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

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**ABSTRACT**

Tissue engineering is a hybrid technology that combines biology and materials science. Tissue engineering and regenerative medicine are among the most promising cutting-edge applications that require the development of biomaterials to assemble functional constructs that restore, maintain, or improve damaged tissues or whole organs. Hence, these areas are focused on the development of biomaterials that mimic the physicochemical microenvironments of cells and tissues. This field of medicine holds great promise for improving the health and quality of life for millions of people by developing functional substitutes for damaged organs and tissues, including bone repair, cardiac tissue, cartilage, and lung tissues. Development in tissue engineering can eliminate the problems related to organ transplantations, such as rejection or lack of suitable donors. The advancement of tissue engineering has also given a promising opportunity for better clinical practice in treating dental patients especially in the fields of endodontic and periodontal tissue as well as whole tooth regeneration. Dental pulp contains highly proliferative cells that can be activated upon injury and undergo proliferation and differentiation toward osteoblastic phenotypes to provide for dentin repair. The easy access, proliferation capacity, and multidirectional *in vivo* / *in vitro* differentiation makes oro-facial SCs an important source of SCs for use in regenerative dentistry and medicine. The main aim of the present topic/ chapter is to discuss various components of tissue engineering and regarding its components. Scaffolds being an integral component for tissue regeneration, is discussed in detail regarding its fabrication and applications along with light on medical and dental applications of tissue engineering.

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

1. **INTRODUCTION**

The advent of tissue engineering has been motivated by the challenge of producing tissue substitutes that can restore the structural features and physiological functions of natural tissues *in vivo.* The definition of tissue engineering was demarcated in **1993** by **Robert Langer** and **Joseph Vacanti**. It is defined as “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 tissue engineering paradigm is to isolate specific cells through a small biopsy from a patient; to seed the cells under controlled culture conditions to grow artificial tissue that becomes interconnected porous structures called scaffolds, which are capable of supporting three-dimensional tissue formation; to deliver the construct to the desired site in the patient's body; and to direct new tissue formation into the scaffold, which may be absorbed over time. In most cases, biocompatible, degradable polymers are utilised to induce surrounding tissue ingrowth or to serve as temporary scaffolds for transplanted cells to attach, grow and maintain differentiated functions. This field of medicine holds great promise for improving the health and quality of life for millions of people by developing functional substitutes for damaged organs and tissues, including bone repair, cardiac tissue, cartilage, and lung tissues. The first achievement of tissue engineering is based on the generation of skin substitutes for burned patients.

Tissue engineering has gained importance over a few decades due to the drawbacks in traditional tissue or organ transplant such as insufficient number of donors, traumatic procedures and rejections. Development in tissue engineering can eliminate the problems related to organ transplantations, such as rejection or lack of suitable donors. It also eliminates the use of materials with a relatively low biocompatibility, and therefore, decrease in postoperative complications and tissue rejections is observed. A major advantage of this approach is that tissues can be designed to grow in such a way that they match precisely the requirements of the individual in terms of size, shape, and immunological compatibility, minimising the need for further treatment.

1. **COMPONENTS OF TISSUE ENGINEERING**

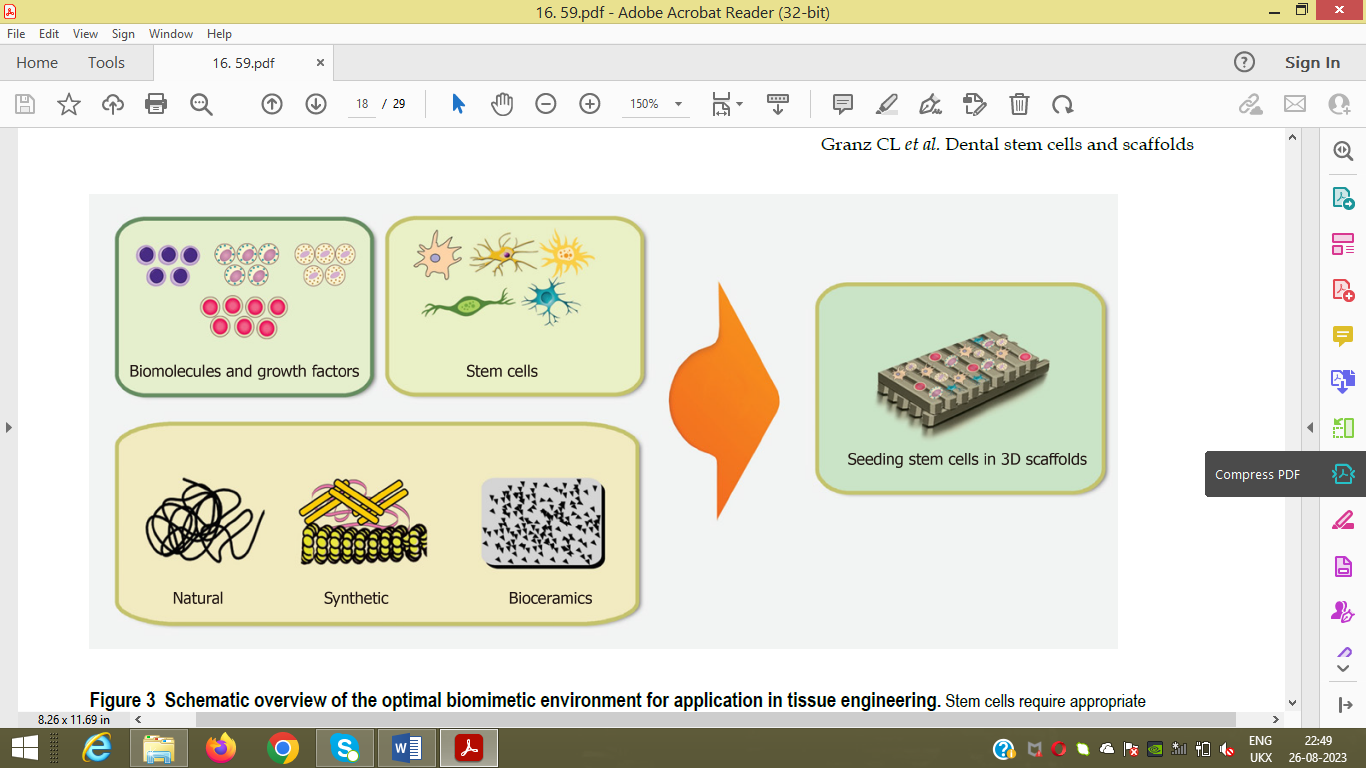
Tissue engineering is classically based on three pillars:

(a) The cells (stem cells/progenitor cells), responsible for synthesizing the new tissue matrix;

(b) The signalling/growth factors necessary to promote and facilitate the functionalities;

(c) The biomaterial scaffolds, necessary for cell differentiation, multiplication, and biosynthesis, that act as an extracellular matrix.

These three things (scaffolds, cells, growth factors) are known as “the tissue engineering triad”, and this system is set up in an appropriate environment in a bioreactor1.



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

1. Stem cells –

Stem cells are undifferentiated cells with self-renewing and clonogenic capabilities, which can differentiate into various cell lineages.

According to the basis of their origin, stem cells are categorized as

1. Embryonic,
2. Induced pluripotent stem cells (iPS), and
3. Adult (tissue-specific) stem cells.

Based on their differentiation potential, stem cells can be classified as

1. Totipotent (the ability to give rise to all types of cells),
2. Pluripotent (the potential of the cells to produce any type of cells in the organism),
3. Multipotent (the potential to give rise to cells of their tissue of origin),
4. Oligopotent (the potential to differentiate into only a few cell types), and
5. Unipotent (the ability to produce one cell type).

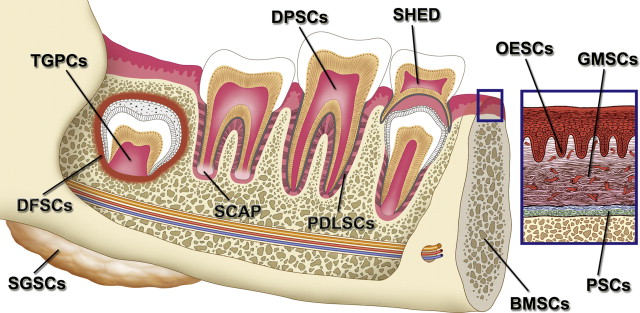
Embryonic stem cells are pluripotent, whereas adult stem cells are limited to differentiating into various cell types of their original tissue (multipotent)2.

