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RESEARCH ARTICLE

TISSUE ENGINEERING IN ORAL AND MAXILLOFACIAL REHABILITATION - A NEW PERSPECTIVE

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Abstract

Objective: To collect and conclude a literature review on the applications of tissue engineering in oral and maxillofacial rehabilitation

Materials and Methods: The review was searched and collected from many sources about tissue engineering. The database of Literature was collected through the search of PubMed, Google scholar and Researchgate databases. The keywords used for the search were tissue engineering, dental applications, prosthodontics, implant dentistry and craniofacial rehabilitation. A manual search to many of the reference lists of the identified articles and the author's article files and recent reviews was made to find additional publications. Those studies that showed new features about tissue engineering were included in this review.

Results: In total 28 literature sources were searched and reviewed. Studies that described new features about elements and strategies of tissue engineering and its applications were included.

Conclusion: We summarized in this study the key elements and strategies of tissue engineering and its applications in oral and maxillofacial rehabilitation.

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Introduction:-

The loss of natural teeth accompanied with loss of the supporting bone/ tissue complex surrounding the teeth can lead to distortion of the appearance, morphology, and function of the soft tissues¹. Replacement of the missing teeth is done commonly with denture or fixed prosthesis like FPD and implant². The severe bone resorption in edentulous areas and bone defects that arise after tooth loss results in further horizontal and vertical bone loss thereby decreasing the effectiveness of dental implants and other prosthodontic treatments³. Bone augmentation technique using autologous bone implant have been used traditionally to restore these bone defects. Although it is a good

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option, it necessitates additional surgery and morbidity. Various allografts and xenografts have been used but are often prone to rejection after implantation due to antigenecity leading to failure of the implant⁴.

Hard and soft tissue tumors, osteoradionecrosis, trauma, and congenital defects leads to significant maxillofacial bone damage necessitating tissue reconstruction⁵. The reconstruction of maxillofacial defects is complex and challenging. Traditionally, bone grafting procedures were used to address these debilitating conditions. Autogenous bone or osteocutaneous free flaps are considered the gold standard for reconstruction of maxillofacial defects, but it increases the risk of complications, thereby increasing morbidity for the patient. These grafting procedures have numerous disadvantages, which includes hematoma formation, donor site morbidity, infection, longer length of hospital stay and high cost⁶.

Currently, durable and synthetic materials or autologous tissue grafts have been the treatment of choice for diseases and trauma of oral and maxillofacial structures. A paradigm shift has taken place to utilize tissue engineering towards the regeneration of these structures. Several prototypes of maxillofacial structures such as temporomandibular joint (TMJ), condyle, cranial sutures, tooth structures and periodontium components can be regenerated through tissue engineering⁷.

The ideal treatment for the replacement of an individual's lost or damaged tissue is the same natural healthy tissue, which has led to the concept of engineering or regenerating new tissue from pre-existing tissue¹. Patient's expectations from healthcare professional may change, with the increasing public awareness about stem cells and tissue engineering. Considering these facts, dental practitioners must be aware of current concepts of tissue engineering for dental applications, their limitations and future expectations⁸.

The aim of this review is to discuss the main elements and strategies of tissue engineering and its applications in oral and maxillofacial rehabilitation.

Tissue engineering:

Tissue engineering is a blossoming field and is considered to be a new frontier in the regeneration of missing oral/maxillofacial tissues, which is truly multidisciplinary integrating various aspects of bio-engineering⁹. The term was coined first in 1985, and in 1993, Dr. Robert Langer and Dr. Joseph Vacanti described tissue engineering as "an interdisciplinary field that applies the principles of engineering and the life sciences towards the development of biological substitutes that restore, maintain, or improve tissue function"⁶. It is possible to regenerate large defects in periodontal tissues and alveolar bone, and to ultimately replace the lost tooth itself through stem cell and tissue engineering strategies. The targeted tissues/organs for such regenerative therapies in dentistry include the bone, craniofacial skeletal muscles, salivary gland, tongue as well as the condylar cartilage of the temporomandibular joint (TMJ)⁹.

Elements of Tissue Engineering:

The concept of tissue engineering is based on three key elements 1. the cells, 2. scaffolds/matrices and 3. the growth factors/biomolecules to recapitulate physiological processes of development and regeneration.

