can be created by culturing cell sources on AlloDerm in vitro with a separation barrier, which was allowed to migrate into the junction space and interact.[37,38] It was then lifted to air-liquid interface for maturation of the construct.

Composite tissue application

One of the best example of composite tissue in maxillofacial region is TMJ, because of composite composition of articular cartilage and subchondral bone in condyle. It was illustrated in a famous study

performed by Alhadlaq et al., where they were used adult bone marrow MSCs.[39] These stem cells were then expanded, induced and differentiated into osteogenic and chondrogenic lineages. It was encapsulated in poly (ethylene glycol) based hydrogels and crosslinked in a mold to have a stratified organization of osteogenic and chondrogenic layers. Finally, the osteochondral constructs were implanted into the dorsum of immunodeficient mice for up to eight weeks. Histological and immunohistological analysis revealed both structural and immunohistochemical differences between the osteogenic and chondrogenic layers, which served as a primitive proof for composite tissue engineered constructs in the craniofacial region. In a similar study trying to regenerate tissue within an osteochondral defect, a gradient scaffold releasing BMP-2 on the osteogenic side of the scaffold and TGF-b1 on the chondrogenic side was fabricated. This construct showed increased osteogenesis and chondrogenesis on the respective sides.[40]

9. CLINICAL APPLICATIONS

Craniofacial and dental tissue regeneration

Regeneration of craniofacial and dental tissues can be possible via three major approaches of tissue regeneration namely; recombinant protein therapy, cell-based therapy and gene therapy, which improves osteogenesis, soft and hard tissue healing and wound strength.

Skin Graft Donor Sites

The revascularization of donor sites can be enhanced by the angiogenic activity of PDGF and TGFß.[19,41] Tissue engineered stem cells and PRP promotes the healing efficacy of skin graft donor sites especially in split thickness skin grafts.

Sinus Lift Grafts

In sinus lift procedures, PRP gel and various growth factors improves the osteogenesis of graft as well as its revascularization.[42]

Ridge Augmentation Graft

The osteogenic and angiogenic capacity of PRP, stem cells and growth factors enables it to incorporate into and on the surface of the grafts in the cases of vertical and horizontal ridge augmentation procedures. The studies demonstrated that it is advantageous for cortical-cancellous block as well as cancellous marrow graft.[42]

Continuity Defects in the Jaws[19,43,44]

Mandibular bone reconstruction remains a significant challenge in the present world. Even though various reconstructive options are available, restoration of continuity, sensation, dentition, soft tissue, function, and aesthetics, is still not achievable. Ideally mandibular reconstruction should restore the anatomical height, contour of the missing part and oral function. Until now, autogenous bone transplantation—especially free vascularized tissue graft is still remains as a ‘‘gold standard of care’’ for mandibular reconstruction. The major problem with autogenous grafts is donor site morbidity. Hence to overcome these limitations and restore function and esthetics, surgeons as well as researchers are in search of a better alternative. This paves the way for tissue engineering in mandibular reconstruction. In TE, cells allows osteogenesis, scaffold for osteoconduction and growth factors for osteoinduction.

PRP also used by incorporating into grafts to promote stable and constant bone regeneration in continuity defects.[19,45] The PRP should be developed prior to fluid infusion which will dilute blood components and significant tissue wounding which will sequester platelets in the wound. PRP can be incorporated into grafts by layering technique, in which small amounts of PRP gel are added to the graft as it is placed and some on the graft surface. About 20 cc's to 35 cc's of PRP are used depending on the size of the graft. It can also applied in gel form which can be made into a bio-resorbable membrane lasts for approximately 5-7 days. This membrane consists of fibrin with platelets enmeshed in it. It can be used over sinus membrane perforations, sinus lift windows and dental implant fixtures.

Periodontal surgery

Periodontal ligament stem cells (PDLSC) from extracted teeth were cultured, expanded and differentiated in vitro to regenerate the periodontal ligament.[46,47] Recently gene transfer approaches have also been used to deliver additional growth factors like platelet derived growth factor and BMP-7, which improves osseous repair by promoting bone regeneration.

Gene therapy for repair of salivary gland function

Nowadays various studies and researches on gene therapy using an adenoviral vector has been directed to salivary gland tissue regeneration, which increase the salivary flow and thereby improves the quality of life in cases of xerostomia, sjogren’s syndrome, post radiation conditions and aging.[48,49]

Extraction sockets

The studies regarding use of tissue engineered membranes and PRP treated sockets showed reduced postoperative swelling, better hemostasis and flap healing, thereby decreases the incidence of dry sockets.[50]

Alveolar Bone Grafting

Tissue engineering provides higher volume ratio of regenerated bone in cases of alveolar cleft, which provides adequate quantity of bone in minimum time and early wound strength. PRP can also be used with bone grafts to promote bone regeneration.