**Dental stem cells -**

Dental stem cells (DSCs) are neural crest-derived cells that can be obtained easily from dental tissues of both adults and children; therefore, they are a reliable, accessible source of autologous stem cells. DSCs are undifferentiated cells that have non-limited self-renewal, multipotent differentiation potential, and colony forming capacity. DSCs can be isolated from the dental pulp of deciduous, natal, and permanent teeth, the periodontal ligament, the apical papilla, the dental follicle, and gingival tissue. One of the unique characteristics of DSCs is their ability to differentiate into mesodermal, ectodermal, and endodermal cell lineages. DSCs from each source are capable of specifically differentiating into various distinct cells, including epithelial cells, odontoblasts, osteoblasts, chondroblasts, adipocytes, vascular cells, endotheliocytes, neuronal cells, glial cells, photoreceptor cells, and muscle cells. Although all stem cells obtained from various sources are named DSCs, their phenotype, differentiation potential (both in *in vitro* and *in vivo* conditions) and functional properties (such as biological response during differentiation and tissue repair) are different.

For instance, stem cells obtained from the apical papilla possess greater proliferation ability, express a higher variety of neural markers, and induce more uniform dentine-like tissues compared to dental pulp stem cells. Furthermore, DSCs isolated from exfoliated deciduous teeth exert a higher capacity for osteogenic regeneration and a greater proliferation rate compared to dental pulp stem cells. DSCs isolated from pulp tissues are the first and most frequent cells evaluated for their odontogenic, osteogenic, and neurogenic differentiation potentials. This heterogeneity of DSCs is effectively modulated by the function of their microenvironment. DSCs obtained from different sources exhibit various patterns of cell surface markers2.

The microenvironment, which is a three-dimensional (3D) structure surrounded by specific cells and ECM, protects stem cells from inappropriate differentiation, cell damage, and apoptosis and governs tissue maintenance, regeneration, and repair. In addition to providing a physical microenvironment for cells, the ECM gives the tissue its mechanical properties (elasticity and rigidity), provides bioactive molecules and cues to residing cells, and establishes an environment to facilitate tissue remodelling in response to dynamic processes, such as wound healing. Stem cell behaviours are reciprocally regulated by the ECM and signals from the surrounding cells and molecules. The induction of odontoblasts, the biological cells of neural crest origin that survive throughout life, occurs during tooth development. However, under appropriate conditions, DSCs can differentiate into pre-odontoblasts and later secretory odontoblasts, which actively participate in reactionary dentinogenesis.



**Figure 2**: Different sources of dental stem cells

1. Growth factors –

Growth factors (GFs) are molecules capable of stimulating a variety of cellular processes including cell proliferation, migration, differentiation and multicellular morphogenesis during development and tissue healing. Growth factors are an important component of tissue engineering as they can provide a large number of biological signalling molecules to a given microenvironment, mobilize seed cells and peripheral cells *in vivo* to adapt quickly to the implantation bed’s environment, and activate tissue repair. With the aim of improving and streamlining the preparation methods, platelet-rich fibrin (PRF) was developed as a source of autogenous blood-derived growth factor concentrate that could be prepared without the addition of thrombin. Growth factors play an important role in regeneration, both naturally and therapeutically. Numerous polypeptide growth factors bind heparin with high affinity, and this binding activity is important in sequestering growth factors in the extracellular matrix (ECM), serving to localize growth factor activity, to prevent growth factor degradation, and in some cases enhancing binding to cell surface receptors4.

More recently these bio-molecules have been directly incorporated within the scaffold structure or into the scaffold biomaterial in a variety of ways. Soluble growth factors can be directly encapsulated or incorporated during the scaffold fabrication process. A widely used method of growth factor delivery in tissue engineering has been simple physical adsorption of biomolecules on the biomaterial or scaffold surface. The growth factors act by binding to the extracellular domain of a target growth factor receptor, which in turn activates intracellular signal transduction pathways. Many growth factors, such as bone morphogenetic protein (BMP), transforming growth factors (TGF), fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF), have been found to be expressed during tooth formation and repair5.

1. Scaffolds –

For the creation of an autologous implant, donor tissue is harvested and dissociated into individual cells, and the cells are attached and cultured onto a proper substrate that is ultimately implanted back at the desired site of the functioning tissue. Because many isolated cell populations can be expanded *in vitro* using cell culture techniques, only a very small number of donor cells may be needed to prepare such implants. However, it is believed that isolated cells cannot form new tissues by themselves. Most primary organ cells are believed to be anchorage-dependent and require specific environments that very often include the presence of a supporting material to act as a template for growth5.

In most native tissues, cells are contained within a tissue specific, three-dimensional (3-D) extracellular matrix (ECM), which comprises a complex network of nanoscale fibres forming highly structured local microenvironments. Cellular communication, transport of oxygen and nutrients, removal of wastes and cellular metabolism require such an environment, where cellular orientation can be polarized and movement of contents can be directional. Hence, in tissue engineering, a 3-D framework is needed to organize the cells into a higher ordered assembly so as to achieve the desired tissue function. Scaffolds can be used as bio-active agent reservoirs combined with the delivery cells, providing a multitude of advantages, such as a safe delivery profile, protection of bio-active agents from biodegradation and the ability to deliver the bio-active agents locally where the cells are attached6.

1. **FABRICATION OF SCAFFOLD FOR TISSUE REGENERATION**

After years of powerful progress, a set of novel tissue culture, replacement and implantation technologies have been developed, allowing fabricating artificial extracellular matrices, namely scaffolds, to bear stem cells, growth factors, or other biological nutrients aiming at repair of tissue function. Scaffolds are bulk bioactive materials with specific porosity and structure to contribute to the formation of new tissues for completing the medical task. Process of tissue engineering relies extensively on the use of porous 3D scaffolds to provide the appropriate environment for the regeneration of tissues and organs.

The scaffold provides the necessary support for cells to attach, proliferate, and maintain their differentiated function. Its architecture defines the ultimate shape of the new grown soft or hard tissue. The key role of scaffolds is to provide temporary mechanical integrity at the defect site until the damaged tissue is repaired or regenerated, and normal biomechanical function is restored. The scaffold also serves as a carrier for cells, growth factors or other biomolecular signals. It is vital for the scaffold to mimic the structure and properties of human tissue to direct the macroscopic process of tissue formation. During the cell regeneration, the scaffold temporarily help in cell regeneration and gradually biodegrades either in the course of the healing process or after, and a new tissue with a desired shape and properties is produced. This degradability property of the scaffold obviates the need to remove the material later and thus, eliminates the side effects resulted from foreign materials left in the body. Successful fabrication of entirely functional scaffolds should be addressed in two levels:

(a) **Microscale** level should contain an environment suitable for cell survival and function and

(b) **Macroscale** tissue construction should permit the coordination of multicellular processes, provide adequate transport of nutrients, and possess mechanical properties.

A number of fabrication technologies have been applied to process biodegradable and bioresorbable materials into 3D polymeric scaffolds of high porosity and surface area. The scaffolds are designed to achieve this evolution in 3D cells by providing mechanical support during tissue repair. In practice, the techniques of the fabrication of 3D scaffolds are subdivided mainly into conventional or rapid prototyping (RP) method, each producing different scaffolds with different characteristics7.

1. **Conventional fabrication technique –**

Conventional techniques of scaffolding fabrication include the construction of porous polymer structures such as substrates for cell adhesion, but it is difficult to obtain complex structures with tunable microscale and macroscale using conventional methods. A significant number of scaffolds have been developed conventionally for drug delivery, but they have subsequently been used in 3D cell culture in the context of tissue engineering.

|  |  |
| --- | --- |
| Method of fabrication | Technique |
| Solvent Casting and Particle Leaching | A solvent combined with uniformly distributed salt particles of a certain size is used to dissolve the polymer solution. The solvent evaporates leaving a matrix containing salt particles. The matrix is then submerged in water, and the salt leaches away to form a structure with high porosity. |
| Freeze Drying | Involves the use of a synthetic polymer that is first dissolved in an appropriate solvent. After dissolution, the polymer solution is cooled under the freezing point, resulting in a solid solvent that is evaporated by sublimation to leave a solid scaffold with numerous interconnected pores. In this technique, when the solution is cooled to freezing point, the solutes can be separated in the ice phase resulting in a small porous structure characterized by a “fence” of matter surrounding the ice. |
| Thermal-Induced Phase Separation (TIPS) | TIPS is a low-temperature method designed to force phase separation via the temperature alternate related to setting the homogeneous polymer solution with a high temperature in a decrease temperature environment to induce phase separation so that a polymer-rich phase, as well as a poor polymer phase, is achieved. |
| Gas Foaming | This technique uses relatively inert gas foaming agents such as carbon dioxide or nitrogen to pressurize modelled biologically degradable polymer with water or fluoroform until they are saturated or full of gas bubbles. This technique usually produces structures like a sponge with a pore size of 30 to 700 *μ*m and a porosity up to 85%. |
| Electrospinning | A standard electrospinning system consists of four main components: a spinner with a syringe pump, a metallic needle, a high-voltage power supply, and a grounded collector. The strength of the electric field exceeds the surface tension of the droplet to produce a liquid jet that is then extended and whipped continuously by electrostatic repulsion until it is deposited on the grounded collector. The solvent evaporates in the process, and the jet is solidified to form into a nonwoven fibrous membrane. |
| Powder forming process | A suspension of ceramic particles in a suitable liquid (such as water or ethanol) called slurry is used to prepare green bodies. Fillers such as sucrose, gelatine, PMMA microbeads and a wetting agent (i.e. a surfactant) are added into the ceramic suspension, and these chemicals will produce porosity when they are evaporated or burned out during sintering. |
| Sol gel technique | This technique is based on the chemical reaction of inorganic polymerisation of metal alkoxides. Using the sol–gel process, it is possible to fabricate ceramic or glass materials in a variety of forms, including ultra-fine or spherical-shaped powders, thin-film coatings, ceramic fibres, microporous inorganic membranes, monolithic ceramics and glasses, and highly porous aerogel materials |

