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Tissue engineering in maxillofacial surgery- A paradigm shift

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Abstract

Tissue engineering is an innovative, rapidly advancing field that has emerged as an exhilarating substitute for maxillofacial reconstruction. It offers a solution for the reconstruction of large tissue defects by using biochemical and biomaterial engineering of the cell to regenerate bio-artificial tissues and organs. The field of reconstruction is one of the main challenges in the modern era for the oral and maxillofacial surgeon. It brings new substitute to supplement existing treatment regimens for reconstruction/regeneration of the oral and craniofacial complex, which includes the teeth, periodontium, bones, soft tissues (oral mucosa, conjunctiva, skin), salivary glands, and the temporomandibular joint (bone and cartilage), as well as blood vessels, muscles, tendons, and nerves. This article reviews the principles of tissue engineering and its various applications in oral and maxillofacial surgery.

Keywords: Tissue engineering, Maxillofacial surgery, Reconstructive surgery, Tissue regeneration.

Introduction

Tissue engineering is a speciality that predominantly aims to replace lost tissue or organ.

The loss of tissue may be congenital or it may be a result of trauma or infection. It has been defined by Langer and Vacanti, in 1993 as an "interdisciplinary field that applies the principles of engineering and the life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function." (1)

Reconstruction in the maxillofacial region is also very unique since this region is a pool of microbial flora and hence prone to more infections. The same has progressed vividly starting from non-vascularised grafts to vascularised free flaps namely fibula, and radial forearm. etc. They are indeed a double-edged sword with both advantages and limitations as well. Limitations include causing donor site morbidity, long term hospital stay.

Here comes the concept of tissue engineering where there had been numerous attempts to engineer many tissues like mucosa, salivary gland, teeth, and TMJ. In tissue engineering biomaterials have been used as a replacement for tissue grafts and flaps.

The key 3 elements which are indeed the backbone of tissue engineering are Scaffold (framework), cell signalling (for cell differentiation promotion), and stem cells. The main objective of this article is to review the materials available for tissue engineering and provide an overview of the uses of tissue engineering.

Principles of tissue engineering

As previously described tissue engineering has 3 components, namely Scaffold, Cell signalling molecules, and stem cells.

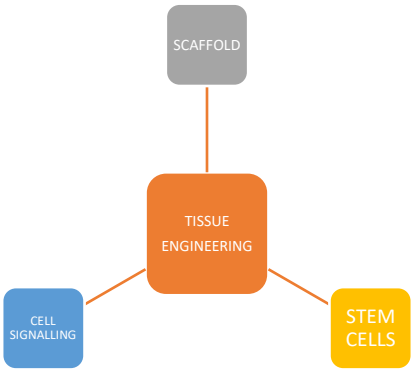

Figure 1: Tissue engineering triad

Scaffold

A scaffold is the one that forms a 3-dimensional matrix or framework.) It is a reservoir of cytokines, and water. The matrix can be made of allo Plast or allograft or xenograft. The scaffold can be an organic or inorganic material. The organic material can be of natural products like collagen, chitosan, or synthetic polymers like polylactide, polyglycol ate, PLGA (Poly lac to glyco late), PCL (Polyca pro lac tone), PEG (Polye thy lene - glycol). Inorganic materials are of various compositions of calcium, phosphate, sodium, potassium, carbonates. Ceramic matrices like β TCP (Beta Tri-calcium phosphate), biphasic calcium phosphate, and hydroxy apatite products are also being investigated (3). Hydrogel polymers are those which are formulated by cross-linking hydrophilic polymers along with a bridging agent (cross-linking polymer). They serve as a matrix for tissue engineering. They are capable of duplicating extracellular topography and delivering bioactive agents that stimulate tissue regeneration. Metal scaffolds are inert alloplastic materials. Metals used are titanium, gold, stainless steel, and cobalt chrome. Currently, rese arch is done on biodegradable metals. Metal nanoparticles when integrated into scaffolds provide increased mechanical strength, better cell adhesion, and enhanced collagen synthesis.

