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Classification and methods of making biological scaffolds

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Abstract

The most important approach to producing bioscaffolds is to regenerate body tissues. Scaffolds must be able to increase the strength of cells and cause biological changes in them. Bioscaffolds allow cells to settle on their fibers and tissues, and they cause the transfer of biochemical factors and the release of nutrients. This study attempts to examine and classify scaffolds by mentioning their characteristics and manufacturing methods. The results of the research show that common methods for producing scaffolds include electrospinning, freeze-drying, 3D printing, phase separation, gas foaming, and particle washing. Bioscaffolds are also classified into four categories: hydrogels, 3D bioprinting, nanofibers, and microscaffolds.

Keywords: Bioscaffold, tissue, classification, effective factor

Introduction

Scaffolds play an important role in TE applications, serving as a temporary platform or template for providing guidance and structural support to develop new tissues. Scaffolds refer to a three-dimensional (3D) porous biomaterial that provides a favorable environment for cells to repair and regenerate tissues and organs. It serves as a template for tissue defect reconstruction while promoting cell attachment, proliferation, extracellular matrix regeneration, and restoration of nerves, muscles, and bones. In addition, scaffolds can transport bioactive materials such as drugs, inhibitors, and cytokines as a mechanical barrier against the infiltrating native tissues, which may disturb tissue restoration and regeneration [24].

Cells in different tissues of the body secrete specific types of macromolecules such as proteins, depending on the type of tissue. These substances form a complex and porous network around the cell, which is called the extracellular matrix, and the cells grow and multiply on it. The cells and the extracellular matrix together are called tissue. All body cells, except those of the circulatory system and some specific embryonic tissues, grow on an extracellular matrix**.** The extracellular matrix that is artificially produced is called a scaffold. Scaffolds are temporary structures for cell support, attachment, proliferation, and differentiation of cells into the desired tissues and organs **.**Eventually, this structure degrades over time at different and adjustable rates, and new tissue grows and replaces it**.** Today, these scaffolds are used in various fields, including drug delivery, gene therapy, and cell therapy. Their main application is in tissue engineering and regenerative medicine [25].

Materials and methods

1. Familiarity with tissue scaffolding and its features

Tissue engineering is a promising field that aims to create functional human tissues and organs using a combination of cells, biomaterials, and biochemical factors. Bioscaffolds play a vital role in tissue engineering by providing a structural framework for cells to grow, differentiate, and organize into functional tissues. Research in the area of bioscaffolds for tissue engineering focuses on developing materials that possess specific properties such as biocompatibility, biodegradability, mechanical strength, and the ability to support cell adhesion, migration, and tissue formation [28].

2. Scaffold considerations in tissue engineering

Scaffolds are one of the three crucial factors used in bone tissue engineering. The word of scaffold is various synthetic and natural material that supplies the necessary foundation for conducting the inserted cells, stimulating cell attachment and cell reproduction in tissue and organ regeneration. Scaffolds also have a function as a transporter to growth factors and stimuli. Perfect scaffolds, should not collapse before new tissue is formed, except to provid an appropriate environment for cell proliferation. Additionally, they shouldn't have immunogenic and toxic restrictions. The main goal in scaffold designing is to create scaffolds that supply cell signaling and mimic the natural environment of tissues. Here we highlight the key features expected of bone scaffold engineering [27].

3. Raw materials in the construction of scaffolding

The following sections will highlight four main categories of raw materials that are commonly used in recent tissue engineering scaffold strategies: collagen, GAGs, bioceramics, and ECM-based materials. Within each section, the most frequently used materials for tissue repair and regeneration purposes will be highlighted. For organizational purposes, although raw materials have been grouped by material type in the following sections, due to the overlap of multiple raw materials in several approaches, tables are arranged by the target tissue application [23].

Biopolymers used in scaffold construction can be natural or synthetic, biodegradable or non-biodegradable. These materials can be divided into three categories: natural, synthetic, and ceramics [25].

a. **Natural polymers:** Natural polymers are obtained from materials found in nature, such as plants, insects or animals [25].

b. **Synthetic polymers:** These polymers are made under controlled conditions. Therefore, their degradation and mechanical properties can be controlled [25].

c. **Bioceramics:** Bioceramics are a group of ceramic materials that are used to repair and strengthen parts of damaged or dysfunctional tissues and organs of the human body [25].

