Scaffolds used in tissue engineering and their applications in medicine and dentistry

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ABSTRACT
Tissue engineering and regenerative medicine are promising trailblazing applications that necessitate the creation of biomaterials in order to create functional structures that replace, preserve, or enhance damaged tissues or whole organs. The primary focus in these fields is on developing biomaterials which replicate the physicochemical microenvironments of cells and tissues. By creating functioning replacements for damaged organs and tissues, like cartilage and osseous tissue, as well as cardiac and lung tissues, this branch of medicine has the potential to remarkably enhance the quality of life of multitude of individuals by improving their health. The progress of tissue engineering has also provided a significant prospect for improving clinical practice in treating dental patients, particularly in the regions of endodontics and periodontial reestablishment, as well as full tooth regeneration. Highly proliferative cells are found in dental pulp, where they can be triggered in response to damage, multiply, and differentiate into osteoblastic phenotypes to support dentin healing. The high proliferation capacity, ability to differentiate in several directions (both *in vivo* and *in vitro*) and the ease of procurement makes the stem cells of oro-facial region an invaluable source. This chapter discusses the various components of tissue engineering. The fabrication and applications of scaffolds are discussed in detail along with the medical and dental implications of tissue engineering per se.

Keywords: Tissue engineering, Regenerative medicine, Stem cells, Extracellular matrix, Guided Tissue Regeneration

I. INTRODUCTION

The difficulty in designing tissue replacements that can replicate the structural characteristics and physiological processes of native tissues *in vivo* has led to innovations in tissue engineering (TE). According to the definition of tissue engineering demarcated in **1993 (Robert Langer),** it is "An interdisciplinary field that applies the principles of engineering and life sciences towards the development of biological substitutes that restore, maintain or improve tissue function or a whole organ".

The TE paradigm isolates particular cells from an individual through a small biopsy, seeding the cells under carefully controlled culture conditions to grow artificial tissue into porous, interconnected structures called scaffolds that can reinforce three-dimensional (3D) tissue formation. These scaffolds can be delivered to any pre-determined site in the individual's body to ensure novel tissue formations that become an integral part of that particular individual's body over a period of time. Biocompatible and biodegradable polymers are often used to promote the ingrowth of surrounding tissue to help the transplanted cells to connect, develop, and preserve distinct procedures. By creating functioning replacements for damaged organs and tissues, like cartilage and osseous tissue, this branch of medicine has the potential to remarkably enhance the quality of life of multitude of individuals by improving their health. Construction of artificial skin for burn victims was the first successful stepping stone in TE.

Over the past few decades, the increased significance of TE is because of the several shortcomings of conventional tissue or organ transplants, such as scarcity of donors, invasive procedures, and rejections. The advancement of tissue engineering can solve these issues. Additionally, it avoids the use of materials with a poor degree of biocompatibility, which results in reduction in tissue rejections and postoperative problems. This method has the significant benefit of minimizing the need for additional therapy by allowing tissues to be created to develop in a way that perfectly matches the needs of the individual in terms of size, shape, and immunological compatibility.

II. COMPONENTS OF TISSUE ENGINEERING

The three integral and important components of TE can be enumerated as:

(a) Cells: Synthesise novel tissue matrix.

(b) Growth factors (GFs): Facilitate novel tissue formation.

(c) Scaffolds: Required for differentiation, multiplication, and biosynthesis of cells. Function like an extracellular matrix (ECM).

These three components (scaffolds, cells, and GFs), commonly known as the “tissue engineering triad," are set up in the suitable environment of a bioreactor[1].

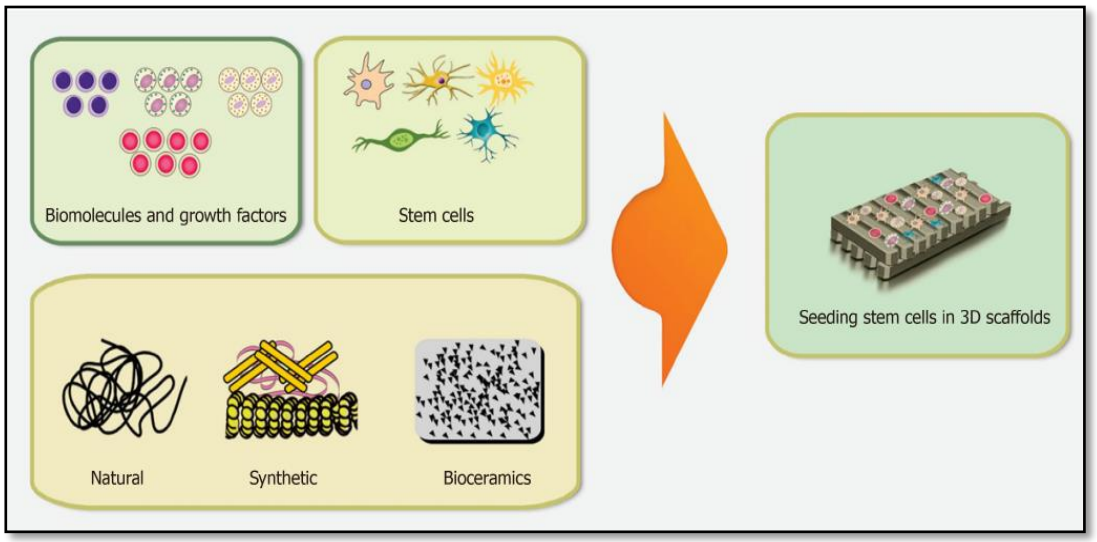

Figure 1: Components of tissue engineering and types of scaffolds used.

1. Stem cells (SCs)–

These are undifferentiated cells and possess self-renewal and clonogenic characteristics. They have the ability to develop into numerous cell lineages. Based on their origin, the stem cells can be categorised as:

i. Embryonic

ii. Induced pluripotent stem cells (iPS)

iii. Adult (tissue-specific) stem cells

Additionally, based on their differentiation potential, the stem cells can be divided into five categories:

i. Totipotent (ability to generate all types of cells)

ii. Pluripotent (ability to generate any type of cell of an organism)

iii. Multipotent (ability to generate cells from the origin tissue)

iv. Oligopotent (ability to differentiate into only some types of cells)

v. Unipotent (ability to generate only one cell type).

Adult stem cells (ASCs) are multipotent whereas embryonic SCs are pluripotent[2].

a. Dental stem cells -

Dental stem cells (DSCs) are derived from the neural crest. The undifferentiated DSCs can self-renew indefinitely, are multipotent, and can colonize. They are distinguished by their ability to develop into all three cell lineages (ecto, endo as well as mesodermal). DSCs of each origin can differentiate into a variety of different cells like cells of epithelium, endothelium, nerves, muscles, blood vessels, bone, cartilage, adipose tissue, photoreceptors, and, odontoblasts. Though Stem cells (SCs) from diverse origins are known as DSCs, they differ in their phenotype, differentiation potential (in vivo as well as in vitro), and biological responses during differentiation.

They can be harvested from the intraoral dental components of an individual of any age group with considerable ease which makes them a dependable source to access autologous SCs. They can be isolated from the dental tissues such as apical papilla, dental follicle, gingiva, and periodontal ligament (PDL) from the teeth of adults, children, and neonates. DSCs isolated from the pulpal tissue are widely tested for their ability to differentiate into odontogenic, osteogenic, and neurogenic cells. Compared to the DSCs of dental pulp, SCs of the apical papilla have a stronger capacity to proliferate, express a broader range of neural markers, and create a better homogeneous dentine-like tissue. Furthermore, compared to the DSCs of dental pulp, DSCs harvested from primary teeth have a higher proliferation rate and greater osteogenic regeneration capability. The role of DSCs' microenvironment effectively modulates their heterogeneity. DSCs derived from diverse sources display a variety of cell surface marker patterns[2].

