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Biopolymer Conjugated Protein Based Hydrogel Scaffolds for Tissue Engineering Application

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ABSTRACT

Tissue engineering is an emerging research area that aims to regenerate the harvested cells/tissues for implantation. Important factors in tissue engineering are cell, growth factors, scaffolds, biochemical and physicochemical factors and methods. The term regenerative medicine and tissue engineering are used synonymously. The important property of the scaffold is to mimic the extracellular matrix. If the matrix is composed of natural proteins and biocompatible polymers, it supports cell proliferation. Biopolymer protein conjugate plays an important role as a scaffold that will provide 3-D structure and bio-similar extracellular matrix structure for tissue regeneration. The biopolymer-protein composite, being biodegradable can be implanted in the body with the cells and slowly biodegrade in the body allowing cells to form tissue or organ. This article focus on a detailed overview of biopolymer-protein based scaffolds used in tissue engineering, their properties, rationale and techniques of development and tissue engineering applications.

1. INTRODUCTION

Many biological materials like silk, cellulose, chitosan, collagen, and gelatin are used traditionally as biopolymers. Modern biotechnological methods like genetic engineering, nanotechnology, tissue engineering, bacterial fermentation, employ these biopolymers in their processes. Currently, many researchers working on biomedical applications are focused on using biopolymers like chitin, chitosan, collagen, starch, polylactic acid (PLA), polyglycolic acid (PGA), hyaluronic acid and its derivatives. A therapeutic approach that combines cells, biomaterials, and microenvironmental factors to induce differentiation signals for cells to develop into surgically transplantable formats and promote tissue repair and/or functional restoration is referred to as tissue engineering. Polymer-protein conjugates are widely used as therapeutics, they display a unique combination of properties derived from both the biologic and synthetic materials, which can be individually tuned to elicit the desired effect. For tissue culture, inherent protein biorecognition can be useful in better cell adhesion and proliferation on scaffolds.[1] This fusion of biological properties and chemical stability or reactivity gives protein−polymer conjugates a unique position at the intersection of chemistry, biotechnology, nanotechnology, and medicine. The subject of protein−polymer conjugates has been extensively reviewed [2−10] with details on synthetic methods and comprehensive summaries of reported work.

2. Biopolymers

Natural biodegradable polymers are generally termed as biopolymers. Biopolymers are polymers that are generated from renewable natural resources and are often biodegradable and non-toxic. They are large macromolecules composed of single or many repeating monomer units. These polymers are of very high molecular weight and monomer composition influences their material characteristics. Polysaccharides such as starch and cellulose signify the most characteristic family of these natural polymers. Other natural polymers such as proteins can also be used to produce biodegradable materials. [11-18]

The interest in biopolymers is mainly due to their biodegradability. The inherent mechanism of biodegradable materials, which makes them capable of undergoing decomposition into carbon dioxide, water, inorganic compounds, methane, or biomass, is the enzymatic action. This biodegradation can be measured by various procedures and thereby establish the natural process of degradation.[19]

2.1 The ideal properties for biopolymers are:

It should be biodegradable, biocompatible, sustainable and renewable. It should also be non-toxic as it will be in direct contact with the blood. Biopolymers should be non-immunogenic and non-carcinogenic. It should possess properties like bacteriostatic, fungicidal and anti-viral. They should be manipulatable to incorporate medicines and must have good mechanical strength.

2.2 Classification of biopolymers

Biopolymers are generally classified as follows:

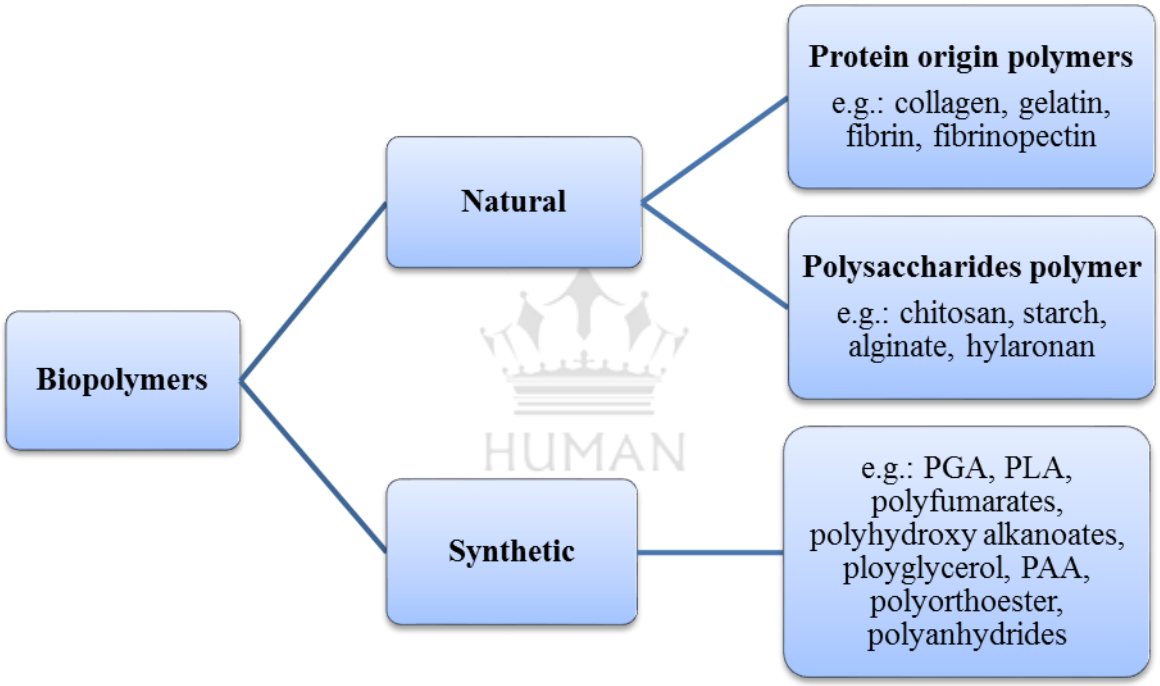

Figure No. 1: Classification of biopolymers

2.3 Applications of biopolymers

The applications of biopolymers are as follows:-

Wound healing: polyethylene glycol and agar, xanthan, methylcellulose, carboxymethyl cellulose, alginate, hyaluronan.

Drug delivery: starch, polyvinylpyrrolidone, polyacrylic acid, chitosan, and acrylic acid.

Dental materials: Hydrocolloids.