Table 1 : Methods of conventional fabrication of scaffolds7,8,9

1. **Rapid prototyping fabrication technique –**

The RP scaffold fabrication technique provides a plethora of potential opportunities for tissue engineering. Firstly, the independent control of macroscale and microscale features allows the fabrication of multicellular structures needed for complex tissue functions. Secondly, three-dimensional vascular beds fabrication will allow support of massive tissue formation that otherwise would have been possible. Thirdly, combining clinical imaging data and 3D fabrication techniques can provide the possibility of production of customized scaffolds as well as mass production of the scaffold designs. The main benefit of these techniques is that they enable the production of customized and patient-specific scaffolds suitable for tissues and organs in question.

|  |  |
| --- | --- |
| Method of fabrication | Technique |
| Stereolithography | A stereolithography system has four main components, namely, a tank with a photosensitive liquid resin, a transferable built platform, a UV laser for radiating resin, and a dynamic mirror system. The process begins with a UV laser by depositing a layer of photosensitive liquid resin on the platform. After the solidification of the initial layer, the platform is lowered vertically. A second layer is then placed on the first layer; the process is repeated until a 3D scaffold is created. Finally, the uncured resin is cleaned off, and the scaffold is postcured under UV light, therefore, this method overcomes the challenges related to wastage in subtractive fabrication methods |
| Fused Deposition Modeling (FDM) | In FDM technique, a solid polymer is cast into a hot extrusion nozzle to be melted and extruded on the surface of 3D object using a computer controlled extrusion and deposition processes; and the scaffold is made from multiple layers of adjacent microfilaments. |
| Selective Laser Sintering (SLS) | This technique uses laser as the power source to sinter powdered material defined by a 3D model in thin layers. Due to the use of a laser, this technique has been utilized to make various materials, such as polymers, metals, or ceramics. |
| Three-Dimensional Printing (3DP) | 3DP is a process of creating tools and functional prototype features directly from the computer models. 3DP technique is performed by applying the powdered material in layers and the selective fusion of the powder by **"inkjet",** where the adhesive is printed. After continuous deposition of the layers, the unbound powder is taken out, yielding a complex 3D object |
| Bioprinting | Bioprinting is a 3 dimensional printing technique, defined as “using material transfer processes for developing a biological pattern and assembly of relevant materials, cells, molecules, tissues, and biodegradable biomaterials with a prescribed structure to achieve some biological functions”. The introduction of solvent-free, aqueous-based systems allows the direct printing of biomaterials on three-dimensional scaffolds for transplantation with/ without seeded cells. |
| 4D printing | Four-dimensional (4D) printing is a recently appeared terminology in 2013 and immediately attracts wide attention in different areas. 4D printing adds a new dimension, time, to ordinary 3D printed products, which allows materials responding to suitable stimuli or self-transform after possessing. Transformation code of 4D printed materials is hidden in the exquisite design of its structure and constituents. It offers great potential for customized medical devices given that the dynamic mechanical property of printed material accords with the behaviour of living tissues. In addition, the time-dependent property of 4D printing makes it suitable for long-term application embedded in human body. |

Table 2 : Method of Rapid prototyping of scaffolds7,8,10

1. **IMPORTANCE OF SCAFFOLDS MATRICES IN CELL DELIVERY**

(i) Scaffolds provide growth of cells either seeded within the porous structure of the scaffold or migrating from surrounding tissue.

(ii) Scaffold matrices can be used to achieve cell delivery with high loading and efficiency to specific sites.

(iii) Scaffold must provide a suitable substrate for cell attachment, cell proliferation, differentiated function and cell migration.

(iv) To permit the transport of biological signalling factors, nutrients and wastes to allow for cell survival.

(v) Possess relatively easy process ability and malleability into desired shapes.

(vi) Minimal stimulation of the immune or inflammatory responses invivo.

(vii) Highly porous with a large surface/ volume ratio which provides high cell attachment11.

1. **APPLICATIONS OF TISSUE ENGINEERING IN MEDICINE**

Biomaterials have been used in various areas of tissue engineering to regenerate tissues for augmentation, repair or replacement. These biomaterials have been vastly applied in bladder, tendons, ligament, kidney, liver, heart valves, myocardial patches, bone, cartilage, pancreas, cardiac, islet of Langerhans, vascular and skin**.** Some of these developments are further described below.

1. **Hard tissue**
2. **Bone tissue engineering –**

Bones have the capability to regenerate, remodel and repair in response to injury. In order to avoid using autograft, allograft or xenograft due to infection, immunological rejection, disease transmission and many other reasons, the development and application of 3D porous scaffold with bone mimicking features was considered. Bio-ceramic scaffolds consisting of hydroxyapatite (HA) have been produced and used because it is biocompatible, bioactive, support and promote new bone formation and mimics the mineral component of the natural bone12.

These scaffolds are used to give mechanical stability to the defect site during healing as well as being aimed at supporting osteogenic, osteoconductive and osteoinductive features of the native tissue. Incorporation of calcium phosphate and HAp into polymeric structures has been widely used to improve the mechanical properties of the scaffolds and addition of these has also been performed to imitate the composite structure of bone due to their chemical and crystallographical similarities to the native tissue13.

1. **Cartilage tissue engineering –**

Cartilage is a human connective tissue that is stiff and flexible, made up of chondrocytes embedded in a highly hydrated ECM. The different classes of cartilage are hyaline, elastic and fibro with different components of ECM have been developed using tissue engineering techniques for the purpose of cartilage repair, joining of cells with scaffolds, mechanical stimulation and growth factors.

In cartilage tissue engineering, a variety of naturally derived scaffold materials (e.g., collagen, gelatin, hyaluronic acid (HA), and biodegradable polymers like PHBV) and synthetically derived polymers (e.g., PLA, PGA, and PLGA) can be used. Collagen, the most widely used natural material, is abundantly found in the supportive structures of mammalian bodies. ***Kose et al14.*** have observed cartilage formation using chondrocytes and macroporous PHBV under both *invitro* and *invivo* conditions. ***Svensson et al.***,15 investigating the potential of native and chemically modified bacterial cellulose to support primary bovine chondrocytes, have found that these scaffolds are good cell carriers for tissue engineering of cartilage.

Synthetic polymers are also widely used materials in cartilage tissue engineering. PGA scaffolds seeded with chondrocytes have been shown to form a cartilage tissue that has similar properties to native cartilage after 20 weeks. PLA scaffolds can induce the formation of a cartilage-like tissue. Poly(ethylene oxide) (PEO) has been tested as an injectable matrix material for chondrocyte transplantation. Poly(*N*-isopropylacrylamide) (PNIPAM) is one of the most commonly studied thermoreversible synthetic biomaterials.

1. **Soft tissue**
2. **Nerve regeneration –**

Peripheral nervous system (PNS) axons have the capacity for regeneration after injury, but if an axon is damaged over a significant distance (longer than 6 mm), it cannot regenerate; outside intervention is needed. An ideal nerve conduit should be neuroinductive and neuroconductive. Scaffolds need to assist regeneration of axons through physical guidance and growth factor presentation.

Growth factors, released from microspheres, Schwann cells and neural progenitors embedded in gels within the tubes provide a similar environment to the autografts and show improvements in recovery. Natural or synthetic gels containing collagen or laminin have also been developed. Guidance is a critical issue in highly anisotropic tissues like those of the nervous system. Patterned surfaces and fibrous materials are used to mimic the complex architecture of this tissue.

Many factors influence the choice of materials, such as the degradation rate, swelling, biocompatibility, and possibility of the incorporation of proteins or neurotrophic factors in the ECM can increase the adhesion and growth potential of cells. One of the most successful materials for nerve regeneration has been observed in PGA. Hydrogels have also been studied as scaffolds for nervous system regeneration16.

1. **Muscle regeneration –**

Between the three types of muscle tissue – cardiac, smooth and skeletal – the cardiac muscle regeneration is of particular importance due to limited regeneration capabilities in mammals.

For construction of tissue-engineered products, cardiac muscle cells (cardiomyocytes) have been obtained from human ESCs and human induced pluripotent stem cells. In addition to cell sources, the type of cells, seeding density and composition of the culture medium are parameters that have to be taken into consideration when constructing a heart muscle substitute.