The microenvironment is a three-dimensional (3D) framework that is enclosed by cells and an extra-cellular matrix. This microenvironment shields the SCs from improper differentiation, cellular injury, and apoptosis. It also governs tissue maintenance and regeneration. The functions of ECM include mechanical support to the tissues, providing bioactive cues to residing cells, and facilitating the environment conducive to remodelling tissues in response to events such as wound healing. The matrix and chemical signals from the neighbouring cells influence the behaviour of SCs. For example, odontoblasts are the cells that are derived from neural crest and are biologically programmed to form teeth. However, under right environmental conditions, these DSCs can differentiate into pre-odontoblasts and then further differentiate into odontoblasts.

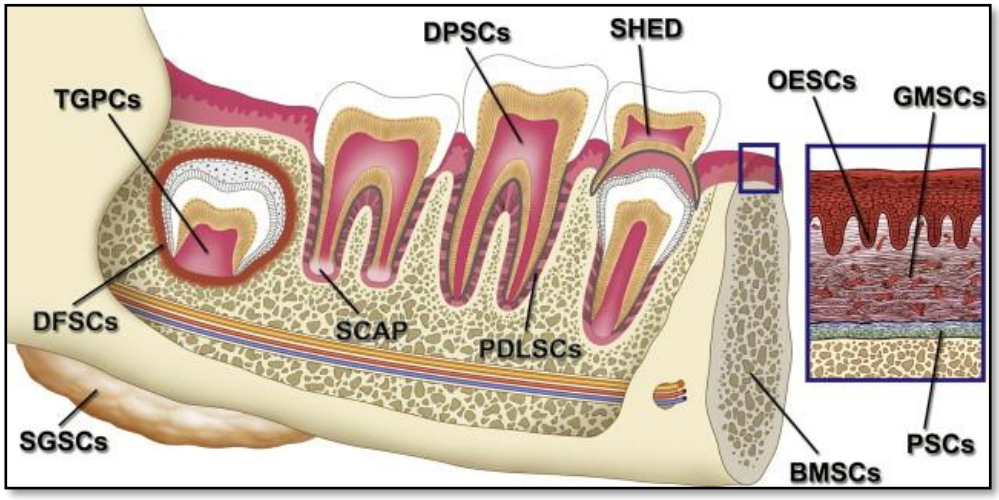

Figure 2: Different sources of dental stem cells

2. Growth factors –

Growth factors (GFs) are chemicals that can stimulate a range of biological activities during development and tissue repair, including cell proliferation, migration, differentiation, and multicellular morphogenesis. GFs are vital to TE because of their ability to offer a variety of signalling molecules to a specific milieu, their ability to mobilize seed cells and the peripheral cells to adapt swiftly to the environment of implantation bed, and their ability to trigger healing. Platelet-rich fibrin (PRF) was created as a source of autogenous blood-derived GF concentrate which can be made without adding thrombin. The goal was to enhance and streamline the preparation technique. Growth factors are essential for biological and therapeutic regeneration. Many polypeptide GFs have enhanced affinity to heparin. This affinity aids in preserving the GFs in the ECM, localizing their acitvity, preventing their degradation, and increasing their binding to the surface receptors[4].

Of late, bio-molecules have been introduced directly inside the scaffold structure or within the scaffold biomaterial in a number of ways. Soluble growth factors can be encapsulated or integrated directly into the scaffolding process. Simple physical absorption of biomolecules on biomaterial or scaffold surface has been a frequently employed technique of GF administration in TE. Growth factors work by attaching to the extracellular domain of the targeted growth factor receptor, activating intracellular signal transduction cascades. Many growth factors were discovered that were expressed in the process of tooth formation and repair. These include bone morphogenetic protein (BMP), transforming GF (TGF), fibroblast GF (FGF), and vascular endothelial GF (VEGF).[5].

3. Scaffolds –

Donor tissue is removed and fragmented into individual cells for the construction of an autologous implant, and the cells are connected and cultured onto an appropriate substrate before being placed back at the desired spot of the working tissue. It may only take a relatively little quantity of donor cells to manufacture such implants because many isolated cell populations may be multiplied *in vitro* utilizing cell culture methods. It is thought, however, that isolated cells are unable to produce new tissues on their own. Most primordial organ cells are thought to be anchorage-dependent and necessitate a growth template[5].

Most natural tissues contain cells inside a tissue-specific, 3D ECM that is made up of a complex network of nanoscale fibers which produce highly organized local microenvironments. Cellular communication, transport of nutrients and O_2, waste elimination, and cellular metabolism all require an environment in which cellular orientation may be polarized and content movement can be directed. To accomplish the intended tissue function, TE needs a 3D framework to arrange cells into an extremely ordered assembly. Scaffolds can be utilized as pools of bio-active agents in conjunction with delivery cells, giving several benefits like a safe distribution profile, protection of bio-active compounds from degradation, and local administration of bio-active agents to specific areas[6].

III. FABRICATION OF SCAFFOLD FOR TISSUE REGENERATION

Following years of significant advancements, a set of novel tissue culture, replacement, and implantation techniques have been developed, allowing the fabrication of artificial extracellular matrices, known as scaffolds,that can carry SCs, GFs, or various biological agents aimed at repairing tissue function. Scaffolds are bulk bioactive compounds with appropriate porosity and structure that aid in the development of new tissues in order to achieve medical objectives. The TE process relies on using the porous structure of the 3D scaffolds in order to create an ideal environment for tissue and organ regeneration.

The scaffold provides the required support for cells to connect, proliferate, and sustain their differentiated function. Its architecture determines the eventual shape of newly formed tissue. The primary goal of scaffolds is to offer temporary mechanical stability at the defect location n until the injured tissue is healed or regenerated and normal biomechanical performance is restored. A scaffold also acts as a vehicle for cells, GFs, and other biomolecular signals. It is critical for the scaffold to imitate the structure and characteristics of human tissue in order to drive the macroscopic phase of tissue creation.

The scaffold provisionally supports cell regeneration. It then biodegrades either during or after the healing process and results in formation of a novel tissue of the required qualities. This biodegradation eliminates the process required to remove the tissue by-products thereby negating any harmful effects of a foreign body type reaction. The successful fabrication of fully operational scaffolds needs to be monitored at two stages:

(a) The **microscale** level: Must include an environment conducive to cell survival and function

(b) The **macroscale** level: Must allow seamless integration of multicellular processes, optimal supply of nutrients, and mechanical properties.

Several techniques have been used to fabricate 3D polymeric scaffolds with high porosity and surface area using biodegradable and bioresorbable materials. The scaffolds' purpose is to provide mechanical support for tissue restoration while the 3D cells undergo this evolution. In practice, 3D scaffold fabrication processes are primarily classified as traditional or rapid prototyping (RP), with each approach yielding different scaffolds with distinct properties[7].

A. Conventional fabrication technique –

Traditional scaffolding fabrication techniques involves developing a porous polymer framework like a cell adhesion substrate. However, it is challenging to build complicated structures to include an adjustable microscale and macroscale using traditional methods. Though scaffolds were initially designed to administer medication, they are now used in 3D cell culture for TE.

