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Review Article

Scaffold: A Concise Review on Biomaterials Used, Fabrication Technologies and Potential Applications

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ABSTRACT

Due to the scarcity of tissue donors, scaffold construction and biomaterial selection are critical components of artificial tissue and bone tissue engineering. This study examines the characterisation, applications, manufacturing processes, biomaterials, and scaffold design issues. Scaffolds must include non-hazardous qualities including biocompatibility and biodegradability for human body as well as the mechanical capabilities needed to support body weight and, depending on the kind of tissue, carry out additional functions. To ensure cell viability and proliferation, scaffold structures like porosity, pore size, and pore shape should be tuned. Natural polymers, synthetic polymers, ceramics, and composites are the different categories of biomaterials. This article has covered various modern and traditional fabrication techniques. In order to match the qualities of the scaffold with the target tissue, the field of bone tissue engineering will need to strike a balance between the method of manufacturing and the choice of biomaterial.

INTRODUCTION

The survival of living organisms relies heavily on their natural ability to heal and repair tissue damage. This damage can occur in various types of tissues, such as hard tissues like bones and teeth, as well as soft tissues including ligaments, muscles, and tendons [1]. Tissue engineering, an exciting and multidisciplinary field, has emerged as a means of forming biological substitutes for storing, regenerating and replacing damage tissues [2]. Scaffolds play a vital role in tissue engineering by giving a supportive structure for cellular growth and regeneration [3].

The Emergence of Scaffold Based Tissue Engineering: In the mid-1980s, the concept of "scaffold-based tissue engineering" was given by Dr. Joseph Vacanti at Children's Hospital. Dr. Vacanti approached Dr. Robert Langer of MIT with an innovative idea to design scaffolds for delivering cells, rather than simply seeding or mixing cells into naturally occurring matrices with limited manipulative properties [3].Creating Highly Porous Scaffolds: Numerous techniques have been developed to fabricate highly porous scaffolds. These methods include fiber bonding, solvent casting/particulate leaching, gas foaming, and phase separation [4].

The main aim of tissue engineering is to create new, biologically functional tissues as well as to heal diseased and rejuvenate or damaged tissue. Globally, there were over 22.4 million orthopaedic surgical procedures conducted in 2017, and by 2022, that figure is expected to reach 28.3 million [5]. For minor injuries, bone tissue can heal on its own when physiological parameters are met. Natural healing, however, is unable to restore the bone's original form or functionality for larger expansions. Owing to these advantages, this treatment is subject to several restrictions, including donor availability, defects that arise during graft extraction, and the recovery duration for the two wounded sites following surgery [6].

The extracellular matrix (ECM)-based biological scaffold materials have demonstrated the ability to promote the constructive remodelling of many tissues in preclinical animal research as well as human therapeutic settings. The extracellular matrix (ECM) that these scaffold materials are made of comes from a range of tissues, including as the liver, small intestine submucosa (SIS),

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blood vessels, skin, nerves, skeletal muscle, and heart valves [7].

Apart from their traditional uses, new research has shown that scaffolds can also help with cell growth and differentiation by releasing different bioactive substances, like fibroblast growth factor (bFGF), transforming growth factor-β (TGF-β), and bone morphogenetic protein-2 (BMP-2), 2, 3. BMP-2 stimulates bone formation in orthopaedic applications. A good scaffold should be able to interact with the cellular component of bone without causing any toxic or immunological reactions. It should also have a suitable porosity that mimics the natural structure of bone tissue, allowing vascular in growth and cellular transportation. Finally, it should have adequate mechanical properties and be biodegradable—an ideal scaffold should break down over time *in vivo*, preferably at a predictable rate [8]. Additionally, the scaffold can be loaded with different medications, such vancomycin, to prevent adverse effects like contamination and inflammation that might manifest after the scaffold is implanted in the body [9].

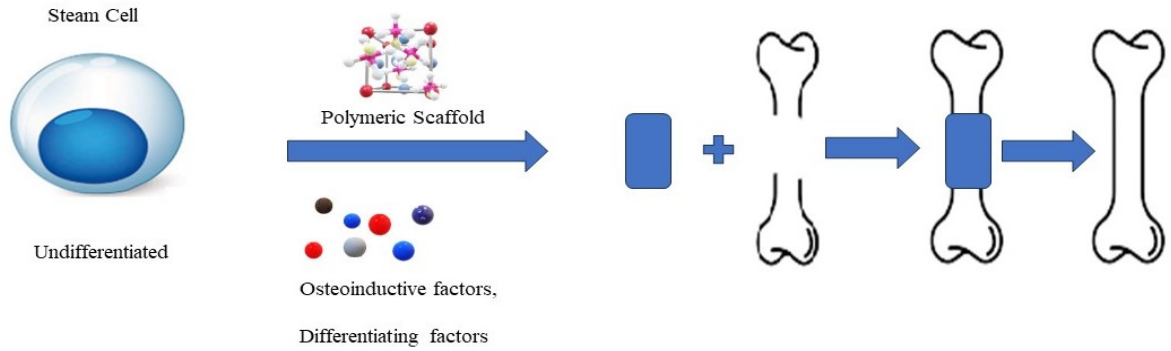

Figure 1: Tissue engineering method for bone repair: undifferentiated stem cells were implanted *in vivo* after being seeded into polymeric scaffolds together with osteoinductive agents and differentiation agents (like dexamethasone) [8].