4. Effective factors in the construction of scaffolding

Today, studies in the field of tissue engineering are expanding widely in Iran, so that tissue engineering has shown its potential for building organs and solving the problem of organ shortage for transplantation. One of the main principles of tissue engineering is the cultivation of cells in a three-dimensional environment similar to the three-dimensional conditions of the body. For this purpose, cells are cultivated on scaffolds. Scaffolds have three-dimensional structures that are made of various materials such as natural and synthetic polymers. Initially, polymers were only used for orthopedic implants, drug delivery, and the manufacture of medical equipment, but subsequent research showed that these materials also have the ability to create a body-like substrate for cells. Tissue engineering scaffolds must have special properties so that they can meet the goals of the researcher in accordance with the body tissue. Having appropriate mechanical, biological, mechanical, and chemical properties are four important factors in the construction and study of scaffolds. Creating these four factors simultaneously is a complex task and requires experience and numerous experiments. Improving any of these properties can negatively affect the other properties. Therefore, optimization and achieving optimal formulation is an important part of the scaffold manufacturing process [26].

5. Characterization of scaffolds in tissue engineering

The selection of tissue engineering scaffold material is directly associated with the effect of the material implanted in the animal or human body. Most importantly, the scaffold materials should have good biocompatibility. Scaffold materials can provide a good adsorption interface for the adhesion of seed cells, in order to facilitate cell proliferation. In addition, the scaffold material must have a certain level of biomechanical strength and maintain a controlled degradation rate once implanted into the animal or human body [31].

6. Types of scaffolds in tissue engineering

Classes of scaffold materials include natural biological scaffold materials, synthetic biodegradable polymer scaffolds materials, composite scaffold materials and nano-scaffold materials. Natural biological scaffolds have good biocompatibility but poor mechanical properties. Synthetic biodegradable polymer scaffolds tend to have strong mechanical properties, but lack good cellular compatibility. Nano-scaffold materials have been widely studied in recent years, but are expensive to produce. Currently, the most widely used scaffold materials are composite scaffold materials. These benefit from the good cellular compatibility of natural biological scaffolds and the strong mechanical properties of synthetic biodegradable polymer scaffolds [31].

7. Preparation of scaffolding from plant tissue

The decellularization of plant-based biomaterials to generate tissue-engineered substitutes or *in vitro* cellular models has significantly increased in recent years. These vegetal tissues can be sourced from plant leaves and stems or fruits and vegetables, making them a low-cost, accessible, and sustainable resource from which to generate three-dimensional scaffolds. Each construct is distinct, representing a wide range of architectural and mechanical properties as well as innate vasculature [32].

Results

Most tissue engineering techniques utilize a three-dimensional porous scaffold seeded with cells to provide a structural support, as shown in Figure 10.2. The success of a scaf-fold typically depends on the characteristics of the starting material and the fabrication methodology. Scaffolds serve as space- holders to prevent encroachment of tissues from the immediate vicinity into the affected site, provide a tem-porary support structure for the tissue that they are intended to replace, create a substrate for cells to attach, grow, pro-liferate, migrate, and differentiate on, serve as a delivery vehicle for cells, and facilitate their retention and distribu-tion in the region where new tissue growth is desired. They also provide space for vascularization, neotissue formation and remodeling to occur simultaneously, along with effi-cient transport of nutrients, growth factors, blood vessels, and removal of waste material [4].To perform all these stated functions, they need basic requirement of being biocompatible (not to produce an un-favorable physiological response), biodegradable (to get broken down eventually and eliminated from the body via natural occurring processes), and their degradation rates should match the healing rate of new tissues. Additionally, they should have mechanical properties consistent with the replaced tissue, optimum architectural properties (pore size, porosity, pore interconnectivity, and permeability) and ease of processing into three-dimensional complex shapes [29].

Properly designed scaffolds are substitute materials mimicking the physical and biochemical properties of the healthy tissue, and providing a 3D environment to promote cell adhesion, proliferation and differentiation to restabilize the physiological function of a tissue. Scaffolds, cells, and growth factors are known as “the tissue engineering triad”. An ideal scaffold for bone tissue engineering should be biodegradable, biocompatible, bioactive, osteoconductive, and osteoinductive [30].