The ideal requisites of a scaffold are as follows

- Biodegradable
- Osteo inductive or osteoconductive.
- Allow signalling of the growth factors
- Provide mechanical support, and rigidity, and enhance cell signalling.
- Act as a template for vascularization, cell proliferation, matrix deposition, and bone regeneration.
- Should be porous for effective migration of cytokines, and water.
- Able to facilitate and nurture cell growth (2 (4)

Collagen has a slow degradation rate making it more susceptible to foreign body reactions. Every material has its side effects too. Ceramic scaffolds have sufficient

strength but are brittle and hence cannot bear much load. Polymer scaffolds have low mechanical strength and hence are used in combinations with ceramic scaffolds. (5)

Recently there are 3-dimensional scaffolds that provide bioresorbable platforms and are also bioactive, pore size can be controlled so that the volume and tissue bulk is also maintained along with the enhanced ability for growth of tissue. (6)

Stem cells

The stem cells can be autogenic i.e. from the patient's own body or can be from another organism too. If the stem cells are from one's own body there is no risk of immune rejection. The stem cells can be classified further into totipotent stem cells i.e they can form all 3 germ layers, pluripotent stem cells i.e they can differentiate into all types of cells in vivo and multipotent stem cells i.e they can form into multiple lineages. The cells can be bone marrow-derived stem cells, mesenchymal stem cells, and osteogenic precursors. The stem cells can also be human embryonic stem cells, human induced pluripotent stem cells, human adult stem cells (mesenchymal stem cells, adipose-derived stem cells, dental pulp derived stem cells, stem cells from exfoliated deciduous teeth, gingiva derived stem cells).

The embryonic stem cells are taken from the inner layer of the blastocyst. It is more challenging to use embryonic stem cells because of immunogenicity, ethical issues, and uncontrolled differentiation. (7,8) They are comparatively more pluripotent and there is research showing embryonic-derived stem cells are superior when compared to bone marrow-derived stem cells (BMDSCs) and other mesenchymal stem cells (MSCs) (5). iPSCs (induced pluripotent stem cells) are the new source of stem cells derived from adult cells by inducing pluripotency genes namely Oct3/4, Sox2, cMyc, and KIF4. (7)

MSCs are the most commonly used stem cells in the craniofacial region. Most commonly they are derived from bone marrow, adipose tissue, umbilical cord, and placenta. They can be differentiated into various types of cells like osteoblasts, chondrocytes, adipocytes, endothelial, cardiovascular, and neurogenic cell types. Both bone marrow and adipose tissue can form cells and tissues of mesodermal origin. For osteogenesis bone marrow derived stem cells are considered highly beneficial due to enhanced osteogenic potential and chndrogenic potential. (23). But stem cells from adipose tissue are considered advantageous as compared with bone marrow-derived stem cells given the bulk of tissue provided and better accessibility. (1, 9) There is new research being done on stem cells taken from cranial sutures, namely Gli1 craniofacial stem cells (GSCs) taken from mice. The clinical applications are yet to start with this type of stem cells (9).

Gingiva-derived stem cells when differentiated can produce adipocytes, chondrocytes, neurocytes, neural cells, and Schwann cells. (3) Stem cells from the apical papilla and Stem cells from human exfoliated deciduous teeth (SHED) are used for bio root engineering. Periodontal-derived stem cells can form collagen, cement oblast-like cells, and adipocyte-like cells. (3)

Signalling molecules

Signalling molecules are the growth factors which are helping in intercellular communication. They are the proteins that bind to the specific receptors that initiate cell signalling leading to different events cell adhesion, proliferation, promotion, differentiation, and migration. (10,11) These molecules regulate the formation of proteins and hence help in tissue formation. Most of the

growth factors form mesenchymal tissues predominantly bone tissue and vascular tissue. They are namely