Tissue engineering: hyaluronan, chitosan, and collagen. [20]

Implants: cellulose and collagen.

Injectable polymeric systems: polypeptides, chitosan.

Skin derivatives: highly purified bovine collagen formalin-fixed skin.

Sutures: Collagen, catgut, brananferulate

Hemostasis: Fibrin sealant and foam, chitosan and poly (N-acetyl glucosamine) gels and chitosan adhesives.[21]

3. SCAFFOLDS

Scaffolding technique has been evolved in the mid-1980s, tissue engineering has continued to evolve as an exciting and multidisciplinary field aiming to develop biological substitutes to restore, replace or regenerate defective tissues.[22]Scaffolds, cells and growth-stimulating agents are generally referred to as the tissue engineering triad, which is the key component of engineered tissues. Typically scaffolds are made up of polymeric biomaterials that provide the structural support for cell attachment and tissue development.

3.1 Properties of an ideal scaffold

Many scaffolds are produced by different biomaterials and are manufactured using an excess of fabrication techniques that have been used to regenerate different tissues and organs in the body. Regardless of the tissue type, other numbers of key considerations are important while designing or determining the suitability of a scaffold in tissue engineering.

3.1.1 Biocompatibility

The most important criterion of any scaffold for tissue engineering is that it must be biocompatible; i.e. cells must adhere, function normally and migrate onto the surface and eventually through the scaffold and begin to proliferate before laying down the new matrix. After implantation, the scaffold or tissue-engineered matrix must elicit a negligible immune reaction to prevent it from causing a severe inflammatory response which might reduce healing or cause rejection by the body.

3.1.2 Biodegradability

The objective of tissue engineering is to allow the body's cells, over time, to eventually replace the implanted scaffold or tissue-engineered construct. Scaffolds are not intended as permanent implants and therefore should be biodegradable to allow cells to produce their extracellular matrix. [23] The by-products of this degradation should also be non-toxic and should be able to exit the body without interfering with other organs. As tissue engineering strategies are entering clinical practice more routinely, the field of immunology is also playing an important role in the research area. [24,25]

3.1.3 Mechanical properties

The scaffold should have mechanical properties that are consistent with the anatomical site where it is to be implanted. It is one of the great challenges to produce scaffolds with adequate mechanical properties in developing bone or cartilage. For these tissues, the implanted scaffold must have sufficient mechanical integrity and strength to function from the time of implantation to the formation of the remodeling process. On the contrary healing rates also varies with age. Many materials have been discovered recently that have good mechanical properties but to the loss of posing less porosity, which has presented to have potential *in-vitro* but has failed when implanted *in vivo* due to lacking the capacity for vascularisation. Hence it characterizes that for any scaffold to succeed there should be a balance between the mechanical properties and porosity.

3.1.4 Scaffold Architecture

The architecture of scaffolds used for tissue engineering is critically important and should have a pore interconnected structure and high porosity to ensure cellular penetration and adequate diffusion of nutrients to cells within the construct and the extra-cellular matrix formed by these cells. Additionally, an interconnected porous structure is required to allow diffusion of waste products out of the scaffold, and the products of scaffold degradation should be able to exit the body without interfering with other organs and surrounding tissues. For any scaffold, a critical range of pore sizes exists (100-500 µm)[26,27] which may vary depending on the cell type used and tissue being engineered.

3.1.5 Manufacturing technology

To become clinically and commercially viable, a scaffold should be cost-effective and should be possible to scale-up from making one at a time in a research laboratory to small batch production. [28]The development of scalable manufacturing processes to good manufacturing practice (GMP) standard is critically important in ensuring the successful translation of tissue engineering strategies to the clinic. [29] This will determine how either the scaffold or the tissue-engineered construct will be stored. The final criteria for scaffold fabrication in tissue engineering are listed above.

3.2 Prerequisites for selection/development of scaffold

i. Tissue to be regenerated should be considered for absorption kinetics of scaffolds. For example scaffold for tissue engineering of the skeletal system should possess a slow degradation rate, to maintain the mechanical strength until tissue regeneration is completed.[30]

ii. It should have processability to form complicated shapes with varying porosity. An ideal pore size should lie between 100 and 500 μm.[31]

iii. It should have mechanical properties that must match the tissue at the site of implantation or tensile forces without inhibiting appropriate biomechanical intimation.

iv. It should also have acceptable biocompatibility and toxicity profiles and having the ability to support cell growth and proliferation.[32]

v. Ideally, an injectable composition should be in liquid/paste form, sterilizable without affecting any chemical characteristics, and have the capacity to incorporate biological matrix requirements to be useful in tissue engineering applications. Upon injection, the mixture should bond to biological surface and cures to a solid and porous structure with appropriate mechanical properties to suit the application. The process of curing should have minimum heat generation and the chemical reactions involved should not cause any harm to cells and tissues.

vi. Biodegradability is often an essential factor since; the scaffold should be absorbed by the surrounding without surgical removal necessity.

vii. The scaffold should have the ability to mimic the native extracellular matrix (ECM), an endogenous substance that surrounds cells, bind them into tissues and provide signals that aid cellular development and morphogenesis.

3.3 Types of scaffolds

3.3.1 Porous Scaffold

Biopolymeric porous scaffolds having higher porosities are exceptionally valuable for tissue designing. Porous scaffolds which are of foam or spongy sort have been utilized in tissue engineering,[33]especially for the development of host tissue, bone regrowth or organ regeneration. Along these lines, the porous system of scaffolds simulates the ECM architecture which enables the cells to associate viably with their environment. Though property of foams and sponges are alike more mechanically stable compared to mesh structures, the use is still confined because of the open spaces present throughout the scaffolds (Fig. 2a).

A foam polymeric scaffolds approach has few potential advantages for proliferating or adherent cell lines, for example,

(a) provides a physical surface on to which the cells can lay their very own ECM,

(b) may restrain cell development of adherent contact-inhibited cells,

(c) gives improved nutrient transport to the center of the device through the porous interconnecting channel network, and

(d) may restrict cluster size by limiting the pore size of the foam and in this manner eliminating out extremely large clusters that can potentially develop a necrotic focus.