Scaffolds made from the nanofibers of PCL and collagen have been evaluated for smooth muscle tissue engineering. The growth of cells were directed by nanofiber orientation, and the cells were able to maintain a typical phenotype shape.

Skeletal muscle has a massive capacity for regeneration without external intervention. Muscle tissue is constantly being destroyed, repaired and remodelled. Although in the case of very hard injury, the regeneration capacity could be limited or lost. Popular biomaterials as scaffolds for the regeneration of skeletal muscle tissue are hydrogels or fibrous meshes. In the development of regenerative methods based on various scaffolds for muscle tissue engineering, electrical stimulation was shown to support better differentiation potential of muscle precursor cells13.

1. **Tendon and ligament regeneration –**

Tendon and ligament injuries are quite common injuries of connective tissue. Surgical repair is the most commonly used method but often leads to degeneration of the frayed tissue. Tendons do not naturally regenerate very well after even minor injuries. Hence, 3D scaffolds are of major importance for regeneration purposes. The 3D scaffolds must provide a suitable bio-environment and adequate mechanical properties such as elastic modulus, toughness and ultimate strength. The natural grafts often fail to bring back the mechanical and structural properties of the original tissue.

The major attention in ligament TE is given to the anterior cruciate ligament (ACL), which stabilizes the knee, because of its poor healing capabilities. For the reconstitution of ACL, the PLLA combined with silk fibers has been tested to mimic the natural mechanical properties and to deliver growth factors. The mechanically strong PLLA nanofibers combined with flexible PCL nanofibers have also been tested for ACL regeneration. This material was able to release growth factors and support the proliferation of human mesenchymal stem cells (hMSC), and regenerative tissues express critical ligament markers13.

1. **Skin tissue engineering –**

The most common and appropriate way of treating skin defects is to use autologous skin grafts. This method includes harvesting skin from the patient and transferring it to the injured area. Besides being a painful procedure, harvesting causes morbidity at the donor site, and it cannot be applied for defects larger than 50% of the total body surface area (TBSA). In addition, large and deep wounds cannot be covered with autologous skin grafts since only areas smaller than 2% of the TBSA are suitable for application of full-thickness skin grafts. Skin tissue engineering products have been designed to cover the injured area, preventing microbial infection and heat and fluid loss, as well as promoting the healing process, no matter how large the wound.

Skin substitutes can be acellular or cellular, carrying either autologous or allogenic cells. Acellular skin substitutions are generally used for the treatment of partial-thickness wounds. Allogenic cellular skin substitutes are generally for temporary rather than permanent use. Grafts with allogenic cells achieve wound coverage and promote wound healing, but allogenic cells do not survive in the patient after the healing process is accomplished. Cells for autologous cellular skin substitutes are obtained from biopsies of the patients’ own tissue or from the outer root sheath of hair follicles. Cells are allowed to proliferate in cell culture and are then applied as epithelial sheets or combined with biopolymers. Autologous cellular substitutes are considered permanent skin substitutes.

Even though scaffold-based skin substitutes have been widely used, they still have several limitations, with vascularization being the most serious problem. The most common approach for vascularization is introduction of VEGF to the scaffold. The limit of the thickness of an artificial skin that can become vascularized easily is said to be around 0.4 mm. Substitutes thicker than this can be applied with a two-step approach that allows the skin substitute adequate time to vascularize in between13.

1. **Vascular tissue engineering –**

The use of natural tissues as blood vessel substitutes is presumed to make the graft more resistant and less immunogenic. Detergents and enzymes are used to remove the cells (decellularization) and their surface antigens to prepare acellular matrices to be used as scaffolds. The reconstitution of biological structures by decellularization is an attempt to mimic the natural vascular architecture because these materials have mechanical, biological and chemical advantages over synthetic materials.

The use of these matrices with repopulation by human cells can be contemplated in the building of perfusable constructs. Collagen has low antigenic, low inflammatory, and almost no cytotoxic responses, and thus represents a preferential natural scaffold material. Elastin confers elasticity and recoiling ability to blood vessels and is the main ECM protein of the arterial wall13.

1. **Cornea tissue engineering –**

The fact that the cornea is avascular is a major challenge that obtained its physiological needs from lachrymal fluids at the front and aqueous humour due to need for donor corneas and rejection. The use of suitable scaffold and the patient’s limbal cells to colonise the substrate was considered in the recent research for a new cornea by tissue engineers13.

1. **APPLICATIONS OF TISSUE ENGINEERING IN DENTISTRY**

Caries, trauma, erosion and periodontal disease are pathologies characterized by the damage and loss of dental tissues and sometimes loss of the whole tooth. Restorations of damaged tooth tissues and substitution of missing teeth with artificial prostheses represent the traditional therapeutic solutions17. Dental implants, such as titanium implants, has the advantage that it can rapidly recover tooth function with a natural appearance18. However, dental implants function through osseointegration, the direct connection between the implant and the surrounding alveolar bone, and lack periodontal and cementum tissues present in naturally formed teeth, which function to cushion and modulate the mechanical stress of mastication. These disadvantages have prompted an ongoing search for alternative methods that would overcome the need for root canal treatment, autologous tooth harvest for auto-transplantation and dental implants.

Scientific advances in stem cell biology and tissue engineering technology have shown the potential use of dental stem cells, combined with biodegradable scaffolds supplied with bio-active agents, such as growth factors, to control the spatial and temporal organization of dental progenitor cell proliferation, differentiation and function. Tooth engineering involving epithelial–mesenchymal interactions could be a model system to evaluate not only tooth development but also the revascularization and reinnervation of the bio-engineered tooth. Therefore, tissue engineering efforts have been focused on the development of new culture systems, part or whole tooth regeneration and vascularization and periodontium regeneration. Natural tooth formation is a highly complicated molecular process, where the spatiotemporal expression and interactions of growth factors, cytokines and transcription factors direct macromorphological (crown size, tooth length) and micromorphological (cusp number and position, root formation) tooth development. Proper control of these interactions is needed in order to generate bio-engineered teeth of specified size and shape.

For the tissue engineering of any dental tissues, the biomimetic of the original tissue should be carefully developed to reproduce the natural tissue, respecting its morphological and functional characteristics. For that, scaffolds should have adequate stability, biocompatibility, and bioprocess control; these characteristics are needful considering cell signalling processes. In addition, cells should have besides adequate environment and should be present high adhesion for allowing their tissue differentiation and no migration, and the nutrient perfusion should reach the cellular component into different scaffold gradients19.

**Scaffolds for regeneration of pulp - dentine complex –**

Despite the high clinical success rate of traditional root canal therapy following removal of a dental pulp (pulpectomy), other consequences need to be considered. For instance:

**1.** In a root canal–treated tooth, the esthetics are of the tooth crown, which are affected through structural loss of tissues or discoloration of the crown by staining from the endodontic filling material.

**2.** The structural integrity of root canal–treated teeth may also be undermined because of loss of tooth structure and subsequent restorative procedures.

**3.** Pulpless teeth lose some of their ability to sense environmental changes, which may lead to the progression of caries being unnoticed by patients.

**4.** Long-term studies have shown that tooth loss is higher for root canal–treated teeth than non-treated because of secondary caries and associated complex restoration problems.

Advances in tissue engineering and biotechnology have opened new directions for designing biological methods for pulp treatment that are aimed at *insitu* regeneration of partial pulp or *denovo* synthesis of total pulp replacement. The approaches include

(1) An effort to harness the pulp’s own regenerative capacity, i.e., induce host cells from the apical region of the pulp to migrate toward the interior of the root canal or

(2) The replacement of the entire pulp tissue by transplantation of *invitro* engineered pulp tissue19.

**Cell homing**

The American Association of Endodontists (AAE) has focused its regenerative endodontics effort on revitalization of dental pulp and continuous root development in immature permanent teeth. The current goal of regenerative endodontics is to restore the vitality of dental pulp in immature, developing permanent teeth and to enable otherwise arrested root development. Studies conducted by them have finally lead to a procedure called cell homing20.

Cell homing is regarded as an active means of recruitment of endogenous cells, including stem/progenitor cells, into an anatomical location. The regenerative technique by chemotactic cell homing represents an alternative approach for pulp regeneration compared with mainstream cell transplantation. Such an approach was also proposed for *in situ* pulp and periodontal tissue regeneration. In this concept, different bioactive cues were adsorbed or encapsulated into biomaterial scaffolds. Upon release of these bioactive cues into endodontically treated root canals, local and/or systemic cells, including stem/progenitor cells, migrated and homed *in vivo* into the root canal, leading to subsequent pulp regeneration21.

Figure 3: Infusion of bioactive cues in scaffolds

1. Bioactive cues can be adsorbed, tethered, or encapsulated in biomaterial scaffolds. Upon release of bioactive cues, such as from endodontically treated root canals, local and/or systemic cells, including stem/progenitor cells, can be homed *in vivo* into an anatomic compartment that serves as a native scaffold22.