Implant surgeries

Tissue engineered bone constructs promotes early bone healing and strength via osseointegration in the cases of implant placements.[51] Autogenous growth factors in the PRP gel also contributes the positive outcome, which was illustrated in various studies available till date.

Distraction osteogenesis

Tissue engineered constructs and mixtures of autonomous bone graft with PRP helps to fill the distraction gap and restore severe atrophic mandible during distraction osteogenesis.[52]

PRP in patients with anti coagulants

The biological and therapeutical improvement achieved by PRP can avoid hemorrhagic or thromboembolic complications in cardiac patients under anticoagulant while performing oral surgeries, which simplifies its systemic management as well.[53]

MSC-based immunotherapy

MSC induce neoangiogenesis by releasing cytokines and growth factors (paracrine effect) so that intralesional stem cell therapy can be used as an option for oral submucous fibrosis. Increased free radical scavenging by antioxidants removes senescent cells from the lesions. Reversal of hypoxia in the diseased tissue stimulate stem cells to transform into new fibroblasts and removes disintegrated collagen fibers, which significantly improves the mouth opening and gradually decreases the burning sensation/ blanching. As PDLSCs, SCAP and dental pulp stem cells possess immunosuppressive properties by increasing tissue-protective regulatory T cells (Tregs), suppress inflammatory cell infiltration and proinflammatory cytokine secretion, induce angiogenesis and increases ECM formation, its systematic delivery can be used for treatment of BRONJ and to enhance oral ulcer healing.[54,55]

10. Gene Therapy

It is an emerging concept in which genetic information is transferred into target cells, which synthesize the endogenous protein encoded by the gene.[43] The process of transfer of functional genetic information into the target cell is known as transduction. Here the recombinant vector (virus) containing therapeutic DNA enters the target cell via a receptor-mediated process, and then into its nucleus, where it may integrated into host genome or remain extrachromosomal. These transduced cells can produce and secrete growth factor encoded by the DNA. In reviews and studies gene therapy has been reported for mineralized tissue formation and thereby re-construction of mandibular continuity defects in animals and repair of mandibular condyle and temporomandibular joints. So far the gene transfer in tissue engineered devices in maxillofacial surgery has been performed so far only in a few approaches at the preclinical level.

Gene therapy can be of viral and non viral. In viral gene therapy, adenoviral vectors are commonly used to transfect craniofacial tissues. After removal of pathogenic gene sequences from these virus particles, their ability to deliver genetic material to host cells is used to transfect the target cells with the respective gene. It can efficiently transfects both replicating and quiescent cells and large amounts of genetic information can be inserted into themsp.[56] A variety of viral vectors including adenoviruses (AVs), adenoassociated viruses (AAVs), and retroviruses such as lentiviruses (LVs) are available[mlk69]. Adenoviruses can be easily manipulated and produced in high titers. While adenoviral vectors remain in the cytoplasm of the target cells, retroviral vectors integrate their DNA permanently into the genome. In this way, viral gene transfer using retroviral vectors is more effective than using adenoviral vectors. But the major concern with viral gene therapy is risk of an immune response from the expression of viral antigens on the surfaces of transfected cells, which led to the emergence of non-viral gene therapy.

Even though the nonviral gene therapy can deliver larger genes with minimal immunogenicity, it has lower invivo transfer efficiency. One of the major technique of non-viral gene therapy to regenerate cranial bone defects is the use of cationic liposomes by delivering BMP-2 plasmid cDNA.[58] Cationic macromer poly(ethylene imine) has also been used to deliver BMP-4 plasmid DNA in a sustained and localized manner from poly(lactic-co-glycolic acid) scaffolds within critical size cranial defects. It can also performed indirectly by invitro transfection of the cells and re-implanting it into the defect along with TE scaffold material. However the direct technique is simpler, it has lower transfection efficiency and nonspecific cellular targeting. The indirect technique requires additional harvesting and culturing procedures, at the same time it avoids the risk of direct implantation of viral vectors into the patient and disturbing the host genome.