Depending on the choice of solvent and phase separating conditions, foams can be controlled to form either random or oriented pore architectures.[34] Improvement in the structure and increased pore interconnectivity of the porous scaffold is required for the development of artificial blood vessels[35] or peripheral nerve growth. Three-dimensional shapes are required which lead to the advancement of modern extrusion technologies and techniques for adhering porous membranes to desirable shapes. [36] Ideal pore sizes vary for various cells and tissues. [37] Porous scaffolds can be manufactured with specific pore size, porosity, surface-area-to-

volume ratio, and crystallinity. Porous controlled-release systems contain pores that are sufficiently huge to empower the dissemination of the medication. [38]

Synthetic biodegradable polymers, for example:- Poly L-lactic corrosive (PLLA), Polyglycolide (PGA), Poly lactic-co-glycolic corrosive (PLGA), polycaprolactone (PCL), [39] poly (D, L-lactic corrosive) (PDLLA), polyester elastomer (PEE) in light of poly(ethylene oxide) (PEO), and poly(butylene terephthalate) (PBT) [40] are utilized as porous scaffolding materials.

For upgraded authority over porosity and pore distance across when contrasted with most creation strategies, a dissolvable throwing, and particulate filtering system were created. Electrospinning is a cutting edge technique for making porous scaffolds made out of nano- and microscale biodegradable fibers.

3.3.2 Hydrogel Scaffold

For a decade, hydrogels have been used as scaffolds to guide the growth of new tissues which played an ever-increasing role in the revolutionary field of tissue engineering. The design and application of biodegradable hydrogels have dramatically increased the potential impact of hydrogel materials in the biomedical field and enabled the development of exciting advances in controlled drug delivery and tissue engineering applications.[41]

Hydrogels consist of naturally derived macromolecules that have potential advantages of biocompatibility, degradability, intrinsic cellular interaction, etc. Hydrogels have structural similarity to the macromolecular-based components in the body and are considered biocompatible (Fig 2b).

Hydrogels are made either from synthetic or natural polymers, which are crosslinked through either covalent or noncovalent bonds.[42] Hydrogels in tissue engineering must meet several design criteria to function appropriately and promote new tissue formation.

These criteria include both physical parameters (e.g., degradation and mechanics) as well as biological performance parameters (e.g., cell adhesion). Degradation rates of tissue scaffolds must be matched to the rate of cellular processes to optimize tissue regeneration.[43, 44]

Therefore, the degradation behavior of all biodegradable hydrogels should be well defined, reproducible, and tunable via hydrogel chemistry or structure. Biocompatible hydrogels are

currently used in cartilage wound healing, bone regeneration, wound dressing,[45] and as carriers for drug delivery.[50] Hydrogel with growth factors can act directly to support the development and differentiation of cells in the newly formed tissues.[46] Hydrogels with high water content favors rapid nutrient diffusion which is favorable for promoting cell migration and angiogenesis.[47] The hydrogel scaffolds have received intensive study for their use in the engineering of replacement connective tissues, primarily due to their biochemical similarity with the highly hydrated glycosaminoglycans (GAGs) components of connective tissues.

Examples of hydrogel-forming polymers of natural origin are:- collagen, gelatin, fibrin, hyaluronic acid (HA), alginate and chitosan.

The synthetic polymers are :-Poly(lactic acid) (PLA),Poly(propylene fumarate) (PPF)-derived copolymers,PEG-derivatives, and Poly(vinyl alcohol) (PVA).

3.3.3 Fibrous Scaffold

The development of nano-fibers has enhanced the scope for fabricating scaffolds that can potentially mimic the architecture of natural human tissue at the nanometer scale.

Present there are three techniques available for the synthesis of nanofibers: electrospinning, self-assembly, and phase separation.

Electro-spinning is the most widely studied technique and also seems to exhibit the most promising results for tissue engineering applications. Nanofibers synthesized by self-assembly and phase separation have had relatively limited studies that explored their application as scaffolds for tissue engineering. The high surface-area-to-volume ratio of the nanofibers combined with their microporous structure favors cell adhesion, proliferation, migration, and differentiation, all of which are highly desired properties for tissue engineering applications (Fig 2c).[48, 49]

Nanofibers used as scaffolds for musculoskeletal tissue engineering including bone, cartilage, ligament, and skeletal muscle, skin, vascular, neural tissue engineering, and a vehicle for the controlled delivery of drugs, proteins, and DNA.[50]

Natural polymers and synthetic polymers explored for the fabrication of nanofibers such as collagen, gelatin, chitosan, HA, silk fibroin, PLA, polyurethane(PU), PCL, PLGA,

poly(ethylene-vinyl acetate) (PEVA) and poly (L-lactide-co-caprolactone) (PLLA-CL) are fibrous scaffolds in biomedical application.

The blending (or mixing) technique is a common choice for the nanofiber functionalization. However, most of the polymer nanofibers do not possess any specific functional groups, and they must be specifically functionalized for successful applications.

The most popular and simplest nanofiber modification methods are physical blending and coating.

Surface grafting polymerization has also been used for attaching ligand molecules and adhesive proteins on the nanofiber surface for the application of affinity membrane and tissue engineering scaffold, respectively. Drugs, growth factors, and genes can be directly mixed into the polymer solution and electrospun to prepare drug carriers with controlled release properties.[51]

3.3.4 Microsphere scaffold

Microsphere-based tissue engineering scaffold designs have attracted significant attention in recent years. *M. Singh et al.* [52][53] initially used a microsphere-based approach for tissue engineering scaffold. Microsphere scaffolds are having a spatial extension and temporal duration control which provides the stiffness gradients for interfacial tissue engineering.

Microsphere scaffolds are increasingly used as drug delivery systems and in advanced tissue engineering applications such as gene therapy, antibiotic treatment of infected bone, and so forth.[54]

The influence of nanotechnology on scaffold design and the possibility of sustained-release formulations of growth factors via microspheres are showing promising developments. Microsphere scaffolds are generally a polymer matrix used for drug encapsulation for the release of drugs at a relatively slow rate over a prolonged period (Fig. 2d)[55]

Polymers with the low molecular weight used in developing porous microspheres for the rapid release of the drug, while polymers with high molecular weight for developing microspheres for a slower drug release profile can be achieved due to its dense nature.[56]

Injectable microspheres have also been developed for the controlled delivery of drugs.[57]

Microspheres as building blocks offer several benefits, including ease of fabrication, control over morphology, physicochemical characteristics, and its versatility of controlling the release kinetics of encapsulated factors.[58]

The methods used to produce microsphere-based scaffolds have utilized heat sintering [59,60],solvent vapor treatment [61,62],solvent/nonsolvent sintering method [63,64]ornonsolvent sintering technique.