Discussion

Some key areas of research in bioscaffold development for tissue engineering are as follows:

Material selection: To make bioscaffolds, researchers investigate a range of natural, synthetic, or hybrid biomaterials. Natural biomaterials including collagen, fibrin, chitosan, and alginate offer biocompatibility and bioactive characteristics. Poly (lactic acid) (PLA), poly (glycolic acid) (PGA), and their copolymers are examples of synthetic materials that offer customizable surface characteristics, predictable degradation rates, and mechanical stability. The benefits of both natural and synthetic materials are combined in hybrid materials.

Scaffold construction techniques: Different fabrication processes, including electrospinning, 3D printing, electrohydrodynamic jetting, and solvent casting, are employed to create bioscaffolds with regulated topologies and pore configurations. These methods allow complicated tissue geometries to be created and original tissue microenvironments to be mimicked.

Surface modification: To strengthen cell adhesion, facilitate tissue creation, and improve cell-material interactions, researchers investigate surface modification approaches. Chemical functionalization, the immobilization of bioactive molecules, and the addition of particular physical cues such as topography, stiffness, and electrical conductivity are examples of surface changes.

Biomimetic approaches: By adding particular ECM constituents, such as fibronectin, laminin, and growth factors, biomimetic bioscaffolds replicate the natural extracellular matrix (ECM) of tissues. Replicating the niche milieu necessary for cell proliferation, differentiation, and the formation of functional tissues is the aim.

Composite and hierarchical scaffolds: Researchers explore the development of composite and hierarchical scaffolds that combine multiple materials, such as polymers, ceramics, and metals, to achieve a combination of mechanical strength, bioactivity, and controlled degradation rates.

Decellularized scaffolds: Decellularization techniques involve removing cellular components from tissues while preserving the ECM. Decellularized bioscaffolds can serve as a scaffold template to support the seeding and differentiation of cells, leveraging the natural ECM structure and biochemical cues.

***In vitro* and *in vivo* evaluation:** Researchers evaluate the functionality and biocompatibility of bioscaffolds *through in vitro* cell culture experiments and *in vivo* animal studies. These assessments assess cell adhesion, proliferation, differentiation, tissue integration, and immunological responses [28].

Conclusion

The concept of a ‘scaffold’ has been used in tissue engineering for more than twenty years, being an integral concept of the latter field. In fact, other than cells and signaling molecules, the scaffold is considered as one of the three important constituents that enhance cell growth in tissue engineering (Lanza *et al*., 2020). Scaffolds provide the framework and initial support for cells to adhere and tissue to grow, eventually defining the size and shape of the tissue in question [1].

Collagen scaffolds are being replaced by ultra-vaporous scaffolds obtained from biodegradable polymers. Biodegradable polymers degrade as new tissue is formed and residues remain in the body. The main challenge with using such polymers is creating biodegradable structures with the desired adhesive properties, proper porosity, and mechanical properties. These scaffolds should undergo degradation according to the rate of tissue formation and be cytocompatible with the host tissues. Scaffolds can be presented as implants or injections to deliver cells, drugs, and genes into the body. The scaffold provides a substrate for cell attachment, proliferation, differential function, and migration. Polymer scaffolds can take the form of a three-dimensional (3D) porous matrix, a nanofiber matrix, a thermosensitive sol-gel transition hydrogel, or a porous microsphere. Multifunctional scaffolds are prepared based on organic, inorganic, and mixed materials (organic-inorganic). The scaffolding materials must be biocompatible, tissue compatible, biodegradable, and capable of designing an appropriate scaffold with the required functionalities [2].

For this aim, the present review focuses on the role of the scaffold in tissue engineering to be considered not just as a passive support for cell seeding but as an active platform that can effectively contribute to tissue regeneration and host integration [3].

and one of its key points specifically focuses on the definition of a viable and instructive scaffold for cell seeding, proliferation, migration, and differentiation in the case of stem cells. This statement implies a careful selection of: (i) the material(s) for scaffold fabrication, either synthetic or naturally derived, (ii) the most suitable technique that allows to deal with a substrate morphologically and mechanically similar to the ECM to be replaced, thus avoiding any mismatch with the surrounding tissue, (iii) possible surface treatments that can confer a positive biochemical profile to elicit a significant biological response, and (iv) drugs and/or growth factors to be loaded into the scaffold and subsequently released to enhance the final performance, avoiding, for instance, any side effect that can limit the therapeutic efficacy [3].