**(B1)** Current root canal treatment of diseased dental pulp

**(B2,B3)** Removal of substantial enamel and dentin structures because obturation of gutta percha requires unobstructed access

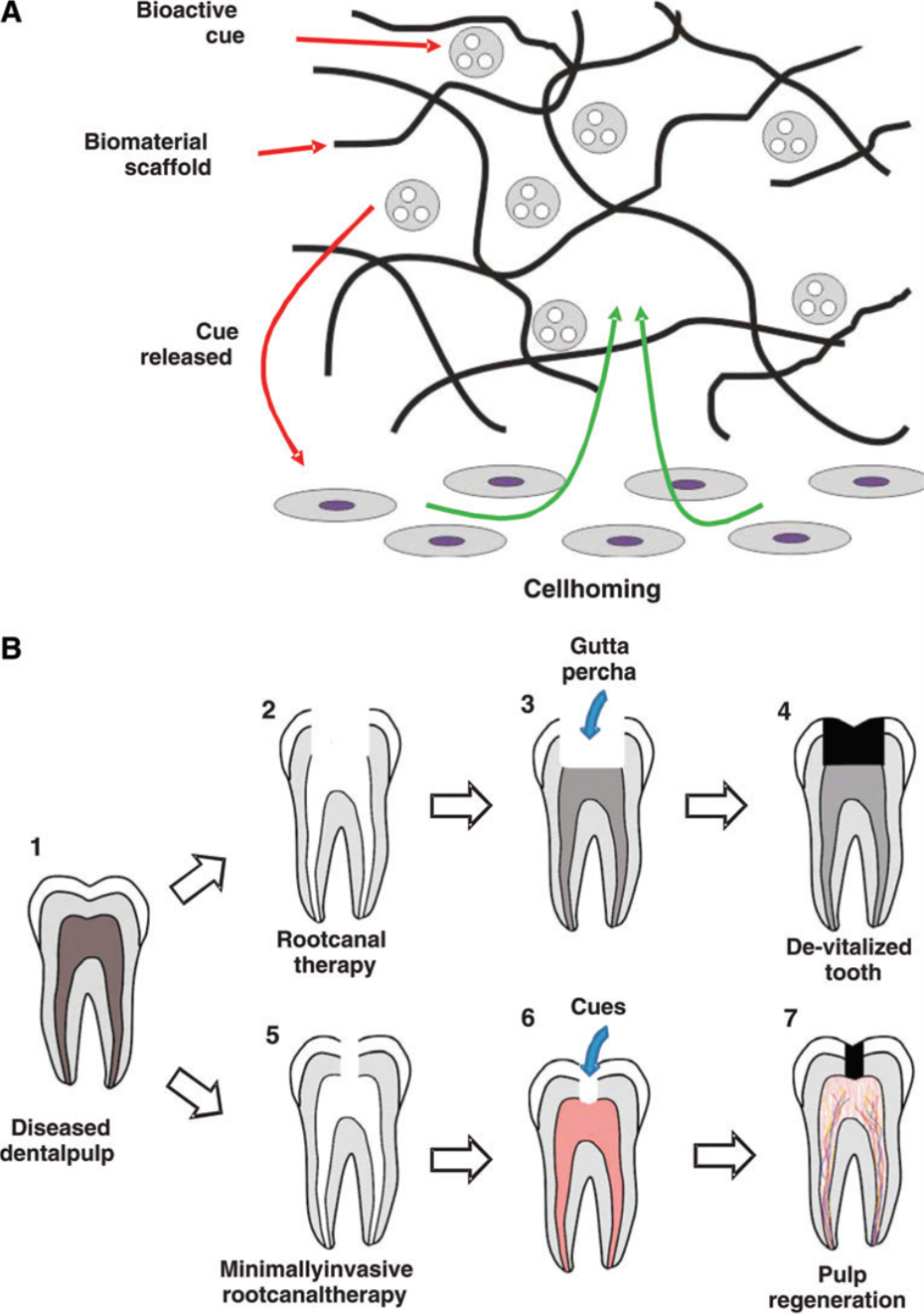
**(B4)** De-vitalized tooth

Figure 4: Advantage of cell homing over conventional RCT

**(B5,B6)** Instead, the diseased dental pulp is treated with a revised, minimally invasive root canal therapy as the delivery of injectable bioactive cues does not require unobstructed access to pulp chamber and root canal. Although residual inflammation in endodontically treated root canal and peri-apical region are anticipated to present challenges for pulp regeneration, chemotaxis-induced angiogenesis as shown in the present may provide the potential for native defense mechanisms that may counteract residue infection in the root canal.

**(B7)** Vital tooth with regenerated dental pulp.

**Scaffolds for periodontal tissue engineering –**

The goal of periodontal regenerative techniques is to restore damaged periodontal tissues, for example, due to trauma, tumor, or inflammation; by promoting bone regeneration and/or restoring tooth attachment. The complex structure of the periodontium presents a challenge in periodontal tissue engineering, because it comprises both soft and hard tissues that all need to be restored around the tooth to achieve functionality. In addition, to achieve regeneration of the periodontium, not only the migration and differentiation of specific cells to the wound area and a substrate promoting formation of new tissue growth are important, but also signaling molecules and growth factors to regulate the process as well as a vascular network bringing nutrients and oxygen to the cells are required in the establishment of new tissues19.

**Scaffolds for gingival tissue –**

Reconstruction of gingival tissues is a challenging treatment in periodontology. The final goal of periodontal therapy is regeneration of the lost tissues, but reaching this goal is difficult because improper treatment will lead to scarring which has adverse effects on function and esthetics.

Guided gingival tissue regeneration:

One of the treatment modalities in periodontal therapy is guided tissue regeneration (GTR). The concept of guided tissue regeneration is based on preventing the migration of epithelial and gingival connective tissue cells into previously diseased root surface. A basic part of GTR is placement of a physical barrier for prevention of apical migration of the epithelium and gingival connective tissue cells. The turnover of soft tissue is faster than bone formation, hence barrier scaffold is used to prevent the infiltration of soft tissue. Barrier scaffolds also stabilize the graft materials and preserve graft and reduce the rate of graft resorption. The guided tissue regeneration is used for periodontal tissue engineering and periimplant diseases treatment.

In regenerative dentistry, one of the most important necessities in a tissue engineering scaffold is facilitating bone or new gingival tissue formation. These scaffolds are named as **conductive scaffolds**. This matrix facilitates cell penetration, attachment, proliferation, and differentiation. There are two main types of gingival scaffolds:

1. Non resorbable gingival membrane:

There have been different non-resorbable gingival membranes for regenerative purposes. Among different materials, polytetrafluoroethylene (PTFE) scaffold was first developed in 1958 and widely used in bone regeneration. It is composed of an inner cell occlusive area and outer cell adherent. Hence these scaffolds can selectively exclude migration of epithelial and gingival connective tissue cells.

Expanded polytetrafluoroethylene (ePTFE) based scaffolds –

* One of the best materials for gingival regeneration.
* Subtype of ePTFE scaffolds is titanium reinforcement that is indicated when the defect anatomy is not supported.
* Disadvantages –

1. High frequency of early spontaneous exposure to oral environment, compromising their effectiveness.
2. Should be removed after 5 – 8 weeks.

Non restorable polytetrafluoroethylene (nPTFE) based scaffolds –

* Barrier material that only small molecules can penetrate into it.
* Risk associated with soft tissue cells or bacterial penetration is relatively low.

Drawbacks of ePTFE and n-PTFE scaffolds.

1. Inhibit healing of soft tissue fenestration defect.
2. Lesion size and inflammation intensity increases with time.

Dense polytetrafluoroethylene (dPTFE) based scaffolds –

* Reduces the mortality rate of patients due to elimination of the need for primary closure after bone grafting.
* Preserve hard and soft tissue in extraction sites19

1. Resorbable gingival membranes:

**Collagen –**

Widely used and promising biomaterial which is naturally a fibrous protein, extracted by various techniques of animal tissues. Resorption rate of this scaffold ranges between 6–8 weeks to 6-8 months depending on their technical characteristic. Collagen attracts and activates gingival fibroblast cells and stimulates the fibroblast DNA synthesis. Collagen scaffolds accelerate flap stability, vascularization, and epithelization. Using collagen scaffolds, fenestration defects are healed almost completely. Inflammatory cell infiltration inside and around collagen scaffolds is relatively low.

***Wang et al.23*** proposed concepts for immobilization of scaffold with alkaline phosphatase, bioactive glass or hydroxyapatite nanoparticles in collagen scaffolds to control degradation and enhancement of osteogenic potential. Studies conducted by ***J.Kozlowska and A.Sionkowska24*** has shown that degradation rate of non–cross-linked collagen scaffolds is higher than cross-linked collagen scaffolds.Disadvantages of these scaffolds are their limitations for providing the space for wound and limited regeneration after their use. Other limitations include lack of control on resorption rate and risk of disease transmission and ethical issues.