Method of fabrication	Technique
Solvent Casting and Particle Leaching	To dissolve a polymer solution, a solvent coupled with evenly dispersed salt particles of pre-determined size. The solvent evaporates and leaves behind a matrix of salt particles. After submerging the matrix in water, the salt leaches out, resulting in a structure that incorporates great porosity.
Freeze Drying	A synthetic polymer dissolved in a suitable solvent is used. The resultant solution is cooled below its freezing point, leading to a solid solvent. This is then sublimated to leave a solid scaffold that has multiple linked pores. At the freezing point, solutes can be isolated in their solid states to result in a small porous structure with a “fence” around them.

Thermal-Induced Phase Separation (TIPS)	It is a low-temperature approach to force phase separation by setting a homogeneous polymer solution at a high temperature in a lower-temperature environment, resulting in a polymer-rich phase alongside a poor polymer phase.
Gas Foaming	This approach pressurizes simulated biologically degradable polymers with water or fluoroform until they become completely saturated. Inert gas foaming agents like CO_2 or N are used. This approach often yields sponge-like structures with 85% porosity and a pore size of 30 to 700 μm.
Electrospinning	A typical electrospinning system has four major parts: a spinner with a syringe pump, a metallic needle, a high-voltage power source, and a grounded collector. The intensity of the electric field surpasses the surface tension of the droplet, resulting in a liquid jet that is constantly stretched and whipped by electrostatic repulsion until it lands on the grounded collector. During this process, the solvent evaporates and the jet solidifies to produce a nonwoven fibrous membrane.
Powder forming process	Green bodies are made from a slurry, which is a suspension of ceramic particles in an appropriate medium (water, ethanol). Fillers that consist of sucrose, gelatine, PMMA microbeads, and wetting agents are added to the ceramic solution. When the medium evaporates or burns out during sintering, porosity is generated.
Sol gel technique	The chemical process of inorganic polymerisation of metal alkoxides underpins this approach. Ceramic or glass materials may be manufactured in a number of shapes using the sol-gel technique.

Table 1 : Methods of conventional fabrication of scaffolds[7,8,9]

B. Rapid prototyping fabrication technique –

The RP scaffold construction technology opens up an array of possibilities for tissue engineering. For starters, the ability to independently regulate macroscale and microscale characteristics enables the creation of multicellular structures required for complicated tissue functions. Secondly, the manufacturing of 3D vascular beds will enable supporting vast tissue growth which may not be conceivable otherwise. Third, by merging clinical imaging data and 3D fabrication processes, it is possible to create custom-made scaffolds as well as bulk production of these designs. One fundamental advantage of such approaches are that they offer to create personalized and patient-tissue-specific designs.

Method of fabrication	Technique
Stereolithography	This has four major components: a tank containing photosensitive liquid resin, a transportable platform, a UV laser to radiate resin, and a dynamic mirror system. A UV laser is used to deposit a coating of photosensitive liquid resin on the platform. When the primary layer has solidified, the platform is lowered vertically. The initial layer is then covered with a second layer, and the procedure is continued till a 3D scaffold is formed. Then uncured resin is washed away, and the scaffold is post-cured under UV light. This step overcomes the issues associated with waste in subtractive production processes.
Fused Deposition Modeling (FDM)	Computer-controlled extrusion and layering are used to cast a melted polymer from a heated extrusion nozzle which is dispensed on a flat surface. The pre-programmed design is constructed from many layers of nearby microfilaments.
Selective Laser Sintering (SLS)	This method sinters powdered material identified by a 3D model in very thin layers using a laser for the power source. Because of the usage of laser, this technology is used to create a variety of materials such as polymers, metals, and ceramics.
Three-Dimensional Printing (3DP)	This is a method of swiftly establishing tools and functioning prototype features based on computer models. Material in its powder form is electively fused with an adhesive in an “inkjet”. Any excessive unprocessed powder is removed after the fabrication of 3D structure is completed.
Bioprinting	This method involves three-dimensional printing and is defined as "developing a biological pattern and assembling relevant materials, cells, molecules, tissues, and biodegradable biomaterials with a prescribed structure to achieve some biological functions." Fabricating structures for transplantation is now possible with solvent-free aqueous bases. These materials can be directly printed onto 3D scaffolds either with or without the seeding cells.

4D printing	Four-dimensional (4D) printing is a new word that emerged in 2013 and has sparked widespread interest in a variety of fields. 4D printing adds an additional dimension, time, to traditional 3D printed items, allowing materials to respond to appropriate stimuli or self-transform after possession. The excellent design of 4D printed materials' structure and components conceals the transformation code. Because the dynamic mechanical characteristic of printed material corresponds to the behaviour of real tissues, it has significant promise for tailored medical devices. Furthermore, because 4D printing is time-dependent, and is appropriate for long-term use implanted in the human body.

Table 2 : Method of Rapid prototyping of scaffolds[7,8,10]

IV. IMPORTANCE OF SCAFFOLDS MATRICES IN CELL DELIVERY

(i) Scaffolds allow cells to develop by seeding them within the porous framework of the scaffold or by migrating from adjacent tissue.
(ii) Scaffold matrices may be employed to produce high loading efficacy in cell transport to specified areas.
(iii) The scaffold must offer an appropriate substrate for cell adhesion, proliferation, differentiation, and migration.
(iv) To allow for the movement of biological signaling factors, nutrients, and wastes in order for cells to survive.
(v) Be reasonably easy to process and malleable into desired forms.
(vi) Minimal *in vivo* activation of immunological or inflammatory responses.
(vii) Extremely porous. Have a greater surface-volume ratio which allows optimal cell adhesion[11].

V. APPLICATIONS OF TISSUE ENGINEERING IN MEDICINE

Biomaterials have been utilized to regenerate tissues for augmentation, repair, or replacement during various TE applications. These biomaterials have found widespread use in bladder, tendons, ligaments, kidney, liver, heart valves, myocardial patches, bone, cartilage, pancreatic, cardiac, islet of Langerhans, vascular, and skin applications. Some of these advances are enumerated here.

A. Hard tissue

a) Bone TE –

In reaction to trauma, bones have the potential to regenerate, remodel, and repair. The creation and implementation of 3D porous scaffold with bone imitating properties was studied in order to avoid employing autograft, allograft, or xenograft owing to infection, immunological rejection, disease transmission, and a variety of other reasons. Hydroxyapatite(HA)-based bio-ceramic scaffolds have been developed and employed because they are biocompatible, bioactive, support and stimulate new bone development, and imitate the mineral component of normal bone[12].

These scaffolds are utilized to provide mechanical stability to a healing site, and also to support the osteogenic, osteoconductive, and osteoinductive properties of an original tissue. Calcium phosphate and hydroxyapatite (HAP) have the ability to mimic the interconnected osseous framework due to their crystallographic and biochemical resemblances to the natural tissues. Due to this reason, they are extensively incorporated into the base materials to enhance mechanical properties of the polymeric scaffolds[13].

b) Cartilage TE –

Cartilage is a human connective tissue (CT) made of chondrocytes encased within an extremely well hydrated ECM. Hyaline, elastic, and fibro cartilage with various ECM components have been created employing tissue engineering techniques for repair of cartilage, connecting cells via scaffolds, mechanical stimulation and stimulation of GFs.

TE employs scaffolds of diverse characteristics. These can be listed into 3 categories.

1. Naturally available – collage, gelatine, hyaluronic acid (HA)
2. Biodegradable polymers
3. Artificial polymers – PLA, PGA and PLGA

Among these, collagen is an extensively used substance of natural origin. According to ***Kose et al.,[14]*** regeneration occurred via chondrocytes and microporous PHBV, both *in vivo* and *in vitro* environments. A study conducted to analyse the ability of native as well as chemically altered bacterial cellulose to support primary chondrocytes reported that the altered scaffolds were good cell transporters for TE cartilage[15].