The scaffold acts as a template in which cells and growth factors are implanted to imitate the extracellular matrix to maintain and restore tissue function. High porosity, pore interconnectivity, biocompatibility, biodegradability, and mechanical properties are indispensable properties that must be considered when designing the scaffold [4]. Hence, the scaffold selected for engineered tissue should mimic the ECM of that specific tissue. Selecting appropriate cells, isolating and expanding targeted cells, and selecting suitable biomaterial for scaffold designing are factors that thrive in tissue engineering. However, a solitary polymer cannot achieve every single property of a scaffold, so the desired property can be attained by mixing it with a variety of polymers. Along with the selection of material, process technique or fabrication method also provide a more significant impact on the features of the resultant scaffold [4].

Scaffolds play an important role in TE applications, serving as a temporary platform or template for providing guidance and structural support to develop new tissues. Scaffolds refer to a three-dimensional (3D) porous biomaterial that provides a favorable environment for cells to repair and regenerate tissues and organs. It serves as a template for tissue defect reconstruction while promoting cell attachment, proliferation, extracellular matrix regeneration, and restoration of nerves, muscles, and bones. In addition, scaffolds can transport bioactive materials such as drugs, inhibitors, and cytokines as a mechanical barrier against the infiltrating native tissues, which may disturb tissue restoration and regeneration [4].

Requirements of Scaffold [9]

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. Therefore, to fulfil the function, scaffolds should meet some specific requirements. At first, scaffolds must provide appropriate conditions to promote cell viability, proliferation and differentiation. A sufficiently high porous architecture of scaffold and an adequate pore size are necessary to facilitate cells seeding and diffusion throughout the

whole structure of both dividing cells and nutrients. A large surface area is desirable for cell adhesion, therefore, the volume of the pores should be relatively high (50–90%) Scaffold should be characterized by a high open porosity in which the pores are connected with each other and with the surface of the material. Interconnected porous structure of scaffold is required for cell penetration, tissue ingrowth, nutrient and waste transport. The presence of isolated pores prevents the diffusion of gases and fluids between cells. Most research shows that the best bone tissue ingrowth occurs in materials with a pore size of 100−500 µm,

however, the high porosity and pore size affects the strength scaffold parameters. Therefore, it is necessary to evaluate the optimal value of the mentioned properties Biodegradability and biocompatibility are important features for tissue engineering. The ideal scaffold made from a suitable biomaterial should be absorbed by the surrounding tissues without the necessity of a surgical removal and minimal degree of immune and inflammatory. It is important that the rate of degradation must be coincided as much as possible with the rate of tissue formation. That means that while cells fabricates their own natural matrix structure

around themselves, the scaffold is able to provide structural integrity within the body and eventually break down leaving the neotissue, newly formed tissue which will take over the mechanical load To provide tight integration with surrounding tissue, scaffold should be characterized by high bioactivity which is responsible for chemical bond formation. Osteoconductivity is another desirable scaffold property which support the regeneration process of damaged bones by assisting cells to adhere to the graft surface and to proliferate Scaffold should have mechanical properties compatible with the anatomical location and it must be strong enough to allow surgical handling during implantation. Production of scaffolds with appropriate mechanical and physical properties is one of the biggest challenges in tissue engineering.

Scaffolding Fabrication Methods

An array of processing techniques has been developed for tissue engineering and consequently, has been applied to BTE (Table 2). Ideally, the optimal fabrication technique can produce repeatable scaffolds with a controlled hierarchal porous structure, as the geometry of the pores structure has profound effects on both mechanical and biological response of bone tissue. [62] Nowadays, the fabrication technologies that can facilitate inclusion of cells and growth factors are highly fashionable for optimal scaffold creation. [63] It is certain that advanced processing techniques which facilitate the production of highly customizable scaffold geometries for patient specific implants, are required for specialized clinical needs. Current methods for producing bone tissue scaffolds can include: i) electrospinning, ii) freeze drying, iii) 3D printing or additive manufacturing (AM), iv) phase separation, v) gas foaming and vi) particulate leaching (Table 2). Some of these techniques can be broken down into further subcategories, for example AM can be subdivided into fused deposition modelling (FDM), direct ink writing (DIW), stereolithography (SLA), digital light processing (DLP), and selective laser sintering (SLS) [5].