**Poly(lactic-co-glycolic acid) (PLGA) –**

PLGA is a synthetic copolymer of polylactic acid (PLA) and polyglycolic acid (PGA) with excellent biocompatibility, controllable biodegradability, and mechanical properties. PLGA is relatively hydrophilic which makes it suitable as gingival scaffolds. In gingival tissues, PLGA scaffolds have promoted the proliferation of human periodontal ligament cells (PDLCs). It is demonstrated better spread on the collagen-coated scaffold compared with the uncoated scaffold because of indication of excellent biocompatibility.

PLGA induces the osteogenic differentiation of PDL cells. ***Sadeghi et al.25*** demonstrated better spread on the collagen-coated scaffold compared with the uncoated scaffold because of indication of excellent biocompatibility. Controlling degradation rate of PLGA is achieved by adjustment of its composition ratio of lactide (LA)/glycolide (GA). Furthermore, biodegradable and biocompatible nature of PLGA polymers and the benefit of easy manipulation have made PLGA an acceptable candidate for periodontal GTR scaffolds.

**Oxidized cellulose mesh barriers –**

Another class of resorbable hemostatic materials with acidic nature that has positive effects in GTR procedure. The oxidized material converts to a gelatinous mass and incorporates the blood clot to form a scaffold. The main part of this scaffold is resorbed within 1 week after surgery.

Disadvantages –

Limited wound space and delayed healing of the bone because of acidic nature of scaffold.

**Chitosan –**

Widely used biomaterial in tissue engineering produced by deacetylation of chitin which is a polysaccharide. It is shown that chitosan and hyaluronic acid composite scaffolds have potential for use in periodontal regeneration. Chitosan is osteocompatible and has an osteoconductive property. Chitosan has some advantages such as antimicrobial effects, hemostatic properties, wound healing potential, and biocompatibility. Studies conducted by ***Qasim et al.26***  shown that chitosan and hyaluronic acid composite scaffolds have potential for use in periodontal regeneration.

**Alginate –**

Frequently used in gingival tissue engineering which is a natural polysaccharide obtained from seaweed extracted of brown algae (Phaeophyceae) including *Laminaria hyperborea* and *Laminaria digitata*. The extract is filtered and calcium chloride is mixed with the filtrate in order to precipitate alginate. Alginate composition, molecular weight, purity, and concentration used in the scaffolds play the biggest role in providing mechanical strength, biocompatibility, cell adhesion, proliferation, and osteogenic differentiation. Alginate poses no toxicity to cells and thus is biocompatible in the physiological area, making it suitable for several biomedical applications.

**Fibrin –**

Natural biomaterial that is isolated from the patient’s blood. Fibrin scaffolds have potential to prepare suitable environment for angiogenesis. They are biocompatible and biodegradable. Other advantages include high seeding efficacy and cell distribution in a uniform pattern and adhesion capability. Fibrin hydrogels are a kind of subtype fibrin scaffolds that are used in tissue engineering of liver, skin, cardiovascular, and bone tissues. Their disadvantages include low mechanical properties, fast degradation, and gel shrinkage.

Fibrin glue is prepared from purified fibrinogen and thrombin, and its function in tissue engineering is as a delivery vehicle and as a scaffolding matrix. It has been used in tissue engineering of maxillofacial bone and periodontal tissue. For modifying the mechanical properties of fibrin glue, it can be incorporated with polymers such as gelatin, hyaluronic acid, and chondroitin 6 sulfate.

**Scaffolds that promote enamel mineralisation**

Enamel results from highly complex, orchestrated, and specific natural process and, in the early stage, the enamel organ guides all process. By weight, mature enamel is ∼95% mineral, ∼1%–2% organic material, and ∼2%– 4% water. In the earlier stages, it is possible to find 25%–30% of protein. It is highly probable that proteins play an important role in the process of enamel mineralization. Amelogenin and enamelin are principal proteins present in developing enamel. Mature enamel is compounded by hydroxyapatite. Proteins and peptides constitute less than 0.1% by weight. There are traces of lactic acid, citric acid, carbohydrates, and lipids.

Common repair of defective enamel using materials such as amalgam, composite resin, and ceramics, which do not have a chemical component like crystal structures and physicochemical properties observed in the enamel. Because of that great difference, the performance of reparative material is generally below of natural enamel. Because of the high specific nature of enamel, evidentially, it is very difficult to find a reasonable method to improve an artificial production of this material. Polymeric composites based on hydroxyapatite are more commonly used as scaffolds for enamel biomimetic structures.

The principal biomaterials used for enamel scaffolds are the following:

* Decellularized tooth bud
* Nanohydroxyapatite
* Polymers/hydroxyapatite composites. The common polymers are poly(l-lactic acid) (PLLA), poly(glycolic acid) (PGA), poly(lactic-co- glycolic acid) (PLGA), poly(ethylene glycol) (PEG), polycaprolactone (PLC).
* Collagen/apatite
* Silk
* Polysaccharides–cellulose, alginate, hyaluronic acid, chitosan.
* Gelatin19

**Biomimetic methods**

In this method, the scaffold is generated firstly by natural enamel and after by newly formed hydroxyapatite. This looks like a dynamic scaffold quasi-bi dimensional. Biomaterials for biomimetic procedures are prepared in the laboratory under very close physiological microenvironment conditions found in ECM. The system design should have a special membrane that allows only calcium ions to enter the system in a one-way form and synthetic amelogenin protein or ECM should to be trapped between the two layers of membranes to initiate mineralization. Talking in the simple way, enamel remineralization is like building something layer by layer. On the eroded enamel surface, amelogenin derivates or its derivate peptides have to apply and wait for a time to grow hydroxyapatite.

***Chung27*** used triplet repeats of asparagine–serine–serine peptide (3NSS) to regulate enamel remineralization. The biomimetic process provides hydroxyapatite recrystallization and greatly 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. The new compound is based on a complex between ELP and amorphous calcium phosphate deposited on acid-etched enamel surface. Images from SEM presented pilled and prismatic formation of hydroxyapatite near a natural enamel.

***Mukherjee29*** performed a biomimetic in vitro approach using full-length amelogenin (rP172) and leucine-rich amelogenin peptide (LRAP) to promote remineralization of enamel to improve biointegration and mechanical strength. ***Dogan et al.30*** worked with shADP5, a 15-amino acid-long amelogenin-derived peptide, which was shown to promote cell-free and rapid formation of mineral layer on demineralized human root dentin. The results showed high peptide-guided remineralization of white spot lesions in the enamel from human teeth samples.

**Self-assembling peptide methods**

Professor ***Collin Robinson*** and colleagues from the University of Leeds develop a manner to reproduce the enamel matrix inside enamel lesions and promote remineralization using self-assembling peptides. This kind of biomolecules act over spontaneous folding of proteins and the formation of DNA double helix. In this technique, eroded enamel is filled with monomeric P114, which diffuses through the pores of the hypermineralized plate into the subsurface lesion body. Then, the peptide monomers spontaneously form a 3D matrix via hydrogen bonds. After that, crystallization around the matrix starts and calcium phosphate from saliva crystallizes around the matrix, forming new enamel. This peptide acts like a scaffold. The process filling is fundamental because there is a necessity to reach at bottom of eroded hole for high efficiency remineralization.

**Regeneration of enamel using hydroxyapatite as basement method**

This methodology is based on the use of hydroxyapatite nanoparticles, hydroxyapatite nanocompounds, calcium phosphates, and compounds as cement for plugholes in eroded enamel. The principal application is product toothpaste with up to 5% concentration.

**Natural or semisynthetic scaffold with stem cell methodology**

The use of natural scaffold with stem cell always needs an enamel matrix derivative (EMD) to promote reseed and differentiation. The first step of the process is to stimulate the cell proliferation and ligament formation. EMD protein precipitation is governed by physical and chemical characteristics of the material used as grafts or scaffold used. Besides, temperature set to 37°C, and the ideal pH control should be between 3.9 and 4.2.

**Synthetic scaffolds**

Generally talking, the use of synthetic scaffolds for enamel restoration is based on natural mineralization of hydroxyapatite from natural or artificial saliva. The principal problem of this methodology is its inherent limited biosorption of scaffolds and the more complex study for the toxicology because of newly synthesized molecules; besides, the process is very time dependent as observed in the biomimetic deposition of hydroxyapatite.

**Scaffolds for dental cementum**

Cementum is a critical part of the tooth, attaching the tooth to the underlying bone and repairing minor damage to the root. As the cementum is located below the gingival tissue, a complete repair of cemental defects is not possible. Hence, an extensive research is being carried out in discovering suitable methods for dental repair using tissue engineering scaffolding. The challenge lies in a biomaterial that needs to be produced to allow good porosity, mechanical strength and that encourages cell proliferation within the scaffold; all while ensuring biocompatibility and that the product is not rejected from the body, causing adverse reactions.