In cartilage tissue engineering, synthetic polymers are another often utilized material. After 20 weeks, PGA scaffolds seeded with chondrocytes create cartilage tissue with characteristics comparable to native cartilage. PLA scaffolds have the ability to stimulate the creation of cartilage-like tissue.

One of the materials explored for chondrocyte transplantation is poly (ethylene oxide) (PEO). Similarly, poly (N-isopropylacrylamide) (PNIPAM) is extensively researched for its thermoreversible properties.

B. Soft tissue

a) Nerve regeneration –

PNS axons demonstrate the ability to recover following nerve damage. However, when an axon is injured across a substantial length (more than 6 mm), it loses its regenerative capacity and requires external interference. A neuroinductive and neuroconductive nerve conduit is desirable. Scaffolds must help axon regeneration by providing physical support and growth factors.

Growth factors produced by microspheres, Schwann cells, and neural progenitors mimic the autograft conditions and enhance healing. Natural or synthetic collagen or laminin gels have also been created. Guidance is crucial in particularly anisotropic tissues such as the nervous system. To replicate this complicated architecture, patterned surfaces and fiber materials are employed.

Many aspects impact material selection, including degradation rate, swelling, biocompatibility, and the ability of incorporating proteins or neurotrophic agents in the ECM to boost cell adhesion and growth potential. PGA has been identified as one of the most effective materials for nerve regeneration. Hydrogels have additionally been considered as neural system regeneration scaffolds[16].

b) Muscle regeneration –

Due to mammals' restricted regeneration capacities, cardiac muscle regeneration is of special relevance among the three kinds of muscle tissue - cardiac, smooth, and skeletal.

Human ESCs and human induced pluripotent stem cells were used to generate heart muscle cells (cardiomyocytes) for the development of tissue-engineered products. Factors to be considered while building a heart muscle replacement include cell sources, type of cells, their seeding density and configuration of the culture medium used.

Scaffolds consisting of PCL and collagen nanofibers have been tested for smooth muscle tissue engineering. Cell development was guided by nanofiber direction, and the cells maintained a normal phenotypic shape.

Skeletal muscle has an enormous capability for regeneration in the absence of external intervention. Muscle tissue is continually destroying, repairing, and remodeling. However, in the event of severe damage, regeneration capacity may be reduced or lost. Hydrogels and fibrous meshes are popular biomaterials used as scaffolds for regenerating skeletal muscle tissue. Electrical stimulation was proven to enable higher differentiation potential of muscle precursor cells in the development of regeneration approaches based on diverse scaffolds for muscle tissue engineering[13].

c) Tendon and ligament regeneration –

Injuries to ligaments and tendons are fairly frequent and these are commonly managed by surgical restorative procedures. Unfortunately, this frequently results in ragged tissue deterioration. Under normal circumstances, tendons do not tend to recuperate even after a slight injury, and therefore, 3D scaffolds play a crucial role in their regeneration by offering an appropriate bio-environment and acceptable mechanical qualities like elastic modulus, toughness, and strength. Natural grafts frequently do not succeed in restoring the mechanical and structural qualities of original tissue.

Because of its poor healing capacity, the anterior cruciate ligament (ACL), which stabilizes the knee, receives a great focus in ligament TE. The PLLA coupled with silk strands has been studied for ACL restoration to approximate natural mechanical characteristics and to provide growth factors. ACL regeneration has also been explored using mechanically robust PLLA nanofibers mixed with flexible PCL nanofibers. This substance was shown to be capable of releasing GFs and promoting the proliferation of human mesenchymal stem cells (hMSC), and regenerating tissues expressed essential ligament markers[13].

d) Skin tissue engineering –

Autologous skin grafts are the most common and effective method of repairing skin abnormalities. This treatment involves removing skin from the normal healthy region and applying it on the wounded region. In addition to being a painful process, harvesting also produces morbidity at donor site. As a result, this procedure is not useful in conditions where the skin is damaged over more than half of the total body surface area (TBSA).

Furthermore, larger and deeper wounds are not amenable to treatment by autologous skin grafts as only a negligible area of the normal skin (<2% of TBSA) is suitable to receive a full-thickness skin graft. In such instances, skin TE solutions can be fabricated to sheath the injured skin. This offers the advantages of avoiding bacterial invasion, heat, and fluid loss, and aiding the process of healing, regardless of the size of wound.

Acellular or cellular skin replacements can contain autologous or allogenic cells. Acellular skin substitutes are often utilized to repair partial-thickness wounds. Allogenic cellular skin replacements are often intended to be temporary rather than permanent. Allogenic cell grafts provide wound covering and enhance wound healing, but these grafts perish after the repair process is complete. Biopsies of patient’s own tissue or the outer root sheath of hair follicles are used to acquire cells for autologous cellular skin replacements. In cell culture, cells are allowed to grow before being placed as epithelial sheets or mixed with biopolymers. Permanent skin substitutes are autologous cellular substitutes.

Even though scaffold-based skin replacements have been widely employed, they still have significant drawbacks, the most important of which is vascularization. The most typical method for vascularization is to introduce VEGF into the scaffold. A minimum of 0.4 mm thickness of the artificial skin is required for quicker establishment of vasculature. When a thicker graft is required, it can be placed in two instalments, which affords the initial layer sufficient time to establish circulation[13].

e) Vascular tissue engineering –

It is assumed that using natural tissues as blood vessel replacements will make the transplant more resistant and less immunogenic. To create acellular matrices for use as scaffolds, detergents and enzymes are employed to remove the cells and their surface antigens. Decellularization is used to recreate biological features in order to emulate natural vascular architecture since these materials offer mechanical, biological, and chemical advantages over synthetic materials.

The employment of such matrices in the construction of perfusable structures with repopulation by human cells is an option. Collagen has minimal antigenic, inflammatory, and cytotoxic reactions, making it a preferred natural scaffold material. Elastin, the major ECM protein of arterial wall, gives blood vessels flexibility and recoiling ability.[13].

f) Cornea tissue engineering –

Cornea is avascular and obtains its physiological needs from lachrymal fluids at the front and aqueous humour due to which it poses a challenge to replace it from donors which mostly results in transplant failure. The utilization of an appropriate scaffold and the patient's limbal cells to colonize the substrate was examined in recent tissue engineering research for a new cornea[13].

VI. APPLICATIONS OF TISSUE ENGINEERING IN DENTISTRY

Dental caries, traumatic dental injuries, and erosion of the tooth enamel and dentin are some of the pathologies that can lead to destruction of dental tissues. In a few instances, it could also result in the loss of a whole tooth completely. Traditional therapeutic solutions include repairing the damaged dental tissues or replacing the lost tooth with an artificial prosthesis[17]. Dental implants, such as titanium implants, have the advantage of rapidly restoring tooth function with a natural appearance[18]. They function via osseointegration, but lack the natural periodontium which buttresses masticatory forces. These drawbacks have spurred an ongoing search for alternatives to root canal treatment, homologous tooth harvesting for auto-transplantation, and prosthetic replacements.

Technological advancements in stem cell biology and TE have demonstrated the possible usage of DSCs in conjunction with biodegradable scaffolds containing bioactive mediators like GFs to regulate the temporal and spatial organization of dental progenitor cell proliferation, differentiation, and function. Tooth engineering incorporating epithelial-mesenchymal interactions might be used as a model system to assess tooth formation and also revitalization and reinnervation of bio-engineered teeth. In recent times, efforts of TE have focused on the creation of novel culture methods, partial or complete tooth regeneration, vascularization, and periodontal regeneration. The spatiotemporal expression and interactions of GFs, cytokines, and transcription elements direct macromorphological (crown size, tooth length) and micromorphological (cusp number and position, root formation) events in the development of teeth. To create bio-engineered teeth of a specific size and form, these interactions must be properly controlled.