Gene therapy can be accomplished by invivo or exvivo approach. Invivo approach has the advantage of direct injection into target site but it carries the risk of non specific cellular targeting. Even though the specific cellular targeting is possible with exvivo approach, this lengthy approach carries the risk of contamination. Exvivo transfected cells are not immunologically privileged, but still express viral antigens on their surface enabling the host response following implantation.[5]

11. Bioreactor Systems

Even though the bioreactors were initially developed to test biomaterials, recently it can also available for extracorporal tissue growth. These are devices in which biological or biochemical processes, or both, are performed under controlled conditions of pH, temperature, pressure, oxygen supply, nutrient supply, and waste removal, yielding the required amount of the tissue in return of autologous material and a scaffold as

inputs for a defined culture period.[59] The design of bioreactors can be of two types namely closed and open system (using conventional dishes and flasks). In order to obtain reproducible and standardized results, the culture conditions such as temperature, pH, nutrient, and oxygen supply should be adjustable. Recently various new techniques like fluorescence microscopy, micro-computerized tomography, oxygen and substrate monitoring, flow determination etch were used to maintain controlled conditions.

12. Transportation of Regenerated Tissues

The maintainance and transportation of stem cells and regenerated tissues technique is critical for maintaining the cells viability and preventing the contamination. Several devices have been tested as safe and efficient transportation containers. Various transport medium were used which includes Dulbecco's Modified Eagle Medium high glucose (DMEM(H)), DMEM low glucose, DMEM/F-12, Keratinocyte serum free medium (K-SFM) and α-Minimum essential medium(MEM). Transportation containers were developed by Nozaki et al.[60] (2008) and Oie et al.[61] (2014). 101 These containers consisting of an incubation vessel for temperature, a sealing apparatus for air pressure and four packaging chambers for sterility.

13. Stem Cells Banking

Stem cell banking is the process of storing stem cells for future stem cell based regenerative therapy. It includes enrollment, collection and isolation of stem cells, cryopreservation, and receipt of sample certificate. Lifecell is India's first and largest stemcell bank, where as Stemade is India's first private dental stem cell bank. Stem cells can be isolated from the cryopreserved PDL, pulp tissues, apical papilla, bone marrow, fat or lining epithelium for future regenerative therapies. Tissue samples containing stem cells were placed in a screw top vial with an appropriate media to nourish it during transport and should reach the processing storage facility within 40 hours. Then it was trypsinized and passaged to yield colonies of stem cells. The required cell type can be manipulated by utilizing suitable inductive signals and growth factors to the stem cells.

14. Advantages

- Replace degenerated or damaged tissues
- Promotes bone regeneration
- Rapid bone healing
- Increases the functionality and therapeutic outcome.

15. Disadvantages[62]

- All the factors in TE should be biocompatible and safe
- Very expensive as the process is very labour-intensive.
- Tumourigenity
- Graft rejection
- Immunogenity
- Cell migration.

16. Future Perspective

Nowadays there are some questions arising regarding the ultimate results of TE. The future of TE lies in the hands of close collaborations between engineers and surgeons. So a better understanding of the function and local environment of defective structures must be required for a appropriately engineered construct. Existing reconstructive techniques were based on macroscopic characteristics whereas TE based upon microscopic characteristics of the defects that have to be restored.

BMSCs are the most common cells used in tissue engineering. Recent advances were undergoing by incorporating genes to make the stem cells more pluripotent. Another advancement in research for TE is recombinant technology that has prompted the idea of gene transfer to the seeded cells which allows for overexpression of the required growth factors and make it less dependent on host tissue factors. The rapid prototyping technique is recently developed to produce specific scaffolds with characterized architecture, which will reduce the risk of inflammation in vivo by the acidic pH change caused by the degradation of biodegradable polymers. Ceramics found to be more successful due to osteoconductivity, easy access, and absence of immunological reaction. Advancing technologies in designing and fabricating scaffolds such as bioreactors and rapid prototyping may help to create cell environments that resemble natural human tissues.

Ex vivo gene therapy is a novel approach in TE, which avoids the need for expensive and difficult production of recombinant cells or growth factors and promotes bone healing. In this method, progenitor cells are transduced by gene therapy to express bone forming factors. Therefore the combination of genetically modified cells with scaffolds has been increased bone formation in vivo.

17. CONCLUSION

TE is an enchanting way of producing living tissues for patients who have lost an important tissue or part of it. It forms an excellent and engrossing alternative for maxillofacial reconstruction in near future. Various studies were already undergone in this field. As per the studies, the most commonly used cells are BMSCs and scaffolding biomaterials are synthetic ceramics. In most of the reviews the commonly used growth factor for tissue regeneration was BMP 2. The studies showed that the presence of these three components showed high proliferation, attachment and differentiation of cells in vitro and new bone formation in vivo. Nano-scaffolds and composite polymer-ceramics are more recently developed alternative to conventional scaffolds. New technologies like recombinant gene transfer, bioreactors, rapid prototyping etc were on the way of development in the field of TE, which may help to find out a final solution for the challenges faced during the process of tissue regeneration.

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