**Various biomaterials used for cementum scaffolds**

**Nonrigid biomaterials**

These biomaterials are capable of dissolving in water, and so they need to be mixed with other materials such as HA or CaP, to be able to form a three-dimensional structure. Commonly non-rigid or soft biomaterials come in the form of hydrogels, which can be injected rather than have to be implanted into tissue. Due to hydrogels non rigid property, they are a good biomaterial for injecting into areas such as the cementum. When injected into the desired location hydrogel changes from a solution to a gel, its softness allowing envelopment of surrounding tissues.

**Poly ethylene glycol (PEG)**

PEG can have a linear or branched chain structure, it can become flexible when altering the cross-linking properties, enabling properties of the PEG to be best suited to allow tissue growth and cell enveloping. PEG can be cross-linked with covalent PEGs with reactive chain ends, giving a high water content, enabling hydrogel formation. PEG does not inflict an immune response within the body and has good biocompatibility. The degradation rate of PEG varies and occurs in the presence of oxygen, rates can be altered by lowering the temperature or implementing antioxidants into it.

**Hyaluronic acid**

Hyaluronic acid is a linear polysaccharide glycosaminoglycan found naturally in the ECM of most vertebrates and can be found in the cementum in small amounts. Hyaluronic acid interacts with growth factors, cellular and extracellular structures, aiding the tissue to maintain structure and homogeneities. It is biocompatible and rarely produces an immunological reaction, however it degrades rapidly and has low mechanical strength. Adding a hydrophobic group to the hyaluronic acids chemical group, slows degradation and increases the mechanical stability of scaffolds.

**Chitosan**

Chitosan is a natural linear polysaccharide, it is both biodegradable and biocompatible, serving as a good biomaterial that will not cause the human body to reject or have a cytotoxic reaction to it. Chitosan has very flexible usability, which can be formed into hydrogels, nanofibers, sponges, and bead-type scaffolds. Collagen type 1 has been used alongside chitosan, seeding epithelial-mesenchymal cells into its matrix, concluding that the collagen and chitosan combination allowed mesenchymal-derived dental pulp stem cells and HAT-7 epithelial cells to migrate and differentiate. If chitosan is used as a hydrogel, it needs chemical alteration, as chitosan would not dissolve in the body, reducing the usability of the scaffold.

**Silk**

Silk proteins are natural proteins, with good cellular biocompatibility and are biodegradable, however they have small pore size and mechanical property control. Additionally, the main fractions of natural *Bombyx mori* silk (heavy chain and light chain) fibroin have variable physical and mechanical properties and can be isolated using formic acid for further scaffold synthesis. Silk-based scaffolds were tested with combinations of enamel matrix derivatives (EMD) and/or induced pluripotent stem cells (iPSCs), this was to study how it could perform in repairing a periodontal defect. Silk-fibroin scaffolds with CaP coating have shown high porosity, good biodegradability, and improved bone contact, allowing stimulation of bone MSCs, with the combination of iPSCs and EMD encouraging cementum formation, alveolar bone and PDL.

**Rigid biomaterials**

Rigid biomaterials can act to sustain loads from teeth movement, acting as a structural substitute, however a rigid biomaterial may not be necessary as it could be too rigid to allow the biomaterial to attach to the native cementum of the tooth. If a scaffold were made to incorporate not just cementum formation, but alveolar bone formation also, rigid materials would need to be utilized to enable structure recreation at a defect site.

**Polylactic acid (PLA)**

PLA is a synthetic polymer with excellent biocompatibility and biodegradability. PLA releases lactic acid when degrading *in vivo*, which can be removed naturally by metabolic activities in the body. Hydrolysis degrades PLA without the need for enzymes. Lactic acid, the building block of PLA, can be easily sourced due to its natural presence in the environment and its production via the fermentation process to acquire lactic acid from renewable sources such as sugar cane.

**Polylactic-coglycolide (PLGA)**

PLGA is a synthetic polymer, which is formed from the combination of PLA and PGA, through ester bonding. These two polymers are combined because when used individually, their mechanical properties are poor, and the degradation rate is too fast to serve as a scaffold for bone-like structures, potentially causing premature scaffold collapse. ***N.*** ***Tanataweethum, W. Liu, W. Goebel, D. Li and T. Chu31*** combined PLGA with CaP, to reduce the inflammation when implanting these scaffolds. The ratio of PLA to PGA can be altered, resulting in different properties, such as slower or faster degradation rates, to suit the required application.

**Polyglycolic acid (PGA)**

PGA is a synthetic biomaterial which quickly degrades, is hydrophilic and highly crystalline, but has low mechanical strength. When degradation occurs, the metabolic processes remove the glycolic acid produced and result in it being excreted through the urine. PGA has been shown to be a great biomaterial to culture DPSCs, giving a high-density tissue with great collagen deposition when compared to collagen and alginate hydrogels, of which cell growth was insufficient for these materials.

**Scaffolds for engineering tooth – ligament interfaces**

The periodontium complex is a highly hierarchical organ composed of interlinked hard and soft tissues, including cementum, periodontal ligament (PDL), and alveolar bone, and as such represents an anatomically unique structure. The function of the periodontium is to biomechanically support the teeth by absorbing and transferring compressive stresses into the mandible and surrounding cranial structures caused by masticatory function. The periodontal ligament inserts into the cementum by the so-called Sharpey’s fibers, which form various angles with the root surface that reflect the distribution of the physiological load, thus exemplifying the role of the periodontal ligament tooth anchorage and masticatory force distribution.

Periodontitis is an inflammatory disease initiated by the infiltration of bacteria, leading to the destruction of soft and hard tissue. Current clinical techniques are efficient at controlling the inflammatory aspect of the disease and rely on the mechanical removal of plaque from the tooth surface, and implement purpose designed instruments for scaling and root debridement. These therapies are successful at limiting the progression of the disease by reducing the inflammatory response caused by the presence of bacteria. However, it only results in partial soft tissue reattachment, forming an inferior connection known as the long junctional epithelium, without the formation of new periodontal ligament attachment.

The concept of **“Guided Tissue Regeneration” (GTR)** was introduced in the early 1980s. This technique relies on three principles:

(1) Wound stabilization,

(2) Space maintenance allowing bone ingrowth, and

(3) Selective cell repopulation of the root surface.

This surgical procedure combines root debridement with the insertion of an occlusive membrane over the defect. The membrane, either resorbable or non resorbable, enables selective cell repopulation of bone and periodontal progenitor cells to the defect void, while restricting the infiltration of rapidly migrating gingival epithelial cells. This creates space for the repopulation of the defect with bone and periodontal ligament cells that have the capacity to regenerate the tissue, at the expense of gingival tissues that lack this ability.

GTR technology has critical limitations that result in limited clinical reproducibility, poor predictability, and a lack of truly functional regeneration. Even though, the literature shows that GTR can be efficient in selected cases, predictable periodontal regeneration remains elusive. Recent advancements in the field have advocated for the utilization of tissue engineered constructs, which could potentially circumvent the aforementioned limitation of GTR and achieve appropriate periodontal regeneration19.

**Scaffolds for periodontal regeneration:**

**Monophasic scaffolds**

The concept of tissue engineering is based on the implantation of a resorbable structure, referred to as the scaffold. Monophasic scaffolds are the most basic application of tissue engineering methodology toward periodontal regeneration. The effective implementation of a scaffold requires key physical attributes, such as a unique and precise geometry (possibly patient specific), potential cellular and growth factor delivery, capacity for extracellular matrix (ECM) formation, and vascularization along with timely degradation *in vivo*.

The vast majority of tissue engineering approaches using monophasic scaffolds targeting periodontal regeneration are directly or indirectly inspired by GTR, and hence the scaffold is designed to maintain both space and wound dimension, while allowing for selective cell repopulation. Although the utilization of monophasic scaffold has shown some efficacy toward periodontal regeneration, the complex architecture of the native tissue requires a highly coordinated spatiotemporal healing response to achieve functional regeneration. To this end, scaffold compartmentalization via the design of multiphasic structures has been advocated to facilitate such a complex regeneration process.

**Multiphasic scaffolds**

The novel development of additive biomanufacturing via 3D printing and associated technologies initiated a new phase in scaffold manufacturing with advanced architectural design. Computer-aided design models integrated with improved additive biomanufacturing technologies allowed the fabrication of scaffolds possessing variable architectures to a micro- and mesoscale. Subsequently, greater control of structural arrangements enabled mimicry of periodontal compartmentalized tissues and thus resulted in the emergence of a new generation of “multiphasic scaffolds.”

These multiphase constructs are envisioned as a solution to many of the insufficiencies of earlier attempts at periodontal regeneration using monophasic scaffolds, particularly regarding spatiotemporal control over regeneration to improve integration of multi tissue structures. Based on the number of phases included in the scaffold architecture, multiphasic scaffolds are divided into biphasic (i.e., PDL and alveolar bone compartments) and triphasic (i.e., PDL, alveolar bone, and cementum compartments).