- Bone morphogenic proteins (BMPs)
- Fibroblast growth factor (FGF-2)
- Insulin-like growth factor (IGF)
- Platelet-derived growth factor (PDGF)
- Transforming growth factor beta (TGF-β)
- Vascular endothelial growth factor (VEGF)

Bone morphogenic proteins are the most commonly used growth factors. They are derived from group of TGF β. They induce bone formation by recruiting the mesenchymal cells. Osteogenic BMPs are BMP-2, BMP – 7, BMP-6 AND BMP-9. Chondrogenic BMPs are BMP-2, BMP-4, BMP-6, BMP-7, and BMP-9. BMP – 2,4,6,7 are the most predominantly used BMPs. They are the most vividly used growth factors in craniofacial region. BMPs are potent stimulators of denovo osteosynthesis. They are used in cleft palate repair, implant site development like (sinus lift procedures, ridge augmentation, ridge preservation,), and mandibular reconstruction. (1), (9).

Platelet-derived growth factors (PDGFs) are the glycoproteins secreted by degranulating platelets and endothelial cells which activate cell membrane receptors on target cells which induces mitogenesis, and angiogenesis. (6) They are used in periodontal applications, and ridge augmentation. (1)

Fibroblast growth factors (FGF) help in hard tissue regeneration, not in soft tissue regeneration. (1) They are also used in periodontal regeneration.

Vascular endothelial growth factors (VEGF) are the signalling protein that induces both angiogenesis and vasculo genesis and also enhance neova scularisation and vessel growth from old vasculature. They promote osteogenesis by synergistically acting with BMP. (9)

Insulin growth factor (IGF) are used in combinations with other growth factors like PDGF and VEGF. They are used in periodontal applications which increase clinical attachment level, increase bone fill, and improvement in probe depth. (1)

Transforming growth factor – β (TGF- β) help in osteogenesis. They enhance osteogenesis migration and proliferation. (5)

These tissue engineering elements are seen in Platelet-rich fibrin (PRF), Platelet-rich plasma (PRP), and BMAC (Bone marrow aspirate concentrate).

Platelet-rich plasma (PRP)

PRP is fractionised plasma of autologous blood. The plasma concenteration in PRP is high than the baseline level. After adding anticoagulant to the patient's blood, centrifuging the blood in 2 stages PRP is obtained. PRP causes activation and degranulation of the platelets, which causes the release of growth factors, thus enhancing stem cell activity, wound healing, angiogenesis, and tissue repair. Alpha and delta granules are those storage granules that are derived from platelets. The alpha granules had many growth factors like VEGF, PDGF, TGF β, IGF, FGF, and EGF (Endothelial growth factor). PRP can be stored for 8 hours and before using they need to be activated by adding 5 mL of 10% calcium chloride with 5000 units of topical bovine thrombin. (14) PRP can control inflammation, improve capillary growth, enhance healing and tissue regeneration, boost up host defense by immediately recruiting neutrophils and macrophages, fibroblasts, and endothelial cells. (15) PRP has many uses in maxillofacial surgery. It can be used in post extraction sockets, post-surgical extraction of third molars, sinus floor elevation and ridge augmentation. (16)

Prataap et al. used autologous PRP post extraction, and found that soft tissue healing was improved, post

extraction pain was remarkably reduced and incidence of alveolar osteitis also decreased notably.(17) Al Morassi et al had performed a systematic review on use of various management strategies in temporomandibular disorders and have concluded that PRP along with arthroscopy and PRP combined with arthrocentesis proved to be beneficial as compared with conservative methods and other injectables like corticosteroids and hyaluronic acid. (18)

Platelet rich fibrin (PRF)