Particle aggregation methodology is proposed to fabricate bilayered scaffolds for osteochondral tissue engineering to achieve an improved integrative bone and cartilage interface which has been needed for this application.

Poly(lactic acid-co-glycolic acid) PLAGA microsphere scaffolds are in the range of trabecular bone, demonstrating the potential of the porous microsphere matrix to be used as a scaffold for load-bearing bone tissue engineering.[65]

The sintered microsphere matrix shows promise as a bone regeneration scaffold.

The advantage of the sintered microsphere structure is its pore interconnectivity and desirable three-dimension pore size. The gel microsphere matrix and the sintered microsphere matrix were designed using the random packing of PLAGA microspheres to create a three-dimensional porous structure for bone regeneration.[66]

Composite microspheres are also used for the fabrication of polymer-ceramic matrices for bone applications.[67]

Chitosan microsphere scaffolds have been produced for cartilage and osteochondral tissue engineering.[68]

3.3.5 Polymer-Bioceramic Composite Scaffold

The development of composite materials for tissue engineering is attractive since their properties can be engineered to suit the mechanical and physiological demands of the host tissue by controlling the volume fraction, morphology and arrangement of the reinforcing phase.[69]

Ceramics used in fabricating implants can be classified as nonabsorbable (relatively inert), bioactive or surface reactive (semi-inert),[70]and biodegradable or re-absorbable (non-inert).[71]

Alumina, zirconia, silicon nitrides, and carbons are inert bioceramics.

Certain glass ceramics and dense HAP are semi-inert (bioreactive), and examples of re-absorbable ceramics are aluminum calcium phosphate, coralline, Plaster of Paris, Hydroxyapatite (HAP) and Tricalcium Phosphate (TCP) (Fig. 2e). [72]

Ceramics are known for their good compatibility, corrosion resistance, and high compression resistance.

Drawbacks of ceramics include brittleness, low fracture strength, difficulty to fabricate, low mechanical reliability, lack of resilience and high density. In recent years, humans have realized that ceramics and their composites can also be used to augment or replace various parts of the body, particularly bone. Thus, the ceramics used for the latter purposes are classified as Bioceramics.

Polymers by themselves are generally flexible and exhibit a lack of mechanical strength and stiffness, whereas inorganic materials such as ceramics and glasses are known to be too stiff and brittle.

The combination of polymers and inorganic phases leads to composite materials with improved mechanical properties due to the inherent higher stiffness and strength of the inorganic material.

Secondly, the addition of bioactive phases to bioresorbable polymers can alter the polymer degradation behavior of the scaffolds.[73]

Complications in the development of polymer bioceramics composite scaffold are

(i) maintenance of strength and the stability of the interface during the degradation period and replacement by the natural host tissue and

(ii) matching resorption rates to the repair rates of body tissues developed for hard tissue implants and tissue engineering scaffolds, due to their excellent biocompatibility, bioactivity, and biosorption in calcified tissue.

Highly porous polymer/ceramic composite scaffolding appears to be a promising substrate for bone tissue engineering due to its excellent mechanical properties and osteoconductivity.[74]

PLGA/HAP composite scaffold has excellent biocompatibility with hard tissues and high osteoconductivity and bioactivity.[75]The composite scaffolds supported uniform cell seeding, cell ingrowth, and tissue formation.

The major inorganic component of natural bone; bioceramics, including calcium phosphate(CP), HAP, and TCP are composite with PLLA, collagen, gelatin [76], chitosan and modified chitosan[77]are widely used as scaffolding materials for bone repair.

3.3.6 Acellular Scaffold

Acellular tissue matrices can be prepared by manufacturing artificial scaffolds or by removing cellular components from tissues by mechanical and chemical manipulation to produce collagen-rich matrices.[95-97]These matrices slowly degrade on implantation and are generally replaced by the ECM proteins secreted by the in growing cells.

The ultimate aim of any decellularization protocol is to remove all cellular material without adversely affecting the composition, mechanical integrity, and eventual biological activity of the remaining ECM. The decellularized biological scaffold was introduced to obtain a physiological matrix scaffold that resembles native blood vessels (Fig 2g).[81]

Acellular tissue matrices have proven to support cell ingrowth and regeneration of genitourinary tissues, including urethra and bladder, with no evidence of immunogenic rejection. Ureteral acellular matrices were utilized as a scaffold for the ingrowth of ureteral tissue in rats.

Acellular bladder matrix has served as a scaffold for the ingrowth of host bladder wall components in rats. Since the structures of the proteins (e.g., collagen and elastin) in acellular matrices are well conserved and normally arranged, the mechanical properties of the acellular matrices are not significantly different from those of native bladder submucosa.[82]The matrix was prepared by mechanically and chemically removing all cellular components from bladder tissue.[83] To engineer tissues successfully, the selection of scaffolds is critical. Although various synthetic biodegradable polymer scaffolds have been developed and improved by

mimicking biological structures, comparing to other scaffolds, acellular scaffolds have the following advantages.

(i) Acellular scaffolds retain their correct anatomical structure even after the decellularisation process.

(ii) Acellular scaffolds retain native ECM architecture and possess the cell adhesion ligands.

(iii)The decellularisation process considerably reduces immunological responses by completely removing cellular components.

(iv)The decellularisation process facilitates similar biomechanical properties as those of native tissues that are critical for the long-term functionality of the grafts.

Various extracellular matrices have been utilized successfully for tissue engineering in animal models and products incorporating decellularized heart valves, Small Intestinal Submucosa (SIS), and urinary bladder have received regulatory approval for use in human patients.[84]

As the scaffold is composed of ECM proteins ideally found in the body thus no immunological responses are observed. When derived from a vessel, the three-dimension architecture is very similar to that of the original, thus conferring appropriate mechanical and physical properties, which is essential in identifying and predicting optimal cell environments to develop scaffolds for preliminary analysis and implantation. Naturally, derived materials and acellular tissue matrices have the potential advantage of biological recognition. Polymer coating of a tissue-derived acellular scaffold can improve the mechanical stability and enhance the hemocompatibility of the protein matrix. Such hybrids can be complex structures such as heart valves, for example, fabricated from decellularized porcine aortic valves and dip-coated with a biodegradable polymer.[85]