The Structure of polymeric scaffolds are endowed with a complex internal architecture and porosity that provide sites for cell attachment and maintenance of differentiation function without hindering proliferation.27 Ideally, a polymeric scaffold in tissue engineering should have the following characteristics: i) Holding appropriate surface properties promoting cell adhesion, proliferation and differentiation; ii) Biocompatiblity; iii) High porosity, and a high surface-area to volume ratio, with an interconnected pore network for cell growth and flow transport of nutrients and metabolic waste, iv) Sufficient mechanical properties and any *in vivo* stresses [6].

Scaffolds serve as temporary or permanent artificial extracellular matrices (ECM) to accommodatecells and support 3D tissue regenerations. They can serve as cellular systems or as delivery vehicles forcells and drug in cell and tissue regeneration; thus, the cellular material must be capable of adequatelycolonizing the host cell to meet the needs of regeneration and repair. Scaffold serves as a key componentin the three- dimensional (3-D) structure for cell interactions. From the 20 centuries, biomaterials havebeen used in the fabrication of medical implants. A scaffold can serve as a carrier for delivering propercytokines and growth factors to the site of repair [1–4]. A scaffold can serve as a carrier for deliveringproper cytokines and growth factors to the site of repair [2, 3]. The fundamental concept underlying tissue engineering is to combine a scaffold with living cells orbiologically active molecules to form a “tissue-engineering construct” that, in the presence of adequateblood supply, promotes the repair or regeneration of tissues [5, 6]. The properties of scaffolds mainly depend on their composition. A pertinent selection of the bioma-terial component of the scaffold is a censorious step in making a successful engineered graft. Scaffoldshould be such that it aids in the growth, migration and organization of the cells, while the tissue isbeing formed. Depending on which tissue is to be regenerated, the required scaffold’s material andits properties will be customised. Biodegradability is often an essential factor since scaffolds shouldpreferably be absorbed by the surrounding tissues without the necessity of a surgical removal [7].

An appropriate scaffold must be capable to repair body tissues with minimum requirements, for cell growth, vascularization, proliferation, and host integration, and finally, materials should be degraded naturally during or after the healing process. However, a scaffold has specific characteristics related to the biological aspect, structure, and chemical composition [8].

Thus, 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 [8].

In practice, the techniques of the fabrication of 3D scaffolds are subdivided into a conventional or rapid prototyping (RP) method (Table 1), each producing different scaffolds with different characteristics. 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. 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 [8].

Introduction

The survival of living organisms depends mainly on their self-healing capabilities in response to tissue damage. Tissue damage refers to any alteration in the structure of a tissue, being a hard or soft one. Hard tissues include bones and teeth, while soft tissues refer to any tissues connecting and supporting different body structures and organs, such as ligaments, muscles, and tendons. Tissue damage could be brought by chemical, mechanical, or even pathological causes. To reverse tissue damage, our bodies are programmed to initiate a self-healing mechanism known as 'tissue regeneration' [10].

when the damage is so severe that the body's own self-healing mechanism cannot cope with the rate of cellular demise, or when the tissue is of the non-replicating type, tissue/organ transplantation is the sole solution. However, several limitations arise with transplants, including the limited numbers of donors and possible transplant rejection [6]. Such limitations had paved the way to look for a more feasible approach serving as a complementary system to conventional therapy. One proposal involved the regeneration of new tissues in place of the defective ones to restore normal tissue/organ function. This marked the introduction of the concept of 'tissue engineering (TE)' [10].

Initially, scaffolds were solely used as supporting matrices. However, as the field of TE advanced over time, other functions had emerged. For instance, by carrying appropriate growth factors and signaling cues, they could signal cell differentiation and facilitate tissue regeneration. Incorporating drug molecules within the scaffold could be one way to directly deliver the drug to the targeted injury site in suitable amounts. Scaffolds possess various biological, structural, and chemical properties that need to be carefully tuned according to the properties of the affected tissue. This is easily achievable via selecting the appropriate fabrication technique [10].

Scaffolds, typically made of polymeric biomaterials, provide the structural support for cell attachment and subsequent tissue development [11].