The biomimetic design must recreate the actual tissue while preserving its morphological and functional properties for tissue engineering of any dental tissue. Scaffolds must have enough stability, biocompatibility, and bioprocess control to do this; and these traits are critical when considering cell signaling pathways. Furthermore, cells should have a suitable environment as well as increased adhesion for tissue development and no migration, and nutritional infusion must have access to the cell component into distinct scaffold gradients.[19].

a. Scaffolds for regeneration of pulp - dentine complex –

In spite of high clinical outcomes, the rate of standard root canal treatment (RCT), other implications must be addressed. For instance:

1. The esthetics of the dental crown in an RC-treated tooth are damaged by mechanical tissue loss or crown discoloration due to staining from the endodontic filling material.
2. The structural integrity of an RC-treated tooth may be jeopardized due to tooth structure loss and later restorative operations.
3. Because pulpless teeth lose a certain amount of their ability to detect environmental changes, the advancement of caries may go unreported by patients.

4. Long-term research has indicated that an RC-treated tooth lose more than non-treated teeth due to subsequent caries and related difficult restorative challenges.
TE and biotechnology advances have led to innovative opportunities to develop biological approaches for pulp treatment targeted at in-situ rejuvenation of partial pulp or *de novo* synthesis of entire pulp replacement.
(1) An attempt to harness the pulp's natural regenerative potential, i.e., stimulate host cells from the apex of the root to migrate inside the root canal; or
(2) Replacing the whole pulp tissue via transplantation of *in vitro*-designed pulpal tissue[19].

b. Cell homing

In the field of regenerative endodontics (RE), the American Association of Endodontists (AAE) is attempting to regenerate dental pulp, emphasizing the continued and complete growth of the tooth root in an injured young permanent tooth. The present objective of RE is to regain the life of dental pulp in growing permanent teeth and to allow root formation that might otherwise be halted. Their research has resulted in a method known as cell homing.[20].

Cell homing is defined as an active method of attracting endogenous cells, including stem/progenitor cells, to a specific anatomical region. When compared to traditional cell transplantation, the chemotactic cell homing regenerative procedure provides an alternate way for pulp regeneration. A similar strategy was proposed for in situ pulp and periodontal tissue regeneration. Various bioactive cues were absorbed or incorporated into biomaterial scaffolds in this approach. When these cues were released into RC-treated tooth root canals, local and/or systemic cells, including stem/progenitor cells, moved and homed *in vivo* into the root canal, resulting in pulp regeneration.[21].

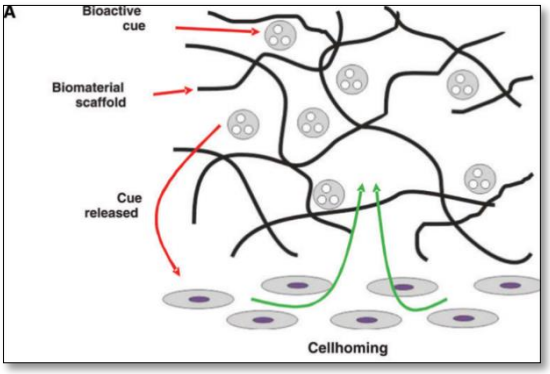

Figure 3: Infusion of bioactive cues in scaffolds

(A) Bioactive cues in biomaterial scaffolds can be adsorbed, anchored, or encapsulated. Local and/or systemic cells, can be concentrated *in vivo* into an anatomic segment that acts as a native scaffold following the release of bioactive signals, such as from RC-treated canals[22].
(B1) Conventional RCT of diseased dental pulp
(B2,B3) Obturation of gutta percha necessitates clear access, and hence significant enamel and dentin structures must be removed.
(B4) De-vitalized tooth

(B5,B6) Because the distribution of injectable bioactive cues does not need unimpeded access to the pulp chamber and root canal, the damaged tooth pulp is treated using a redesigned, less invasive root canal therapy. Although residual inflammation in the RC-treated tooth root canal and the peri-apical area is expected to hinder pulp regeneration, the chemotaxis-induced angiogenesis might potentially aid in offsetting any remaining infection.
(B7) Vital tooth with regenerated dental pulp.

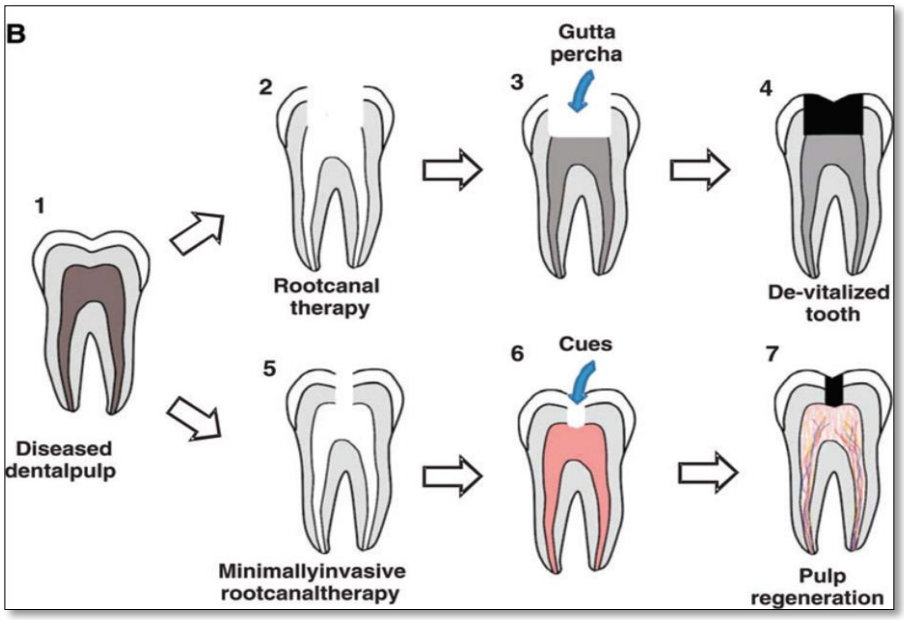

Figure 4: Advantage of cell homing over conventional RCT

c. Scaffolds for periodontal tissue engineering –

Periodontal regenerative treatments aim to repair damaged periodontal tissues, such as those caused by trauma, tumors, or inflammation, by stimulating the regeneration of bone and/or restoring tooth attachment. The periodontium's complicated structure provides a difficulty in periodontal TE since it consists of both soft and hard tissues that must all be rebuilt surrounding the tooth to attain functioning.

Furthermore, the formation of novel tissue requires relocation and proliferation of specific cells to the site of injury and a substrate that supports the development of novel tissue along with signalling molecules and GFs to form a vascular system that supplies O_2 and nutrients[19].

d. Scaffolds for gingival tissue –

Gingival tissue reconstruction is a difficult periodontal procedure. The ultimate aim of periodontal therapy is tissue regeneration, but this is difficult to achieve since inappropriate treatment causes scarring, which has negative consequences on functionality and esthetics.