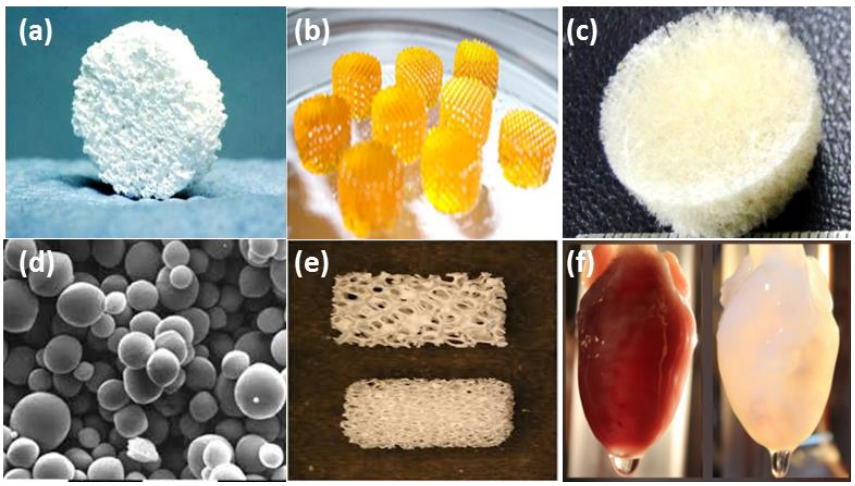

Figure No. 2: Types of scaffolds

a. porous scaffold, b. hydrogel scaffold, c. fibrous scaffold, d. microsphere scaffold,

e. polymer-bioceramic composite scaffold, f. acellular scaffold

3.4 Reason for developing scaffolds

a. Allow cell attachment and migration.

b. Deliver and retain cells and biochemical factors.

c. Enable diffusion of vital cell nutrients and expressed products.

d. Exert certain mechanical and biological influences to modify the behaviour of the cell phase. [86]

3.5 Advantages of biopolymer scaffolds

a. Metals lack degrade ability in a biological environment thus biopolymer is used though metals have an added advantage of having a superior mechanical property when compared with biopolymer.[87]

b. Inorganic/ceramic materials such as HAP or CP, though having good osteoconductivity are studied for mineralized tissue engineering, but restricted due to poor processability to form a highly porous structure and brittleness.

c. A natural ECM or its derivatives may not be the ideal scaffold for tissue engineering applications as tissue engineering should be an accelerated regeneration process compared to

the natural development process. Mature tissue matrix often does not possess highly interconnected macro- or micropore structures to allow for quick and uniform cell populations throughout, which is essential for a tissue engineering/repair process. Hence certain artificially designed scaffold features (such as porosity, pore size, interpore connectivity, etc.) are necessary for optimal tissue engineering applications (accelerated tissue regeneration). Possible immune rejection and pathogen transmission when a natural ECM is used is still a debated issue.[88]

4. Techniques used in tissue engineering(Scaffold fabrication methods)

Different methods have been described in the literature for preparing porous structures that can be employed as tissue engineering scaffolds. Some are enlisted below:-

4.1 Nano/Microfiber Sheet

Presently for the synthesis of nanofiber:- electrospinning, self-assembly, and phase separation are three techniques available in which electrospinning is the widely studied technique.[89]

The future of fabricating scaffolds that can potentially mimic the architecture of natural human tissue at the nanometer scale is enhanced due to the development of nanofibre. The high surface area to volume ratio of the nanofiber combined with their microporous structure is an added advantage thus helps in cell adhesion, proliferation, migration, and differentiation which are highly desired properties for tissue engineering applications.[90]By applying an electric potential to a polymeric solution in the electrospinning process, fibers ranging from 50 nm to 1000 nm or greater can be produced.[91, 92][93]At the tip of a capillary tube the solution is held and electrical potential which is applied provides a charge to the polymer solution. Mutual charge repulsion in the polymer solution induces a force that is directly opposite to the surface tension of the polymer solution.[94, 95]An increase in the electrical potential causes the potential to reach a critical value, at which it overcomes the surface tension forces to cause the formation of a jet that is ejected from the tip.[96-98]The components of a system assemble themselves spontaneously via an interaction to form a larger functional unit is referred to as “self-assembly”. Direct specific interaction and/or indirectly through their environment can lead to spontaneous organization. Recent technological developments have given a great impetus on studying the materials on a nanometer scale.

4.2 Solvent casting/particulate leaching method

It is one of the traditional methods of scaffold manufacture this begins with the dissolution of polymer with the organic solvent.[102] The technique uses particulate porogen to form sponge/ foam –like scaffolds. The polymer is hardened as the solvent evaporates, water is then used to dissolve the porogen which is salt like sodium chloride.

Disadvantage: time-consuming leaching step, which significantly increases scaffold preparation time.

4.3 Phase separation/emulsification

These include emulsification/freeze-drying liquid-liquid phase separation.[103, 104]The fabrication of highly porous PLGA scaffolds uses emulsion freeze-drying.[105] The processing method includes forming an emulsion by homogenization of a polymer solution (in an organic solvent) and water mixture, rapidly cooling the emulsion to lock in the liquid state structure, and remove the solvent and water by freeze-drying. The phase separation process is used if we need porosity greater than 90% and pore size ranging from 20 to 200 µm.

The drawback of this method is the closed pore structure in the resulting matrix.[106]

4.4 Gas-Foaming Process

Gas frothing takes out the utilization of solvents conveyed in dissolvable casting/particulate filtering techniques (Fig. 2B). This procedure forms a permeable structure through the nucleation and development of gas bubbles scattered all through a polymer. Pressure forming is first used to make solid discs of scaffold material.

For example, poly(lactic-co-glycolic corrosive), inside a heated mold. Following this, it is saturated with carbon dioxide by introducing to high weight CO_2 gas (5.5 MPa) for 72 h at room temperature, before dissolvability of the gas in the polymer is quickly diminished by lessening CO_2 weight to climatic dimensions ($P0CO_2$). This causes the CO_2 gas to cluster together, making pores. Porosities of up to 93% and pore sizes of up to 100 mm can be obtained utilizing this procedure. (112-113) It is difficult to control the pore network and pore sizes by gas foaming.