For the scaffold design, some requirements must be fulfilled such as water permeability, porosity, protein affinity, biocompatibility, biodegradability, availability of reactive functional groups for direct reactions with living tissue and for chemical modifications and ease of preparation [12].

To favor cell attachment, scaffolds must have a large, accessible surface area and high internal surface area to volume ratios, capable of allowing sufficient cell growth to replace the damaged tissue or organ [12].

In addition to these features, a key feature for the use a biomaterial in scaffold production is the availability of chemically and biologically active molecular groups and suitable linkers which may be recognized by the cells, allowing the cell-scaffolds interaction to occur. Such characteristics can be achieved by appropriated selected synthetic and natural polymers [12].

Synthesis and manufacturing process of scaffold

Hydrogels

Hydrogels are a special class of polymer matrix, that can be defined as a crosslinked polymer network capable of adsorbing and retaining a large amount of water or fluid within its 3D structure. Hydrogels can be manufactured by physical and chemical processes, for their use in tissue engineering. Hydrogels produced by chemical methods have greater durability compared to those produced by physical methods. For the production of hydrogel scaffolds, techniques such as leaching of particles, solvent casting, lyophilization, phase separation and gas foaming are used to provide scaffold architecture to the hydrogel through the formation of pores.138,139 The agent that promotes pore formation is inserted before the crosslinking process and removed after this step. Hydrogels are also widely used for the construction of scaffolds using 3D printing [12].

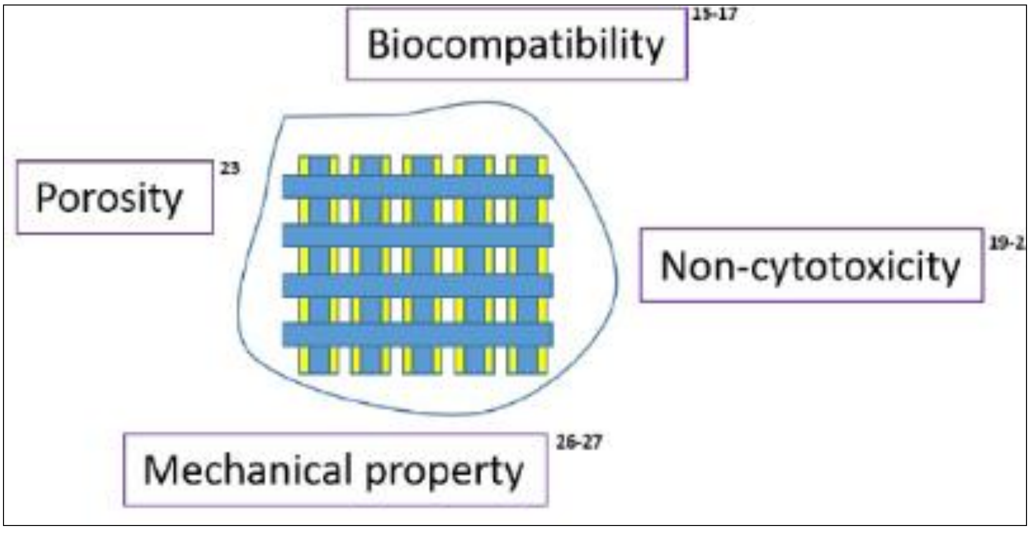

Fig 1: Main features of scaffolds [13]

The main aim of tissue engineering is the fabrication of functional replacements for damaged tissues or organs. Scaffolds play a crucial role intissue engineering, because they represent an alternative to the conventional implantation of organs and tissues. The main goal of scaffolds is to provide appropriate base for tissue growth and cell proliferation. Biomaterials play a critical role in tissue engineering. For the preparation of scaffolds a great number of different natural or synthetic materials have been studied and proposed. The most frequentlyemployed polymers of natural origin in biomedical applications are polysaccharides (alginate, chitosan, starch, cellulose) and proteins (collagen, silk fibroin), due to their bioresorbability, low toxicity, and low manufacture and disposal costs. Moreover, they offer a wide range of advantages for tissue engineering applications such as biological signalling, cell adhesion, cell responsive degradation and re-modelling [15].