Guided gingival tissue regeneration:

Guided tissue regeneration (GTR) is a therapeutic technique used in periodontal therapy. The idea behind directed tissue regeneration is to prevent epithelial and gingival connective tissue (CT) cells from migrating into already damaged root surfaces. A fundamental component of GTR is the creation of a physical barricade to restrict apical migration of epithelial and gingival CT cells. Because soft tissue turnover is rapid compared to osseous tissue, a barrier scaffold is employed to avoid soft tissue invasion, stabilize and maintain graft materials, and slow the pace of graft resorption. The GTR technique is used to treat periodontal TE and peri-implant disorders.

One of the most significant requirements of a TE scaffold in regenerative dentistry is encouraging the growth of novel osseous or gingival tissue. Conductive scaffolds are the name given to these scaffolds. This matrix promotes cell adhesion, proliferation, and differentiation. Gingival scaffolds are classified into two types:

Non resorbable gingival membrane:

There have been many non-resorbable gingival membranes used for regeneration. Polytetrafluoroethylene (PTFE) scaffold, frequently used for bone regeneration, was initially created in 1958. It is made up of an inner cell occlusive region and an outer cell adherent area. As a result, these scaffolds are able to exclude migration of CT cells.

Expanded polytetrafluoroethylene (ePTFE) based scaffolds –

- Most conducive for gingival regeneration.
- Titanium reinforcement are indicated for gross anatomic defects.
- Disadvantages –
    1) Their effectiveness is compromised due to the increased incidence of early exposure to oral environment.
    2) Removal should be done after 5 – 8 weeks.

Non restorable polytetrafluoroethylene (nPTFE) based scaffolds –

- Barrier material is designed to allow molecules of very small dimensions to infiltrate.

- Minimal risk of microbial or soft tissue infiltration.

Drawbacks of ePTFE and n-PTFE scaffolds.

1) Healing of soft tissue fenestration defects are inhibited.
2) Lesion size and intensity of inflammation increases with time.

Dense polytetrafluoroethylene (dPTFE) based scaffolds –

- Does not mandate primary closure following bone grafting which minimises patient mortality rate.
- Preserve hard and soft tissue in extraction sites[19]

a) Resorbable gingival membranes:

i. Collagen –

This is an extensively used biomaterial that is essentially a fibrous protein derived from animal tissues using diverse procedures. Depending on the technical characteristics, the absorption rate ranges from 6-8 weeks to 6-8 months. Collagen attracts and stimulates gingival fibroblasts, as well as stimulating DNA synthesis in fibroblasts. Collagen scaffolds have been shown to improve flap stability, vascularization, and epithelialization. Fenestration flaws are virtually entirely repaired using collagen scaffolds. Inflammatory cell infiltration is modest inside and surrounding collagen scaffolds.

Wang et al.[23] proposed ideas for stabilising and holding the collagen scaffolds in place with alkaline phosphatase, bioactive glass or HA nanoparticles. These additions aided in controlling scaffold deterioration and improving osteogenic potential. Studies conducted by ***J.Kozlowska*** *and* ***A.Sionkowska***[24] demonstrated that the proportion of deterioration is greater in non-cross-linked collagen scaffolds than cross-linked ones. They have limitations in providing adequate room for wound healing, lack of control over the rate of resorption, risk of disease transmission, and other ethical concerns.

ii. Poly(lactic-co-glycolic acid) (PLGA) –

PLGA is a polylactic acid (PLA) and polyglycolic acid (PGA) copolymer with high biocompatibility, controlled biodegradability, and mechanical qualities. Because PLGA is moderately hydrophilic, it can be used to create gingival scaffolds. PLGA scaffolds enhance the growth of human PDL cells in gingival tissues. Because of the good biocompatibility, it spreads better on a collagen-coated scaffold than on the uncoated one as shown by ***Sadeghi et al,***[25]**.**

PLGA encourages osteogenic differentiation of PDL cells. The proportion of degradation of PLGA can be controlled by altering its lactic acid (LA) to glycolide (GA) ratio. Furthermore, properties like biocompatibility, biodegradability and ease of manipulation make the PLGA suitable for periodontal GTR.

iii. Oxidized cellulose mesh barriers –

These belong to the group of resorbable hemostatic materials. Their acidic composition is beneficial in GTR process. The oxidized substance transforms into a gelatinous form and combines with the blood clot resulting in a scaffold, majority of which is absorbed within one week after the procedure.

Disadvantages –

Limited wound space and delayed healing.

iv. Chitosan –

Deacetylation of chitin, a polysaccharide, produces this widely utilized biomaterial in tissue engineering. Chitosan is both osteocompatible and osteoconductive. It possesses antibacterial characteristics, hemostatic qualities, wound-healing capacity, and biocompatibility. Studies conducted by ***Qasim et al.,***[26] had shown that Chitosan and hyaluronic acid synthetic scaffolds have promising applications in regeneration of periodontium.

v. Alginate –

This natural polysaccharide derived extracts of brown algae (Phaeophyceae) is frequently employed in gingival tissue engineering. To precipitate alginate, the extracted substance is filtered and combined with calcium chloride. The properties of alginate have greatest impact on mechanical strength, biocompatibility, adherence of cells, proliferation, and osteogenic differentiation. Alginate is non-toxic and hence physiologically friendly, making it appropriate for a variety of biomedical applications.

vi. Fibrin –

Natural biomaterial extracted from a patient's blood. Fibrin scaffolds have the ability to create an angiogenesis-friendly environment. They are biodegradable and biocompatible. Other advantages include excellent seeding effectiveness, homogenous cell dispersion, and adhesion capacity. Fibrin hydrogels are fibrin scaffold subtypes utilized in TE of the hepatic, integumentary, cardiovascular and osseous tissues. Low mechanical characteristics, rapid deterioration, and gel shrinkage are a few drawbacks.

Fibrin glue is made from pure fibrinogen and thrombin. This serves as a transport vehicle and scaffolding matrix in TE. It was employed in maxilla-facial bone and periodontal TE. Fibrin glue can be modified mechanically by including polymers such as gelatin, hyaluronic acid, and chondroitin 6 sulfate.

Scaffolds that promote enamel mineralisation

Enamel is the outcome of a very sophisticated, organized, and unique natural process, and the enamel organ leads the entire process in early stages. Mature enamel has both organic and inorganic components. It is feasible to identify 25%-30% protein in the early stages. Proteins are very likely to have a key function in enamel mineralization and maturation. Amelogenin and enamelin are the two main proteins found in developing enamel. HA is a component of mature enamel. The other constituents include proteins and peptides (< 0.1%) along with very minimal amounts of lactic acid, citric acid, and phospholipids.

Commonly used materials for repairing faulty enamel include amalgam, composite resin, and ceramics. These lack the qualities of natural enamel which results in significant disparity, the efficiency of reparative materials is often inferior to that of natural enamel. Due to the great specificity of enamel, it is evidently difficult to discover a viable approach to enhance artificial manufacture of this substance. Polymeric hydroxyapatite composites are more typically employed for enamel biomimetic constructions.

The primary biomaterials used as enamel scaffolds are:

- Decellularized tooth bud
- Nano HA
- Polymers/HA composites like PLLA, PGA, PLGA, PEG and PLC.
- Collagen/apatite
- Silk
- Polysaccharides.
- Gelatin[19]

Biomimetic methods

The scaffold in this process is built first by natural enamel and then with freshly synthesized HA. This appears to be a quasi-bidimensional dynamic scaffold. Biomaterials for biomimetic operations are created in the lab under extremely similar physiological microenvironment circumstances to those found in ECM. To induce mineralization, the system should be designed with a unique membrane that permits unidirectional flow of Ca^{+} ions and traps synthetic amelogenin protein or ECM between the two membrane layers. Amelogenin derivates or its constituents must be applied to the eroded enamel surface and allowed to generate HA for a period of time.