PRF is a second-generation autologous preparation of plasma. There is no necessity to add anticoagulants to the patient's blood. PRF consists of fibrin network, cell types, stem cells, and growth factors. PRF releases growth factors slowly and the fibrin network itself has wound healing ability. PRF along with MSCs is a promising solution in hard and soft tissue engineering (e.g., alveolar bone, mandible, calvarium). PRF is packed with endogenous procoagulant agents (e.g., thrombin) apart from calcium chloride, leukocytes, and glycoproteins. PRF is used in bone augmentation, sinus lifting, avulsion socket, guided bone regeneration, pre prosthetic surgery, peri apical surgery and also in tempo romandibular disorders. (15,19,20,21)

Bone marrow aspirate concentrate (BMAC)

BMAC is abundantly loaded with mesenchymal stem cells, growth factors, and cytokines. It comprises increased concentrations of VEGFs, PDGFs, and TGFs. It has both angiogenesis and osteogenesis. It helps in both soft tissue regeneration and hard tissue regeneration. (13)

Types of tissue regeneration in craniofacial surgery

1. Bone regeneration
2. Cartilage regeneration
3. Nerve regeneration
4. Salivary gland regeneration
5. Condyle regeneration

Bone is the most commonly engineered hard tissue. Bone formation occurs by either of the following methods namely osteogenesis, osteoinduction, and osteoconduction. (22) Bone-derived cells from maxillary tuberosity and mandibular ramus are used in bone augmentation and sinus lift procedures. (7) rhBMPs are also used in congenital cleft correction, mandibular continuity reconstruction, and maxillary augmentation. (23)

Prefabricated bio-engineered bone flaps are being studied in mandible repair. In a study done by Warnke et al. rhBMP-7 (recombinant human Bone morphogenic protein - 7), bone marrow-derived mesenchymal stem cells were added to a bio-resorbable polylactic scaffold. These were added in a titanium mesh tray, which acted as an external scaffold in the patient's lattismus dorsi muscle. The patient acted as a bio retractor; after seven weeks the titanium mesh with bone formed was placed in the patient's mandible. After 38 weeks, osteoblastic activity was predominant then it was observed all parts of the mandible has been developed. (3, 7)

Cartilage regeneration in maxillofacial surgery is more Centered on the regeneration of the tempo romandibular joint (TMJ). Bone marrow-derived and periosteum-derived mesenchymal stem cells have chondrogenic potential when used with appropriate scaffolds and growth factors (FGF-2). (3)

There are more researches undergoing to reconstruct nasal cartilage. The stem cells are mostly mesenchymal animal-derived, with scaffolds (poly lactic acid polyglycolic acid copolymer). (23)

There are more applications in peripheral nerve regeneration.

Hu et al in 2016 engineered nerve tissue with the help of adipose tissue-derived stem cells, and polylactide

polymer. Rai et al also cultured Schwann cells from dental pulp stem cells on polylactide. (24)

Salivary gland regeneration

Salivary gland tissue engineering requires cell-to-cell contact, cell contact with the extracellular matrix, and also bio scaffold. Lombert et al in 2016 reported embryonic stem cells and induced pluripotent stem cells in regenerating salivary gland cells.

Both biologic scaffolds like collagen, silk, fibrin, chitosan, alginate, and hyaluronic acid; and synthetic scaffolds like PGA, PLA, and PGLA, and polyethylene glycol and a combination of both biologic and synthetic scaffolds were proposed. 3D scaffolding techniques were tried in rats to stimulate and then maintain parotid cultures. PGLA fibers when coupled with laminin or chitosan promoted apical-basal polarisation and also salivary tight junctions. (25)

Clinical applications of tissue engineering

Tissue engineering in cleft surgery

Cleft palate regeneration demands custom made 3-dimensional scaffold with appropriate stem cells. Resell et al have done in vitro study on TGF-β3 murine cleft model with BMP-2 as a mediator and they successfully showed osteogenic differentiation of palate connective tissue cells. (27) It has been demonstrated by Brunnet et al and Taya et al that with absence of TGF-β3 there is prevention of normal palatal fusion resulting in cleft palate formation. (27,28,29) TGF-b3 (10 ng/ mL) is being constantly investigated in complete palatal fusion, showing promising results. In addition to the by supplementing TGF-b3 human cleft lip fusion has also been induced and paved way for a surgical scarless cleft lip repair (28,30). Umbrella review done by Pendro Hidreque et al comparing autologous graft and rhBMP (recombinant human bone morphogenic protein)2 have shown that rhBMP 2 shows relatively more bone quality and quantity. However, they have also pointed the fact that rhBMP 2 need to be used with caution the reason being it is still off label and more randomised trials should be performed to study the complications of rh BMP (31).