4.5 Rapid-Prototyping Techniques / Bioplotters

Fast prototyping is an innovation that depends on the advanced development of software engineering and assembling industry. [116,117] The primary aim of these methods is their capacity to create complex items quickly from a PC supported plan (CAD) display. This procedure creates parts by ink-stream printing a folio on to successive powder layers. The activity parameters, for example, the speed, stream rate, and drop position can be PC controlled to create complex 3D polymer scaffolds. In any case, the constraint of this strategy is that the goals are controlled by the fly size, which makes it hard to structure and manufacture frameworks with fine microstructures. Mechanical properties of the platforms should be improvised to better the porosity of scaffold[118]Researcher have demonstrated that it is conceivable to frame three-dimensional scaffolds, printing bars of PLLA powder at that point including a dissolvable, to be specific chloroform, conveyed from the ink-stream printer. Weaknesses incorporate the low processing yield (30%) of the PLLA granules and manual situating of the polymer powder bed, a tedious activity. [119]

4.6 Thermally Induced Phase Separation

In this procedure the polymer is first disintegrated in a dissolvable at a high temperature; liquid-liquid or strong liquid stage partition is incited by lowering the temperature. Resulting evacuation of the solidified solvent rich stage by sublimation leaves permeable polymer scaffolds.[120-122]The components overseeing pore estimate are polymer, dissolvable, grouping of the polymer solution and stage partition temperature. This technique, for the most part, produces scaffolds with a pore size of 10-100 µm. The mechanical properties of fabricated scaffolds are greatest by the above method.

4.7 Surface Modification

Recently a variety of synthetic biodegradable polymers has been used as tissue engineering scaffolding materials. The drawback of these scaffolds is their lack of biological recognition on the material surface. Hydrophobic polymers do not provide the ideal environment for cell-material interactions. Hence modification of the surface of polymeric scaffolds has a great scope to explore.[124,125]Various techniques are being utilized to improve surface such as SBF concentration, incubation time, pH value, pre-treatment using an aqueous solution.[126]

4.8 Emulsification/Freeze-drying

Freeze drying starts with freezing of a polymer solution, bringing about the formation of solvent ice crystals surrounded by polymer aggregates (Fig. 2c). The surrounding pressure is at that point diminished using a vacuum, to a level lower than the equilibrium vapor pressure of the frozen solvent (P_0). The solvent is thus triggered to undergo sublimation directly into the gas from the solid phase. At the point when the solvent is sublimated, a dry polymer scaffold with an interconnected permeable structure remains.

Emulsification freeze-drying can likewise be utilized as an essential scaffold fabrication technique. The procedure starts by dissolving polymers/ceramics production in a solvent and afterward blending with water, to acquire an emulsion. The blend is filled in a mold and solidified before the two separates. The frozen emulsion is then freeze-dried to evacuate the solvent and dispersed water, creating pores in a solidified scaffold.

4.9 Soft lithography technique

Soft lithography is a technique for replicating or fabricating structures by using "elastomeric stamps, molds, and conformable photomasks". As it uses elastomeric materials, it is called "soft", most notably poly(dimethylsiloxane) (PDMS). It is used to construct features measured from the micrometer to nanometer scale. In the case of soft lithography, there is no need for complex laboratory facilities and high energy radiation.

There are various advantages over photolithography or electron beam lithography, they include:- It is well-suited for applications in biotechnology, plastic electronics, more pattern transferring methods than traditional lithography and it does not require a photo-reactive surface for creating a nanostructure.

4.10 Laser-assisted Bio Printing (LaBP)

Laser-assisted Bio Printing (LaBP) can be utilized to build multicellular 3D patterns in a natural matrix, and thus generate constructs that are functioning and forming tissue. LaBP arranges small volumes of living cell suspensions in set high-resolution patterns.[127]The researchers after an investigation reported that "generated tissue constructs might be used for *in-vivo* testing by implanting them into animal models". Presently, only human skin tissue have been synthesized, though researchers project that "by integrating further cell types (e.g.

melanocytes, Schwann cells, hair follicle cells) into the printed cell construct, the behavior of these cells in a 3D in vitro microenvironment similar to their natural one can be analyzed".