Porogen leaching process
Porogen leaching is a popular method for preparing porous scaffolds. This method can also be used for improving the structure of the electrospun scaffold. The porogen and the electrospun fibers can be simultaneously deposited on the collector, and then the porogen is removed, which can build macropores inside the electrospun scaffold. These large pores can allow for cell infiltration. This concept has been demonstrated by simultaneously depositing polycaprolactone polymer fiber and sodium chloride particles through a specifically designed coaxial needle [24]. The inner tube held the polymer solution, while the outer annular region held the salt particles. After the salt particles were removed by water immersion, the electrospun scaffold showed a delaminated layer structure, which allowed for cell infiltration into the scaffold. Salt particles can also be codeposited with electrospun fibers using a sieve along with a vibrating orbital shaker [25]. The particles were uniformly dispersed in a hyaluronic acid/collagen fibrous scaffold. After salt leaching, the scaffold contained large pores with cubic shapes, which allowed chondrocyte growth and proliferation inside the scaffold [16].

3D printing
3D printing is the process of making a hardware product from a software file. The production of this product is done by layering raw materials on top of each other until the final object is created, which is called volumetric 3D printing. At present, researches about 3D printing technology are not finished [17]. The techniques currently being used to achieve 3D printing of scaffolds, which involve a layer-by-layer process, include, but are not limited to, direct 3D printing, fused deposition modeling, stereolithography, and selective laser sintering. These techniques have been used to produce scaffolds ranging from millimeter to nanometer sized scaffolds [18]. Advantages of using 3D printing include the ability to fabricate versatile scaffolds with complex shapes capable of homogenous cell distribution, and the ability to imitate the extracellular matrix (ECM). However, the availability of biomaterials with the stability and desired properties for 3D printing of scaffolds is restricted depending on the printing technology used [18].

Electrospinning
Electrospinning is a simple, rapid, and flexible technique capable of fabricating nanofibrous scaffolds. Ultrafine fibers are generated by applying a high-voltage electric field to polymer solution or melt coming out from the tip of a needle, and then deposited on a grounded collector. By adjusting parameters, including polymer concentration, solution viscosity and conductivity, applied voltage, the spinneret-to-collector distance, and humidity, fine fibers with different diameters and architectures can be produced. A variety of synthetic and natural biomaterials can be used to fabricate scaffolds by electrospinning, such as PLGA, PCL, PPC, PHB, collagen, chitosan and fibroin [19].

Solution Blow Spinning
SBS, first described in 2009 by Medeiros, can be considered a combination of ES and melt-blowing technology. The typical SBS setup involves a concentric nozzle with two channels (one for the polymer solution and another for the gas stream), a compressed gas source, and a collector. In the SBS process, a region of low pressure is created around the inner nozzle when the gas flows. According to Bernoulli's principle, the increased velocity of the air in the outer nozzle makes the pressure drop at the nozzle tip, shaping the solution into a structure similar to the Taylor cone that forms in the ES process. The pressurized gas stretches the drop formed at the tip of the nozzle, creating an ultrathin jet when the surface tension of the solution is overcome. As the jet travels, the solvent evaporates, and thin fibers are formed and deposited in a collector [20].

Centrifugal spinning
his is an innovative technique that uses forces associated with centrifugation to mass-produce nano or microfibers. Even though centrifugal force spinning is based on the same mechanism as a cotton candy maker, there have been very few publications proving its efficacy as a nanofiber production method. Polymer solution jets can be drawn from the spinneret without an electric potential difference between the nozzle and collectors; thus, no limitations are placed on using materials with low dielectric constants. By ejecting fibers radially outward onto a collector, this technique is proven experimentally to produce nanofibers with a diameter in the nanometer range. When producing nanofibers by centrifugal spinning, it is required that the polymer is in liquid form. This can be obtained by dissolving the polymer into various solvents or heating the polymer to a temperature where polymer chains start to flow. The polymer is loaded into a special container that rotates the fluid, which is then expelled as nanofibers onto a collector, maintaining a certain gap from the center. In contrast, one form of spinning technique is the centrifugal electrospinning system, which employs a stationary spinneret. The static spinneret is wired to a DC power source, and the polymer solution flow is driven by external pressure. In this configuration, the produced fibers are deposited on a rotating collector. In this approach, fiber alignment is dependent on the intensity of the electric field generated by the charge buildup on the deposited fiber [21].