Chung[27] used triplet repeats of asparagine–serine–serine peptide (3NSS) to control enamel remineralization. The process provides HA recrystallization and significantly enhances nanohardness and elastic modulus in treated lesions. ***Zhou et al.***[28] developed an elastin-like polypeptide (ELP)–assisted process for *insitu* synthesis of artificial enamel in simulated oral fluid.

Mukherjee[29] used full-length amelogenin (rP172) and leucine-rich amelogenin peptide (LRAP) to promote remineralization of enamel, improve biointegration and mechanical strength. ***Dogan et al.***[30] worked with shADP5, a 15-amino acid-long amelogenin-derived peptide and reported high peptide-guided remineralization of white spot lesions in the enamel from human teeth samples.

Self-assembling peptide methods

Researchers at the University of Leeds developed a procedure in which self-assembling peptides were used to replicate the enamel matrix inside enamel lesions. These substances have an effect on the spontaneous bending of proteins and creation of DNA double helixes. Here, eroded enamel is infused with monomeric P114 that diffuses into the underlying lesion body through the pores of hypermineralized plate which then forms a 3D matrix spontaneously via H_2 bonding. Then crystallization begins and the salivary $Ca_3(PO_4)_2$ crystallizes across the matrix, producing new enamel. The procedure filling is critical because high efficiency remineralisation requires reaching the bottom of an eroded hole.

Regeneration of enamel using HA as basement method

This approach employs HA nanoparticles, HA nano compounds, $Ca_3(PO_4)_2$, etc., to plughole damaged enamel. The primary vehicle is toothpaste with a concentration of approximately 5%.

Natural or semisynthetic scaffold with stem cell methodology

When using natural scaffold with stem cells, an enamel matrix derivative (EMD) is always required to facilitate reseeding and differentiation. The procedure begins with stimulating cell proliferation and ligament creation. The physicochemical properties of the substance utilized scaffolds control EMD protein precipitation. Furthermore, the temperature should be adjusted at 37°C, and optimal pH should be between 3.9 and 4.2.

Synthetic scaffolds

These require natural remineralization of HA from saliva to achieve enamel repair. The main issue with this technology is its inherent restricted adsorption of scaffolds and the challenging toxicity analysis due to freshly generated compounds; also, the procedure is extremely time-dependent.

Scaffolds for dental cementum

Cementum is an important component of the tooth, since it connects the tooth to surrounding bone and repairs small root injury. Because the cementum lies beneath the gingival tissue, total healing of cemental defects is impossible. As a result, substantial research is being conducted to develop appropriate approaches for dental repair utilizing TE scaffolding. The difficulty is to create a biomaterial that allows for high porosity, mechanical strength, and cell proliferation inside the scaffold, and also assures biological compatibility and doesn't get rejected by the body, producing unpleasant effects.

Various biomaterials used for cementum scaffolds

Nonrigid biomaterials

Because these biomaterials may dissolve in water, they must be combined with additional materials, such as HA or CaP, to produce a 3D structure. Non-rigid materials are commonly in the form of hydrogels, that can be infused rather than implanted into tissue. Hydrogels are ideal for injecting into tooth cementum where they transform into gel state and envelop adjacent tissues.

Poly ethylene glycol (PEG)

PEG has a continuous or branched chain framework. Flexibility can be modified by modifying its cross-linking properties which makes it ideal for tissue development and cell envelopment. It may be linked together with covalent PEGs, resulting in a greater H_2O content and the production of hydrogels. PEG does not cause an immunological reaction in the body and is biocompatible.

Hyaluronic acid

This is a linear polymer of glycosaminoglycan present in the ECM of most mammals and in trace levels in cementum. It binds with GFs as well as cellular and extracellular components, assisting the tissue in maintaining structure and homogeneity. It is biocompatible and seldom causes an immune response; nonetheless, it degrades quickly and has little mechanical strength. Adding hydrophobic group to the chemical group of hyaluronic acid reduces degradation and boosts the structural integrity of scaffolds.

Chitosan

This is a biodegradable and biocompatible polysaccharide that serves as an excellent biomaterial that is unlikely to cause the human body to reject or have a cytotoxic reaction. Chitosan is a versatile material that may be shaped into hydrogels, nanofibers, sponges, and bead-like scaffolds. Collagen type 1 was employed in conjunction with chitosan, seeding epithelial-mesenchymal cells into its matrix, concluding that this combination enabled mesenchymal-derived dental pulp stem cells and HAT-7 epithelial cells to relocate and evolve. Its non-solvent nature minimises its use as a scaffold without chemical alteration.

Silk

Silk protein molecules have strong cellular biocompatibility and biodegradability. Conversely they have a tiny pore size and strong mechanical control. Furthermore, primary portions of natural Bombyx mori silk fibroin (heavy chain and light chain) exhibit varying physical and mechanical characteristics and may be separated using formic acid to facilitate additional scaffold production. Such scaffolds were evaluated using EMD and/or iPSCs to see how well they might heal a periodontal lesion. Silk-fibroin scaffolds with CaP coating demonstrated great porosity, good biodegradability, and increased bone contact, allowing bone MSC stimulation, with the combination of iPSCs and EMD stimulating formation of cementum, alveolar bone, and PDL.

Rigid biomaterials

Rigid biomaterials can operate as structural substitutes to bear stresses from tooth movement; however, a firm biomaterial may not be essential since it is too rigid to enable bonding to natural tooth cementum. Structure rebuilding at a defect location with both cementum and alveolar bone requires a stiff scaffold material.

Polylactic acid (PLA)

PLA is a synthetic polymer that is both biocompatible and biodegradable. When PLA degrades *in vivo*, it produces lactic acid, which is normally eliminated by metabolic activity. PLA is hydrolyzed without the need of enzymes. The primary component of PLA (lactic acid), is easily available in nature and can be manufactured by fermenting sugar cane.

Polylactic-coglycolide (PLGA)

It is a synthetic polymer created by ester bonding from a mixture of PLA and PGA. Individually, the mechanical properties of these materials are weak. They quickly degrade and may lead to structural collapse. When used as a mixture, the PLA/PGA ratio may be modified to enhance or reduce degradation rates. A combination of PLGA and CaP has been used to minimise inflammation during scaffold implantation[31].

Polyglycolic acid (PGA)

PGA is a synthetic biomaterial with poor mechanical strength that degrades fast, is hydrophilic, and extremely crystalline. When glycolic acid degrades, the metabolic processes eliminate it, resulting in its excretion through urine. It is a superior material to culture DPSCs and provides tissue of greater density and enhanced collagen deposition.

Scaffolds for engineering tooth – ligament interfaces

The periodontium complex is anatomically unique. It interconnects hard and soft tissues of teeth to the surrounding alveolar bone. Periodontitis is an inflammatory condition caused by bacterial invasion that results in the annihilation of soft and hard tissue. Current clinical plaque control methods are limited by partial tissue reattachment without actual renewal of PDL attachment. Hence, a novel idea known as**"**Guided Tissue Regeneration**" (**GTR**)** was introduced. Following root debridement, a barrier membrane was placed over defective root tissue which allowed selective precursor cells into the defects and limits invasion of gingival epithelial cells. This technique has several limitations leading to poor clinical repeatability, predictability, and the absence of completely functional regeneration. Furthermore, reliable periodontal regeneration remains uncertain. The current advances in this area have pushed for the use of tissue-engineered concepts, which may be able to overcome the limitations of GTR and accomplish proper periodontal regeneration[19].