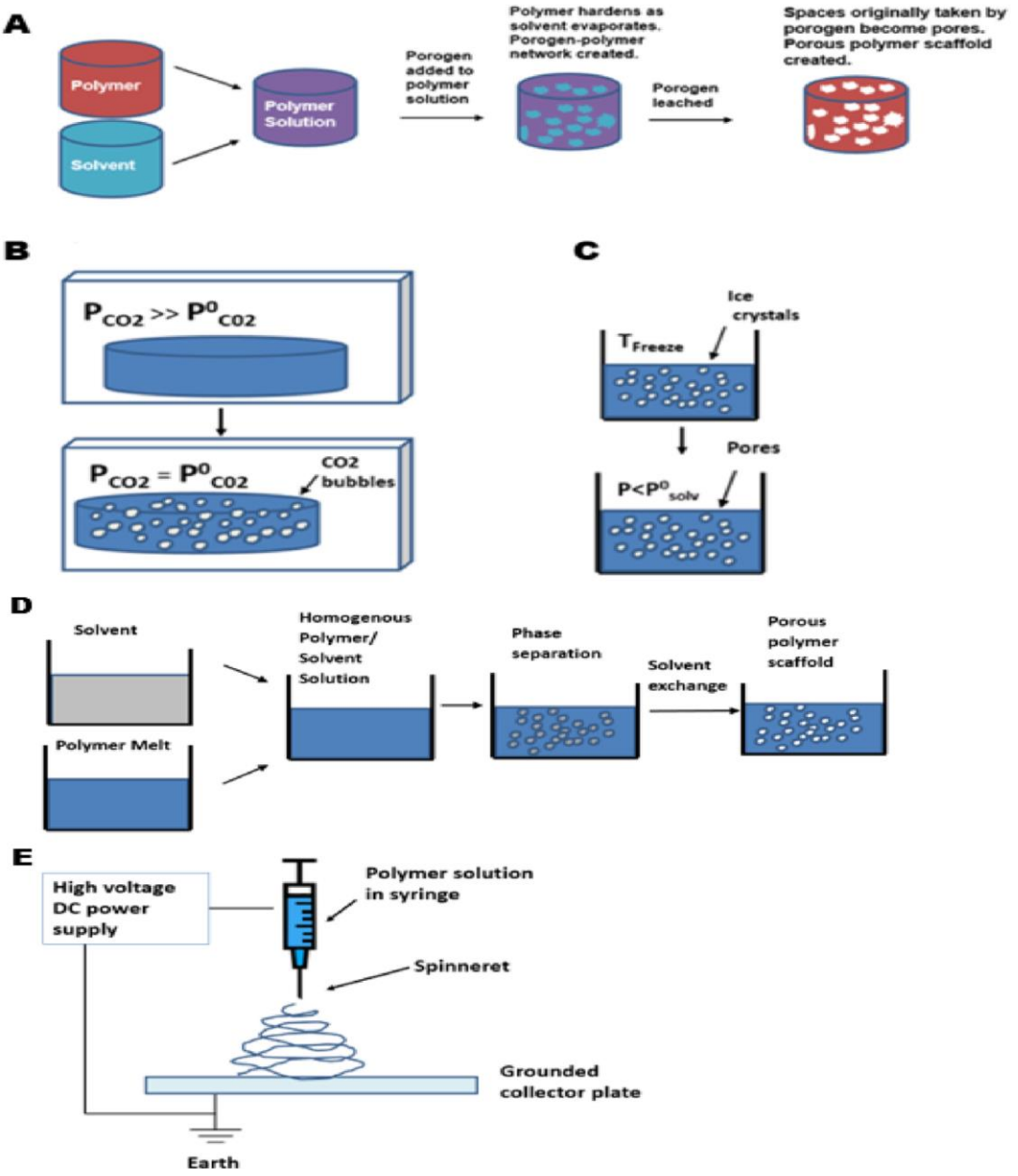

Figure No. 3: Common scaffold fabrication techniques.

(A) Solvent casting-particle leaching process (B) Gas foaming (C) Freeze-drying

(D) Phase separation (E) Electrospinning.

Adapted from Puppi et al. [127]

5. Physicochemical Characterization of scaffolds

Polymeric scaffolds not only serve merely as carriers of cells and inductive factors but also to actively instruct cells and provide step by step guidance of tissue formation. To accomplish this goal, a thorough understanding of the chemistry and physicochemical properties of the tissue to be engineered and the materials used in this process are required. The most important challenge in the field of tissue engineering is developing scaffolds that mimic the architecture of tissue at the nanoscale.

Polymeric scaffolds show excellent potential with mechanical properties and with a wide range of degradation, the qualities which are essential for a range of tissue engineering applications.[128]

Several characterizations are required for the fabrication of successful 3D scaffolds.

5.1 External Geometry

When scaffolds are applied for tissue reconstruction the important factors to consider are physical characteristics. Scaffold with proper physical characters is smart materials that can mimic natural ECM. ECM plays a key role in tissue architecture by providing structural support and tensile strength. Attachment sites for cell surface receptors are related to a wide range of processes relating to cell differentiation, tissue formation, homeostasis, and regeneration.[129,130]

Special emphasis is given in medical applications as it deals with the fabrication and designing of macro- to nanoscale structural architectures. Nano- to macroscale structure geometrically or topologically mimics the native state of ECM in living tissues. Three-dimensional scaffolds are capable of regenerating tissue and organs in their normal physiological shape. Mimicking the ECM using biomaterials would be a logical approach for the engineering scaffold for a variety of tissue types. Efficient control over processes of ECM reconstitution can be achieved by the interaction with polymeric materials as polymer materials permit a most versatile variety of surface characteristics. The importance of scaffold geometry in maintaining highly interconnected porous fabrics of high surface density provides an extremely high surface-to-volume ratio, favoring cell attachment and proliferation.

5.2 Surface Properties

Surface properties include both chemical and topographical characteristics, which can control and affect cellular adhesion and proliferation. The scaffold surface is the initial and primary site of interaction with surrounding cells and tissue. As most cells utilized in tissue engineering are anchorage-dependent, it has been vital that the scaffold should facilitate their attachment. Thus, scaffolds are favorable with a large and accessible surface area.

For example, a high internal surface-area-to-volume ratio is essential to accommodate the number of cells required to replace or restore tissue or organ functions. The surface properties can be modified to enhance the performance of the biomaterials. For instance, by altering the surface functionality using thin film deposition, the optimal surface, chemical, and physical properties can be attained.[131,132]Therefore, surface modification of biomaterials has become an increasingly popular method to improve device multi-functionality, tribological, and mechanical properties. Most of the surface modifications and immobilizations of biomolecules are performed to improve the biocompatibility of the polymeric scaffold; thereby, cells can specifically recognize the scaffold. These biomolecules include adhesive proteins like collagen, fibronectin, Arginylglycylaspartic acid (RGD) peptides, and growth factors like basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), insulin.etc.

The biomolecules can either be covalently attached, electrostatically adsorbed or self-assembled on the biomaterial surfaces to develop brand new materials.[133]

5.3 Porosity and Pore Size

Scaffolds must have an exceedingly porous structure with an open fully interconnected geometry for providing a huge surface area that will allow the cells to grow, uniform cell dissemination, and encourage the neovascularization of the construct. [134]

Following critical parameters are taken into consideration while developing scaffolds:-

average pore size, pore size distribution, pore-volume, pore interconnectivity, pore shape, pore throat size, and pore wall roughness.

It gives a porous biocompatible network into which the encompassing tissue is prompted and acts as a temporary template for the new tissue's development and reorganization. [135] Pore

estimate is additionally a vital issue in such a case that the size utilized are excessively little, pore impediment by the cells will occur, which will counteract cell entrance, extracellular network generation, and neovascularization of the internal territories of the platform. The impacts of pore estimate on tissue recovery has been stressed by examinations showing ideal pore size of 5 μm for neovascularization, [136] 5– 15 μm for fibroblast in development, [137] 20 μm for the in development of hepatocytes, [138] 200– 350 μm for osteoconduction, and 20–125 μm for recovery of grown-up mammalian skin. [139] Pore interconnectivity is likewise basic to guarantee that all cells are inside 200 μm from blood supply to accommodate a mass exchange of oxygen and supplements.[140,141]

5.4 Interface Adherence and Biocompatibility

Biocompatibility is characterized as, materials that cause negligible biological responses. Biocompatibility alludes to the capacity of a biomaterial perform its desired function concerning a medical therapy, without eliciting any undesirable local or systemic effects in the recipient or beneficiary of that therapy. It ought to create the most appropriate and beneficial cell or tissue response in that particular situation and improve the clinically relevant performance of that therapy. Biocompatibility of a framework or grid for a tissue-building item alludes to the capacity to execute as a substrate that will bolster the suitable cell movement, including the assistance of sub-atomic and mechanical flagging frameworks. [142]

Some critical variables that decide scaffold biocompatibility are their science, structure, and morphology, which thus are directly influenced by the polymer combination, scaffold processing, and sterilizing conditions.