Stem cells are a group of undifferentiated cells that have the capacity to differentiate into specialised cells and are classified into 2 groups - 1. embryonic stem (ES) cells and 2. adult cells. In addition to these stem cells which are naturally present in the human body, induced pluripotent stem (iPS) cells have been recently generated artificially via genetic manipulation of somatic cells. ES cells and iPS cells are collectively referred to as pluripotent stem cells because they can develop into all types of cells from all three germinal layers. In contrast, most adult stem cells are multipotent, i.e., they can only differentiate into a limited number of cell types⁹. Tooth also contains several mesenchymal stem cells that have regenerative capacity. Dental derived mesenchymal stem cells include dental pulp stem cells (DPSCs), stem cells from human exfoliated deciduous teeth (SHEDs), stem cells from apical papilla (SCAP), periodontal ligament stem cells and dental follicle progenitor cells⁶. Recent studies have shown that the stem cells from other tissues like bone marrow, fat tissue, and endometrium are also capable of differentiating into odontoblasts or tooth-forming cells².

Scaffolds made of natural /synthetic materials serve as extracellular matrix, and act as a carrier for growth factors. Scaffolds are biomaterials with two-dimensional or three-dimensional architecture that help in migration, proliferation and differentiation of cells by providing an adequate environment¹⁰. Natural (collagen) or synthetic

polymers such as Polylactic acid (PLA), Polyglycolic acid (PGA), Copolymers of PLA and PGA [poly(DL-lactic-co-glycolic acid)], Polycaprolactones and Calcium phosphate ceramics (Hydroxyapatite, Tricalcium phosphate, Beta tricalcium phosphate) are the various biomaterials used as scaffolds¹⁰.

The function of growth factors is to facilitate and promote cell growth, development, proliferation and migration to regenerate new tissue. Bone morphogenetic proteins (BMP); Hedgehog proteins (HHS); fibroblast growth factor (FGF); interleukins; tumor necrosis factor (TNF) and vascular endothelial growth factor (VEGF) are the different growth factors used in tissue engineering, among which the BMPs have been extensively studied and applied for dental regeneration¹⁰.

Strategies of tissue engineering:

Tissue engineering strategies can be categorized into three major classes: 1. conductive, 2. inductive, and 3. cell transplantation techniques¹.

Conductive technique is a passive phenomenon which utilizes biomaterials to facilitate the growth or regenerative capacity of existing tissue. Nyman et al. were the first to use osteoconductive mechanisms successfully in providing a means for selective wound healing by supporting the ingrowth of the periodontal supporting cells, while excluding gingival epithelial and connective tissue cells from reconstruction sites. Another relatively widespread application of a conductive approach, osseointegration of the dental implant revolutionized treatment options in restorative and prosthetic dentistry¹. Branemark et al. were the first to successfully achieve this phenomenon, and its application is relatively simple as the armamentarium does not include living cells or diffusible biological signals. One significant benefit of this method is its ability to form bone in a well-controlled and usual manner¹.

Induction is the second major tissue engineering strategy, in which specific biological signals help in activating cells in close proximity to the defect site. Urist first demonstrated the new bone formation at nonmineralizing or ectopic sites. This process requires polymeric carriers which include primarily collagen of animal origin and synthetic polymers of lactic acid and glycolic acid to transport inductive factors, like bone morphogenetic proteins (BMPs) to the regeneration site. The rate of carrier breakdown dictates the speed and quantity of inductive factors release. The main limitation of inductive approaches is that the inductive factors may not be known for a particular tissue. This situation leads to the third tissue engineering approach, cell transplantation¹.

The last method is cell transplantation, which transplants laboratory-cultivated cells into the desired target. This approach requires interaction between a doctor, engineer, and a cell biologist. First, an individual is biopsied to acquire information regarding the cells present in the individual by a doctor. Then, a cell biologist appropriately reproduces the specific cells from the biopsy sent to the laboratory. Next, a biodegradable polymeric scaffold is fabricated by an engineer, which is ultimately integrated with the cells of interest. Finally, the scaffold is transplanted into the individual by the doctor. The scaffold eventually breaks down after successful cell transplantation, and also guides the successful formation of healthy tissue¹.