Scaffolds for periodontal regeneration:

Monophasic scaffolds

The most fundamental use of TE technology toward periodontal regeneration is monophasic scaffolds. A unique, accurate, and customizable shape, possible cellular and GF transport, ability to from ECM, blood circulation along with prompt breakdown are all necessary for adequate scaffold application.

The great majority of tissue engineering techniques targeting periodontal regeneration that use monophasic scaffolds have evolved from GTR. Therefore, scaffolds are fabricated to retain space and the entire extent of the defect while concomitantly facilitating cellular proliferation. Although monophasic scaffolds are efficient, comprehensive functionality requires a complicated regeneration process necessitating a multiphasic fabrication method.

Multiphasic scaffolds

The advent of additive biomanufacturing using 3D printing ushered in a new era of scaffold fabrication. Combining these with Computer-aided designing (CAD) methods enabled the creation of scaffolds with changeable structures at the micro- and mesoscale. Such multicomponent constructions are seen as a remedy to several shortcomings of previous techniques. Greater structural control permitted mimicking of periodontal segmented tissues, giving rise to a new generation of "multiphasic scaffolds" which can be categorized as:

1. Biphasic – PDL and alveolar bone
2. Triphasic – PDL, alveolar bone and cementum

Tissue engineered alloplastic scaffolds for reconstruction of alveolar defects

Alveolar bone deficiencies can be caused by a variety of factors. The craniofacial area accounts for approximately 1/3rd of all birth abnormalities. Extensive alveolar abnormalities can cause substantial quality-of-life concerns, such as social stigma, nutritional inadequacy, and speech difficulties. Alveolar bone loss typically results in a defect in any one dimension or a combination of the three dimensions. Therefore, reconstruction should be accomplished in all three dimensions to restore masticatory function via dental implants[19].

Additive manufacturing of synthetic biomaterials for alveolar bone regeneration

While several alternative biomaterials may be used for bone TE, bioactive $Ca_3(PO_4)_2$ is biocompatible and has widespread applications. Its compounds have been used as substitutes to autogenous osseous transplants. Till recent times, HA is the most often used substance for osseous replacement. Ceramics have had poor results in bone replacement and their massive prefabricated forms render them difficult to use in small areas such as periodontal osseous defects. However, 3D printing enables creation of tailor-made fabrications of desired dimensions.

Pediatric alveolar cleft defect regeneration

An estimated 75% of patients with cleft lip and palate have an osseous imperfection due to which they have nasal regurgitation via a nasolabial fistula, insufficient osseous support for the teeth, and facial asymmetry. Autogenous bone grafting manages such defects and is frequently done during the mixed dentition period (6-11 years of age). Successful interventions result in structural support for tooth eruption, closure of the nasolabial fistula, better speaking capacity, and augmentation of the dental arch for improved esthetic results. In spite of outstanding long-term results in certain cases, this treatment is accompanied by donor-site morbidity. Graft resorption necessitates re-surgery in about 12-40% of individuals. Additionally, larger clefts have worse outcomes.

Based on encouraging preclinical results in research for reconstructing mandibular abnormalities, use of dipyridamole-loaded bioceramic scaffolds for alveolar defects in translational skeletally undeveloped rabbit models has been studied. The reformative characteristics of 3D-printed scaffolds containing dipyridamole to stimulate dose-dependent bone formation in rabbit alveolar bone were established in this investigation. Furthermore, bone growth extended the whole length of previously formed defect.

Nano-indentation examination of bone quality exhibited improved bone healing compared to bone grafts, with new bone present both inside the scaffold and at the junction between osteotomy and construct. In addition, this newly formed bone was exceptionally cell-rich and vascularized. Additionally, these results indicated that the scaffold's intrinsic osseoconductivity was successful and was aided by dipyridamole's local impacts, but was not swamped by dipyridamole's osteogenic potential.

The premaxillary-maxillary suture remained patent even at dosages one to two logarithmic increments higher than those required to stimulate bone formation in young patients. This is in stark contrast to substances like rhBMP-2, which produce a wide range of side effects, including premature suture closure, and are hence contraindicated in young patients according to FDA recommendations. Long-term studies have since been carried out to evaluate results of subsequent scaffold implantation.

The first results of a six-months study comparing DIPY-coated 3D-printed -TCP to autologous bone transplant were found to be promising. Bidirectional augmentation were visible at 6 months, with significant scaffold degradation compared to 8 weeks. The repair of bone was comparable in experimental and control animals that received an autologous bone transplant. Importantly, craniofacial growth was equivalent between 3D-printed -TCP scaffold and autologous bone graft control animals[19].

Whole tooth reconstruction

Tissue engineering has mostly been involved with regeneration of tooth-ligament-bone interactions in periodontics. However, much study has been undertaken on the exciting concept of bioengineered teeth. Whole tooth TE is based on recapitulating complex, integrated natural events that occur during tooth development. The essential prerequisites of dental TE are the same as for other organ engineering, which entails the fabrication of scaffolds, insertion of cells and/or GFs, and implantation in a human host.

Whole-tooth regeneration entails completely reconstructing the function and morphology of the tooth. A mature PDL fiber attachment is also required to incorporate the resulting tissue into the mandible. Whole tooth regeneration was first attempted by ***Young et al,.***[32] via seeding a PLLA monophasic scaffold with dissociated tooth bud cells of porcine origin in a single-cell suspension. They succeeded in generating several small tooth-like structures that had dentine and enamel-specific proteins. Though this attempt could not successfully fabricate a complete viable tooth, it was the first true example of tissue-engineered tooth-like structures being generated under *in vivo* conditions. These researchers further improved their attempt by merging the *in vivo* and *in vitro* techniques to develop a PGAPLGA hybrid tooth-bone scaffold by integrating osteoblasts and tooth bud cells. This was then combined with the osteoblast-seeded scaffold and implanted. They succeeded in developing tooth-like structures with histologically validated enamel and dentine along with type III collagen between the fabricated tooth and bone[32].

Honda et al,.[33] pioneered single tooth germ regeneration. They established a method to regulate the interaction between cells via seeding of mesenchymal and epithelial cells in a collagen scaffold which led to the formation of enamel-dentine complex. The entire process took significantly lesser time (about five weeks lesser) then the earlier techniques. Additionally, the resultant fabrications closely resembled the original teeth.

Teeth as natural scaffolds for TE

Decellularized scaffolds retain internal form, structure, and biochemical make up of the natural tissue along with the elimination of cellular components which reduces immune-rejection. Decellularized teeth have been used as a bioactive 3D ECM scaffold and resulted in odontogenic differentiation in hPDL stem cells and hDPSCs[34].

To identify the optimum cell mix for entire teeth bioengineering, ***Zhang et al,.***[35] examined *in vivo* decellularized tooth buds seeded with several cell types. After being seeded with human umbilical vein endothelial cells, pig dental epithelial cells, and human dental pulp cells, the recellularized tooth buds showed improved regeneration when compared to the experimental and control groups.

VII. CONCLUSION

The tooth is a complex biological structure that necessitates an intricate cascade of molecular signals and gene expression for its growth and development. Comprehending the molecular mechanisms and combinations of different growth factors and gene expression that reinforce this process is crucial for tooth regeneration. Importantly, one of the major hurdles in stem cell research is successfully differentiating dental stem cells into particular lineages. Tissue engineering based on SCs is a potential strategy for repairing dental and maxillofacial structural integrity. Tissue engineering advancements may potentially aid physicians in the restoration of injured tooth structures with tissue-engineered scaffolds in the future, rather than replacing these structures with acellular lumps of restorative material that have no inherent healing capabilities whatsoever.

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