Several biodegradable polymers such as PLA, PGA, PLGA, polydioxanone(PDO), poly trimethylene carbonate(PTMC), and so on are extensively used/tested on a wide range for medicinal applications as it has good biocompatibility.

Depending on the surface characteristics such as wettability, hydrophilicity/hydrophobicity ratio, bulk chemistry, surface charge and charge distribution, surface roughness, and rigidity polymeric materials are studied for its adsorption, desorption of adhesion, and proliferation on various mammalian cell.

Various surface medications are accessible to streamline the biocompatibility of surfaces in contact with living tissue, to seal in undesirable residues or added substances utilizing a

covering and to manage discharge and additionally assimilation utilizing a specifically porous surface.[143]Recently, oxidized polystyrene surface,[144] ammonia plasma-treated surface [145]and plasma-deposited acetone[146]have improvised the physical and chemical surface interaction thus affecting the cell adhesion and growth of polymeric biomaterials.

5.5 Degradation Rates

Scaffold degradation can occur through mechanisms of physical or chemical processes and/or biological processes that are mediated by biological agents, such as enzymes in tissue remodeling. The biodegradable scaffold gradually degrades in a predetermined period and is replaced by newly grown tissue from the adhered cells. Degradation results in scaffold dismantling and material dissolution/resorption body fluids.[147]

Polymeric scaffolds that undergo bulk degradation tend to break down the internal structure of the scaffold thus reducing the molecular mass.[148]A polymeric scaffold can be described similarly to the dissolution of soap that primarily undergoes surface degradation. The rate at which the surface degrades is usually constant. Therefore, even though the size of the scaffold becomes smaller, the bulk structure is maintained. These types of degrading scaffolds provide longer mechanical stability for the tissue to regenerate.

Biodegradation of polymeric biomaterials consists of cleavage of hydrolytic or enzymatical sensitive bonds in the polymer leading to polymer erosion.[149]

Factors affecting the biodegradation rates of a polymer are:- intrinsic properties of the polymer, including the chemical structure, the presence of hydrolytically unstable bonds, the level of hydrophilicity/hydrophobicity, crystalline/amorphous morphology, glass transition temperatures (Tg), the copolymer ratio, and the molecular weight.

Controllable degradation and restoration rates shall match the rate of tissue growth in vitro and in vivo for biodegradable or restorable materials.

The non-biodegradable polymeric scaffolds are biologically stable, should provide permanent support over time and should ideally function during the lifetime of the patient.

For example, PMMA is mainly used as bone cement in hip and knee replacements, and high-density PE forms the articulating surfaces of hip and knee joints.

5.6 Mechanical competence

The biostability of many scaffolds relies upon various factors, such as quality, flexibility, and assimilation at the material interface and its chemical degradation.

The scaffold ought to have proper mechanical properties and degradation rates with the bioactive surface to encourage the rapid regeneration of tissue. Mechanical strength of the scaffolds structure must be retained after implantation for the reconstruction of hard, load-bearing tissues such as bone and cartilages. Biomaterial framework must withstand heaps and stresses that a new tissue would bear for better tissue building. Hence following rheological parameters must be evaluated:-

(i) Elastic modulus—measured strain in response to a given tensile or compressive stress along with the force;

(ii) Flexural modulus—measured the relationship between bending stress and the resulting strain in response to a given tensile or compressive stress perpendicular under load;

(iii) Tensile strength—maximum stress that the material can withstand before it breaks;

(iv) Maximum strain—ductility of material or total strain exhibited before fracture.

Low strength and rigidity of the polysaccharides restrict use for soft tissue applications.

Fibrous proteins, whose normal function is to provide mechanical integrity and stability to biological structures are is also utilized for tissue engineering. Fibrous proteins are responsible for the transduction of external mechanical forces to associate cells in a manner that improvises tissue growth.[150] The mechanical properties of bulk biomaterials are changed by their processing into scaffolds of various pore sizes and pore orientations and further that these properties will rapidly diminish as a function of implantation time.[189]

The mechanical rigidity of the surrounding matrix, as well as material roughness and physical confinement, determined by three-dimensional microstructure on a subcellular and supercellular scale, respectively, may significantly modulate the outcome of the balance between cell-matrix forces, leading to the remodeling of cytoarchitecture, cell polarization, alteration of downstream intracellular signaling events as well as modification of the balance of cell-cell forces.[151-153]

The major factor affecting the mechanical properties and structural integrity of any scaffolds, is their porosity, for example, pore-volume, size, shape, orientation, and connectivity.

6. Future Scope:

Medical research continues to explore new scientific frontiers for diagnosing, treating, curing, and preventing diseases at the molecular/genetic level. Important advances have been made in the clinical use of medical implants and other devices. Presently, the emphasis is placed on the design of polymeric scaffold, that is, materials that obtain specific, desired, and timely responses from surrounding cells and tissues. The need for alternative solutions to meet the demand for replacement organs and tissue parts will continue to drive advances in tissue engineering. Polymer scaffolds have all the prospective to provide a new means to control the physical and chemical environment of the biological system. Scaffold fabricated with metal/ceramics can overcome the limitation with the strength and toxicity but it creates complications during surgery. We believe that a solitary material will not satisfy the entire design parameters. We need a wide range of materials in various tissue engineering applications. The general difficulties in scaffold design and fabrication allow new exciting application-oriented research in scaffold design which includes polymer assembly, surface topography or chemical cues, nano-/macrostructure, biocompatibility, biodegradability, mechanical properties, directing cell function and induced formation of natural tissue.

7. CONCLUSION

Tissue engineering is a standout amongst the most exciting interdisciplinary and multidisciplinary research areas and is developing exponentially shortly. Scaffold materials and fabrication technologies play a vital job in tissue engineering. A wide scope of the polymeric scaffold was used to date in the tissue engineering area. Scaffolds must meet certain design parameters to be valuable regardless of whether they originate from natural resources or synthetically created. Each of these methods for framework creation is vital in different handling parameters. All these techniques for scaffold fabrication are sensitive to the various processing parameters. Several innovations in the material design and fabrication processes are resulting in the possibility of production of implants with good performance. The scaffold should be surface compatible as well as architecturally suitable with the host environment. To develop a new design for implants and also to understand the behavior of the scaffold in the principles and theories of the biomedical application of the fabrication

process with the polymers would be useful. Nanotechnology may provide strategies that can help to create features on a scaffold in a dimensional range that may be adequate for cells and biomolecules. It is being observed that as the goals of biomedical engineering increase in complexity, there is a need to develop novel scaffold structures.

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