A common feature to all these tissue engineering strategies is that they typically employ the use of polymeric materials. In conductive approaches, the polymer is used primarily as a barrier membrane for the exclusion of specific cells that may disturb the regenerative process. Whereas, inductive approaches typically employ a carrier or vehicle for the delivery of proteins (e.g., BMP) or the actual DNA (gene) that encodes the protein. These molecules then directly (proteins) or indirectly (DNA/mRNA/protein) exert their effects on cells at the anatomic site by promoting the formation of the desired tissue type. Delivery vehicles are also frequently used in cell transplantation approaches. In addition to serving as simple vehicles for delivery of cells, the vehicles also serve as scaffolds to guide new tissue growth in a predictable manner from both the transplanted cells and interacting host cells. A variety of new materials are also being developed for these applications, and injectable materials that allow a minimally invasive delivery of inductive molecules or cells are especially attractive¹.

Applications in Oral and Maxillofacial Rehabilitation:

Whole tooth regeneration:

The regeneration of a whole tooth is considered to be the ultimate goal of regenerative dentistry. It could be performed as a hybrid strategy, where biologically created tissue compartments such as the periodontal ligament or a tooth crown would be combined with a metallic or ceramic implant or where a biologically regenerated tooth root ("bio-root") would be combined with a prosthetic crown¹¹. Efforts in creating a whole tooth from only cells and

tissues (“bio-tooth”) will be very likely in the focus in the following years¹¹. For whole tooth bioengineering, different strategies have been developed:

Reactivation of the Odontogenic Potency:

For the lost tooth regeneration capacity, revitalizing the odontogenic potency may be an interesting approach to induce tooth formation in vivo in the adult. The existence of a successional dental lamina (SDL) carrying the capacity for inducing odontogenesis is the only prerequisite for tooth replacement¹¹.

Tissue Recombination Approaches:

In classical tissue recombination experiments embryonic tooth germs can be dissociated from native site and later re-aggregated at an ectopic sites. Tooth-like organs with mineralized tissues (dentin and enamel) could be grown after temporary ectopic grafting of these cell aggregates, e.g., into the anterior eye chamber, subcutaneously, or under the renal capsule. The final goal of these experiments was to implant the constructs into the jaws of postnatal animals to generate a whole “bio-tooth”¹¹.

Adult Stem Cell Approaches:

When combined with mesenchymal cells, adult stem cells should have an odontogenic competence and should function as a “tooth inducer”¹¹. Young et al. were the first to successfully regenerate tooth structure containing enamel and dentin using tooth buds from the third molars of pigs. Although the size of the tooth was very small, defined tooth structure was present after 20–30 weeks of transplanting biodegradable scaffolds containing pig tooth bud cells into rats¹². Ohazama and colleagues, in 2004 used non-dental adult MSCs in combination with inductive embryonic dental epithelium for regeneration of tooth. They first transplanted them under the renal capsule and later transplanted them in adult jaws. Tooth formation including root occurred and the teeth erupted. In addition, bone was also induced¹³. Volponi Angelova and associates used human gingival epithelial cells combined with embryonic mouse tooth mesenchyme, which yielded an entire tooth outside of an embryo¹⁴. Research will presumably focus on using adult stem cells from dental and non-dental sources to test recombination or co-culturing for their effects on tooth development in the future¹¹.

The identification of an appropriate autologous stem cell source in humans is one of the major hurdles in the clinical application of tooth regeneration technology. In this regard, iPS cells may be an appropriate cell source because they can be differentiated to dental epithelial and mesenchymal cells and can be prepared from the patient’s own somatic cells³.

Bone regeneration:

In dentistry, bone tissue engineering (BTE) has potentially useful applications such as in cases of alveolar bone defects, cleft palatal defects, ridge augmentation and maxillary sinus lift⁸. Mesenchymal stem cells (MSCs) that can differentiate into osteoblasts are considered the best option for bone tissue engineering⁸. Under inductive conditions in vitro, both dental and nondental MSCs are able to differentiate into chondroblasts and osteoblasts¹⁵. Several studies have used ceramic and fibrin scaffolds for bone formation. Porous ceramics have shown favorable results due to their slow rate of erosion as well as low plasticity. However, fibrin is a suitable material due to hemostatic property, angiogenic ability, faster healing, and bone formation process, as well as osteoconductive property². Moreover, platelet-rich plasma is realized as one of the effective growth factors in bone tissue engineering².