**Tissue engineered alloplastic scaffolds for reconstruction of alveolar defects**

The alveolar ridge is present along the inferior surface of the maxilla and the superior aspect of the body of the mandible. This structure comprises of bony sockets in which dental organs are located. As a platform of insertion for the dentition, the alveolar ridge structurally contributes to speech, facial appearance, and mastication. Extensive alveolar defects can lead to significant quality-of-life issues, including social stigma, nutritional deficiency, and challenges with speech.

Various etiologies exist for alveolar bone defects, including congenital defects, infective/inflammatory processes, trauma, and iatrogenic resection of benign and malignant tumors. Approximately one-third of all birth defects affect the craniofacial region. Bone loss in the alveolar region usually leads to one of the three primary types of ridge deficiency: horizontal, vertical, or a complex configuration, where both vertical and horizontal component dimensions should ideally be re-established for adequate restoration of masticatory function through dental implants19.

**Additive manufacturing of synthetic biomaterials for alveolar bone regeneration**

Although there are many different biomaterials, which can be utilized for bone tissue engineering, bioactive calcium phosphates have the broadest use in humans due to their safety profile and biocompatibility that have been well documented for decades. Calcium phosphate (CaPO4) based bioactive ceramics have been utilized as an alternative to autogenous bone grafts in a multitude of clinical scenarios because of their similarities in composition to the inorganic phase of bone. To date, the most commonly utilized material has been hydroxyapatite (HA), which is the predominant inorganic component of bone. HA has proven to be both biocompatible and osseoconductive, making it a logical selection as a bone substitute.

Ceramics have yielded unfavorable outcomes, to bone replacement, providing the impetus to altering/improving construct designs in an effort to improve performance. These bioactive ceramics commonly exist in powder form or in prefabricated bulk shapes, and fabrication of personalized alveolar bone defects with fit-and-fill designs remains an elusive challenge. However, 3D printing technology has evolved, and protocols capable of printing viscous colloidal inks have been established, allowing the fabrication of personalized devices that are capable of fitting and filling bony defects of different size and complex tri-dimensional shape.

A lattice structure presenting tailored surface texture can also be incorporated within the scaffold to facilitate osseoconduction, vascular ingrowth, and scaffold degradation/absorption. Hence, through additive manufacturing methods, customized modifications to scaffold design can be systematically incorporated on the macro-, meso-, micro-, and nanometer level.

**Pediatric alveolar cleft defect regeneration**

Three of every four patients affected by cleft lip and palate have an alveolar osseous defect or an alveolar cleft. Such defects result in the patient presenting with insufficient osseous support of the dentition, nasal regurgitation through a nasolabial fistula, and facial asymmetry. Autogenous bone graft is the gold standard intervention for this bony defect and is commonly performed during the mixed dentition stage (6–11 years of age) of dental development. Successful treatment provides structural support for tooth eruption, closure of the nasolabial fistula, enhanced speech ability, and augmentation of the dental arch for improved esthetic outcome. Despite cases of exceptional long-term outcomes, this procedure is associated with donor site morbidity and requirement for hospitalization. Graft resorption has been well documented, with many studies reporting the need for secondary procedures and revision rates from 12% to 40%, with patients presenting with wider or bilateral clefts having poorer prognosis.

Based on the promising preclinical results seen in experiments reconstructing mandibular defects, the application of dipyridamole-loaded bioceramic scaffolds has also been investigated in critical-sized alveolar defects in translational skeletally immature rabbit models. This experiment demonstrated the regenerative properties of 3D-printed scaffolds with dipyridamole to induce dose-dependent bone growth in the alveolar bone of rabbits. Furthermore, bone formation spanned the length of the established critical-sized defect.

Analysis of bone quality via nano-indentation suggested that scaffold-treated animals had superior bone healing unlike the random bone formation that occurs with bone grafts, new bone was present both within the scaffold and at the interface between osteotomy and construct. The new bone was also highly cellular and vascularized. Furthermore, results suggested that the scaffold’s inherent osseoconductivity was effective and bolstered by dipyridamole’s local effects, but not overwhelmed by dipyridamole’s osteogenic potential.

Of note for pediatric patients, even at doses of one to two logarithmic increases more than that needed to increase bone formation, the premaxillary–maxillary suture remained patent. This is in direct contrast to compounds such as rhBMP-2, which cause a wide array of adverse events, such as premature suture closure, and therefore are contraindicated in pediatric patients as per FDA guidelines. Long-term experiments have since been conducted to assess outcomes following scaffold implantation.

Initial findings from a 24-week experiment directly comparing DIPY-coated 3D-printed β-TCP to autologous bone graft are encouraging. After 6 months, both horizontal and vertical augmentation were visualized with substantial scaffold degradation compared with that at 8 weeks. Bone repair was comparable between experimental animals and control animals treated with autologous bone graft. Importantly, craniofacial growth also appeared comparable between 3D-printed β-TCP scaffold and autologous bone graft control—there was no readily evident increase in asymmetry, ectopic bone formation, or morbidity or mortality associated with DIPY-coated 3D-printed β-TCP scaffold-treated animals19.

**Whole tooth reconstruction**

The interest of tissue engineering within periodontics has predominantly been associated with the regeneration of the tooth–ligament–bone interfaces. Considerable research, however, has also been conducted toward the intriguing notion of bioengineered teeth. Whole tooth tissue engineering is based on recapitulation of the complex and highly coordinated biological events occurring during odontogenesis. The fundamental requirements for tooth tissue engineering are no different to engineering of other organs, which involves scaffold production, the addition of cells and/ or growth factors, and implantation in a human host.

Regeneration of a whole tooth involves the total rebuilding of functionality and morphology of the tooth crown and root, thus implicating multiple tissue groups, including enamel, cementum, dentin, and the neurovascular core known as the dental pulp. To achieve functional tooth regeneration, these structures must be integrated in the mandible, and therefore, mature PDL fibers attachment is, here also, a fundamental requirement.

In 2002, ***Young et al.32*** first endeavored to regenerate the tooth by seeding a PGA/ poly-L-lactic (PLLA) monophasic scaffold with dissociated porcine-derived tooth bud cells in a single-cell suspension, resulting in the formation of multiple small tooth structures, containing dentin and enamel-specific proteins. This signified the first real example of tissue-engineered tooth like structures being formed *in vivo*, and although it was not successful at precisely controlling both the structures and the number of teeth formed, it demonstrated the feasibility of the approach.

***Young*** and coworkers refined their technology by subsequently developing a PGAPLGA hybrid tooth–bone scaffold combining osteoblast and tooth bud cells. In this approach, tooth regeneration was obtained via a combination of *in vitro* culture and *in vivo* maturation before combining with the osteoblast-seeded scaffold and subsequent implantation. This resulted in the formation of histologically verified tooth like structures (dentin and enamel) along with the presence of collagen type III at the interface between the bioengineered tooth and the bioengineered bone32.

The first example of controlled single tooth germ regeneration was performed by ***Honda et al33.*** who 2 years later in 2006 developed an original technique involving controlled interactions between the cells by sequentially seeding mesenchymal and epithelial cells in a collagen scaffold. This resulted in the development of an enamel–dentin complex, established 5 weeks faster than in previously reported investigations. Furthermore, the tooth structures were more similar to native teeth.

**Teeth as natural scaffolds for tissue engineering**

The advantage of decellularized scaffolds is the maintenance of the original organ morphology, internal architecture, and biochemical composition (ECM and remaining growth factor), whereas the decellularization reduces immune-rejection by the removal of the cellular component. In 2014, ***Ravindran et al34.*** used decellularized teeth as a bioactive 3D ECM scaffold. The natural scaffold demonstrated odontogenic differentiation in hPDL stem cells and human dental pulp stem cells, as demonstrated by the formation of dental pulp like tissue with dentin sialoprotein and dentin phosphophoryn expression in a nude mice model.

In another comprehensive work, ***Zhang et al.35*** investigated *in vivo* decellularized tooth buds seeded with various cell types to determine the ideal cell combination for whole teeth bioengineering. Human dental pulp cells, porcine dental epithelial cells, and human umbilical vein endothelial cells were seeded, and the recellularized tooth buds displayed enhanced regeneration compared with experimental and control groups. ***Zhang et al.*** acknowledged the importance of their work, as the first case of decellularized tooth buds capable of whole tooth regeneration.

1. **CONCLUSION**

The tooth is a complex biological organ, whose formation requires an intricate cascade of molecular signals and gene expression. Understanding the biological processes and interactions of various growth factors and gene expression underlying tooth development will be critical for tooth regeneration. Importantly, the successful differentiation of dental stem cells into specific lineages is one of the main challenges in stem cell research. Tissue engineering based on stem or progenitor cells is a promising approach for restoring the integrity of dental and maxillofacial structures. Improvements in the tissue engineering field may also help the clinicians in the replacement of the damaged tooth structure with tissue engineered scaffolds in future, instead of replacing these structures with acellular lumps of restorative material without any healing function.

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