Airbrushing
Airbrushing, a proposed alternative to traditional electrospinning, is a technique capable of synthesizing open structure nanofiber scaffolds at high rates. Cell penetration within the airbrushed scaffolds was found to be more than twice the cell penetration within conventional electrospun networks. The airbrushed polymer network supported cell growth and differentiation [22].

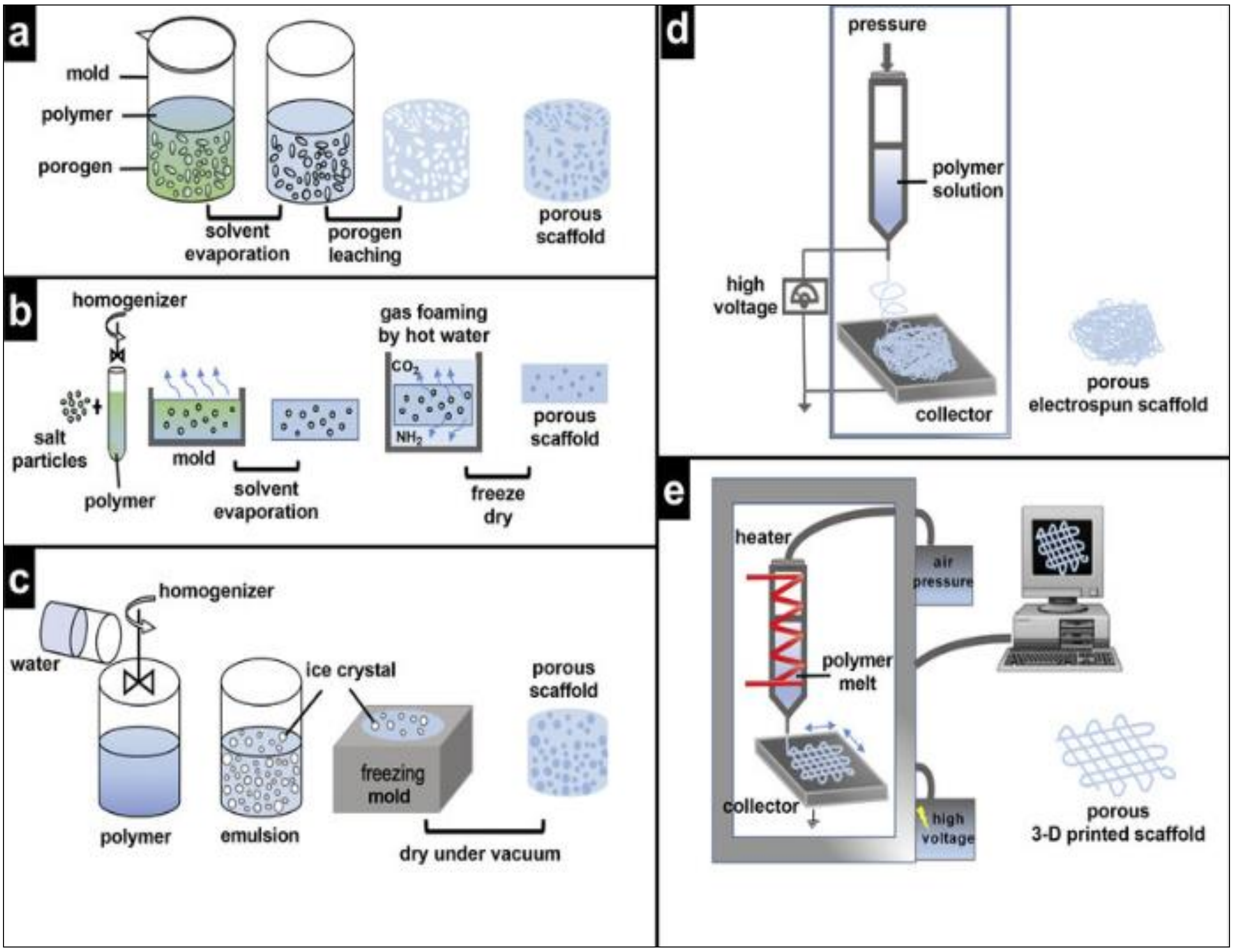

Fig 2: Various porous scaffold fabrication techniques. (a) Porogen leaching, (b) Gas foaming, (c) Freeze-drying, (d) Solution electrospinning, (e) Melt electrowriting and 3-D printing [14].

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