BTE can be achieved using all three tissue engineering strategies. Small bony defects can be regenerated by using both conductive and inductive approaches. When conductive measures cannot sufficiently repair bone, BMPs are then inductively bioengineered. In contrast, cell transplantation approaches offer the possibility of pre-forming large bone structures (e.g., complete mandible) that may not be achievable using the other two strategies¹. These structures may even be completely developed in the lab prior to use in large-scale reconstructive procedure. To overcome the limitations associated with bone autografts and allografts, the bone tissue engineering shows promising results¹⁶.

Oral mucosal regeneration:

Tissue engineering of oral mucosa compared to skin tissue engineering is in its early stages and comparatively less developed. Human dermal matrix and oral keratinocytes were used to develop oral mucosal equivalents which have been used in the surgical reconstruction of the lips, oral vestibule, and tongue and have been proposed for use in tissue engineering of other mucocutaneous structures⁸. Sauerbier et al. cultured oral mucosal keratinocytes in cell

culture flasks and grown on membranes for clinical applications. The tissue engineered oral mucosal grafts have been reported to achieve the specific requirements for clinical procedures such as vestibuloplasty, release of tongue and other pre prosthetic surgeries¹⁷. These studies have suggested that tissue engineered oral mucosal products can be used successfully for oral surgical procedures⁸.

Salivary gland regeneration:

Loss of salivary glands function is reported following the adverse effect of medicines, radiotherapy, and autoimmune diseases, such as Sjogren's syndrome. Caries, mucosal infections, and dysphasia are often associated with decreased saliva, which is known as xerostomia². Saliva substitutes and sialagogues are the currently available therapies that are mostly supportive and are often insufficient⁸. To functionally restore damaged salivary glands, there are two main regenerative approaches³. One approach is to develop an artificial salivary gland using tissue engineering technologies. Another approach is to apply stem cells to the damaged salivary gland tissue³. Tissue engineering of glands could improve treatment but is complicated by the intricate anatomy and histology of salivary glands. Inductive gene therapy has been used to treat salivary gland deficiencies¹.

An alternative treatment is the transplantation of artificial salivary glands in cases of extensive loss of salivary gland tissue¹. Baum et al. developed synthetic salivary gland substitutes from polymer tubes lined by epithelial cells. These devices have the ability to deliver aqueous fluid into the oral cavity when grafted into buccal mucosa¹⁸. These regenerative approaches have the potential to treat patients with insufficient saliva production due to salivary gland tissue dysfunction and/or destruction, thereby treating and preventing the sequelae of hyposalivation.

Craniofacial Regeneration:

Based on the type of tissue being reconstructed, hard (bone) or soft (cartilage) tissue, craniofacial tissue engineering scaffolds and implants can be composed of a specific material or a blend/composite of materials¹⁶. Signal-inducing growth factors and attached proteins can also lead to mechanical property enhancement and cell-cell interaction within the complex. In vivo bone substitutes fabricated by 3-D printing with a common underlying goal of optimizing the mechanical properties, such as compressive strength, to match those of the cranium have been tested and reported in the literature⁵.

Kang et al., in a recent 2016 study, proved that craniofacial reconstruction, specifically biofabrication of the ear and mandible, can be achieved by means of the 3D printing process¹⁹. Wang et al. introduced anti-inflammation functionality into an alginate/nano-hydroxyapatite scaffold with the incorporation of AtsTrin, which suppresses TNF- α signaling. The pro-inflammatory factor by interfering with osteoblastic differentiation was known to stagnate the process of tissue regeneration. Superior osteogenesis was observed by adding AtsTrin to the scaffold, in mouse calvarial defects compared to the control and other experimental groups²⁰. Wang et al. ascertained that the incorporation of bioactive agents can undoubtedly have a stimulatory effect on calvarial bone regeneration in vivo. These positive in vivo results show promise in the future of regenerative medicine as clinicians and research scientists work together in order to produce the most ideal scaffold for human craniofacial defect repair²⁰.