Mostafa et al had studied on nano fiber-based scaffold with Absorbed collagen sponge backbone loaded with BMP 2 on a rodent model for cleft palate. They have observed from 4 weeks bone formation had started to occur in the above scenario. They have pointed that nanofibers show controlled release of the growth factors. (32)

rhBMP 7 successfully regenerates bone at the alveolar cleft which is proved both clinically and radiographically. This is advantageous in a way that it reduces donor site morbidity, hospital stay. (27) Many studies are currently being pursued.

Tissue engineering in TMJ surgery

TMJ disorder management is a never-ending enigma. Tissue engineering could be a boon if articular disc could be replaced. The main aim of tissue engineering is to provoke the regeneration potential of the degenerated disk also by providing functionality, mechanical support. The scaffolds can be either natural (chitosan or alginate) or synthetic (Polylactate, Polyglycol ate, polyamide, poly glycerol sebacate, poly captoprene). (33) The first Invivo study on TMJ disc regeneration by Wu et al that comprised of fibrin/chitosan combination produced by freeze-drying with synovium-derived mesenchymal stem cells (MSCs). The researchers were able to produce homogenous tissue but the cells started decreasing in number after 7 days. (34) Invivo study was done on rabbits by Kobayashi et al where the Collagen sponge scaffold that was seeded with autologous bone marrow

MSCs. After two weeks, connective tissue was formed around the perforated disc. (35)

Many studies were tried by incorporating TGF – β factor to Poly lactate and poly glycolate which could produce more cells, collagen and glycosaminoglycans, but the validity of the strength of the tissue was a question mark. (33) VA Pirani et al had placed tissue engineered TMJ implant on Yucatan minipig. The implants used by the authors were made of costal cartilage which were scaffold free. The mechanical stimuli was enhanced by incorporating TGF-β1, lysyl oxidase, chondroitinase ABC, and passive axial compression. They found the tissue engineered implants were durable, mechanically robust, improve stiffness, prevent gross degenerative changes to TMJ, close TMJ disc defects and also the implant integrated well with the surrounding tissue. Authors claim that regeneration of lost tissue occurs by growth factors which were incorporated and also the stress concentration which had been reduced remarkably. (36)

Future perspectives in tissue engineering

Tissue engineering is an ever-growing field, and is definitely a blessing to maxillofacial surgeon. This technique is currently being approved to be used in alveolar cleft; which could be vividly expanded to cleft palate and lip. TMJ disc regeneration is definitely need of the hour and more in vitro and in vivo studies along with clinical trials should be performed. Salivary gland regeneration should be attempted in patients with aplasia of the salivary gland. Facial nerve and inferior alveolar nerve regeneration should also be attempted. Three-dimensional scaffold should be biotechnologically modified by various novel proto typing methods in such a manner that appropriate growth factors can be incorporated and a better engineered tissue could be provided.

Conclusion

Tissue regeneration has been an emerging technology for a few decades. Maxillofacial surgery is no less in tissue engineering. The appropriate size, shape, and function of the tissue to be regenerated needs to be considered. However, many materials are still emerging which are further investigated to be used as a scaffold, signalling molecules, and stem cells. The material used should never compromise itself in vascularity, or biocompatibility. Tissue engineering is a promising method for the regeneration of various tissues and organs in maxillofacial surgery. However, more research should be pursued in this perspective to provide a better-engineered tissue.

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