Temporomandibular joint regeneration:

Pharmacotherapy, physiotherapy, and surgical intervention are the only treatments available for the management of TMJ disorders. Tissue engineering has a potential application in the treatment of TMJ dysfunction resulting from degeneration and also plays a vital role in regenerating bony condyle or fibrocartilagenous disc⁸. Cartilage generation, however, is more difficult than bone regeneration, in that in most studies although stem cells differentiation is to chondrocytes, the regenerate tissue is not like the natural tissue, structurally and functionally². Many growth factors that could contribute to the joint regeneration were identified². With reference to cells and growth factors, cells extracted from the synovial capsule and fibroblast growth factors, have shown promising results in regenerating the cartilage of the TMJ¹⁰.

In vitro development of TMJ cartilage was first reported by Thomas et al. in 1991, using type I collagen meshes to culture chondrocyte-like cells. The study concluded that the "resultant tissue analog had the clinical appearance and characteristics of the temporomandibular joint disc" and the analog could alternatively be used in vivo for disc repair²¹. This initial research was followed by a series of studies on tissue engineering targeting TMJ tissues. In a study by Abukawa et al., Condylar bone was regenerated using porcine mesenchymal stem cells seeded on biodegradable PLGA scaffolds²². Bailey et al. in his study, compared engineered condylar cartilage made from human umbilical cord matrix (HUCM) stem cells and TMJ condylar chondrocytes seeded onto PGA scaffolds and

concluded that HUCM constructs showed increased levels of biosynthesis and higher cellularity than TMJ condylar cartilage cells²³. Tissue engineering to treat TMJ dysfunction is challenging due to the presence of multiple tissues and the complex anatomy of the TMJ. Additionally, to have a clinical application, the engineered constructs must be biologically and mechanically functional and can remodel according to functional loading stresses.

Implant therapy:

Osseointegrated implants, which were established by Branemark et al., have been used worldwide as a reliable and consistent procedure in dentistry²⁴. Local bone defects and general poor bone quality necessitate bone reconstruction before placement of the implant. Besides this, localized bone loss around the implant fixture, especially in the case of gingival recession represents the clinical challenge which requires further surgical interventions²⁵. Recent surveys revealed that peri-implantitis was considered as a major and growing problem in implantology as the peri-implant soft tissue may have an impaired defense capacity against exogenous irritation due to lack of periodontal ligament²⁴. If implant with PDL is developed, these problems could be resolved, which can be achieved by LIGAPLANTS, a combination of the PDL cells with implant biomaterial²⁶. Implants with periodontal ligament may improve the function of ankylosed implants by providing various biological characteristics of periodontium, such as durability against bacterial challenge and acting as a buffer and sensor of biting force.

In 1990, Buser et al. showed that PDL of the roots served as a source of cells which could populate the implant surface during healing when titanium dental implants were placed in contact with retained root tips. This initial observation suggested the possibility to achieve anchorage of dental implant with periodontal ligament²⁷. Now, tissue engineering has opened a new vista in periodontal regeneration and more so in the treatment of dental implants. Various scaffolds and matrices have proved their ability to regenerate the entire periodontium. Gault et al. in a study involving animal experiments on mice and canine models as well as human clinical investigation, used ligapplants (combination of PDL cells with implant biomaterial) for tooth replacement. PDL formation along with new layer of tissue resembling repair cementum was formed on the ligapplant surface in the canine model. In humans, after surgery, a desmodontal gap, corresponding to PDL space of normal width, was evident around one ligapplant, and the structure of the lamina dura resembled that around a natural tooth²⁸. Ligapplants as tooth replacement has decisive advantages as compared with osseointegration devices, due to their periodontal tissue regeneration. Conclusively, ligapplants have the capacity to induce the formation of the new bone, when placed in sites associated with large periodontal bone defects²⁵.

Conclusion:-

In Dentistry, the future of tissue engineering is most promising, and this new approach is expected to enable regeneration of tissues damaged by the different dental pathologies. With the development of different materials and fabrication techniques, a great deal of progress has been made in the researches, with the purpose of improving the properties of the materials for this field of interest. Methods to improve 3D organogenesis, 3D printing applications, or the appropriate application of stimulatory molecules and drugs should be tested intensively, on the translational level. However, despite all efforts and achieved results in basic and translational research, this approach is still challenging¹¹. Therefore, dentists must be aware of these advances and use the new technologies in daily clinical practice, thereby providing patients with more efficient therapy, and consequently improving their quality of life.

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