MATERIALS AND METHODS

Materials

Natural Polymers

- Collagen
- Chitosan
- Alginate
- Hyaluronic Acid
- Gelatine

Synthetic Polymers

- Copolymer
- Polyesters

Ceramic Scaffolds

- Bio glass
- Calcium Phosphate
- Coral

Composites

- Polymer/Ceramics

Natural Polymers

Natural polymers are appealing because of their biocompatibility and biodegradability when it comes to building 3D scaffolds [10]. It's important to note that different functional groups, polymer concentrations, and polymerization settings may all be used to alter porosity, charge, and mechanical strength. The addition of chemicals, proteins, peptides, and cells can also regulate bioactivity [1].

An increasing number of research investigations have been able to show that the mechanical and biological characteristics of a scaffold substrate, such as its stiffness, biological motif, ligand density, and pace of remodelling, can affect the fate of the regenerated tissue in the long run as well as the behaviour of its cells. In order to create artificial bone, natural polymers such as collagen/gelatine, chitosan, silk, alginate, hyaluronic acid, and peptides are most often researched [11, 12]. Natural fibers and polymers provide benefits in terms of inherent biological patterns that promote tissue penetration and integration as well as cell signalling [13, 14].

Collagen

Collagen, the main building block of bone, is the perfect material to employ for creating 3D scaffolds. As extracellular matrix, collagen

promotes cell proliferation and differentiation and is both biocompatible and biodegradable. Nevertheless, its mechanical qualities are not good. The next investigations employed collagen as the foundation for three-dimensional scaffolds, modifying it by including polymers and other biomolecules to enhance osteoinductivity [11]. The most common protein in human extracellular matrix (ECM) is native collagen. Although collagen has strong biological qualities, it is electrically insulating and has poor mechanical strength [13].

Benefits

1. Biodegradable
2. Various forms of scaffolds.

Drawbacks

1. Handling and disinfection are comparatively challenging [10].

Chitosan

The most prevalent naturally occurring amino polysaccharide is chitin, which is primarily extracted from crustaceans (such as lobsters, crabs, and shrimp) [15]. Chitosan is a polysaccharide material that possesses anti-bacterial properties, biocompatibility, and a positive charge. Given that it resembles the structure of proteoglycans found in the extracellular matrix, this degradable material is appealing for use in tissue engineering applications [10]. However, its insoluble nature in neutral and alkaline solutions limits its tissue engineering applications and complicates its processing conditions. For use as a scaffold in bone, skin, cardiovascular, and corneal tissue engineering applications, carboxymethylated chitosan is widely utilized [13]. It is primarily made up of units, specifically (1-4) glycosidic bonds connecting d-glucosamine residues with a variable number of N-acetyl-d-glucosamine (NAG) groups dispersed at random. Chitosan bears structural similarities to glycosaminoglycans (GAG), an essential component of extracellular matrix (ECM) that interacts with collagen fibers and facilitates cell-cell adhesion [15].

Alginate

The primary source of alginate, a naturally occurring anionic polymer, is brown seaweed. It has characteristics like low toxicity, high resource abundance, low cost, gel-forming ability, biocompatibility, and the ability to imitate extracellular matrixes. It also contains guluronic and mannuronic acids [15]. It's a polysaccharide that can print and crosslink by injection and has a negative charge [10]. Another appealing polymer for 3D scaffolds used in tissue regeneration is alginate. It is soluble in water and, when combined with divalent cations, produces a gel at room temperature, which enables the creation of three-dimensional gels [11]. The addition of collagen and gelatine made up for the biological inertness of alginate, which inhibited cell attachment and migration. These findings demonstrated that the scaffolds' macroscopic channels were coupled. *In vivo* wound healing and re-epithelialization were expedited by sodium alginate, collagen, and glutamate scaffolds, which also decreased skin contraction [16].

Alginate's Characteristics

1. Biocompatible
2. The Inert Nature
3. Simplicity in gelation
4. Non-Toxic
5. Compostable
6. Sustainability
7. Reasonably priced and economical

Hyaluronic Acid (HA)

Extracellular matrix (ECM), that may be separated and employed to support cell growth and differentiation both *in vitro* and *in vivo* [17]. Another substance that has shown promise as a bone scaffold material is hyaluronic acid (HA). It is hydrophilic, nonimmunogenic, naturally occurring, and has also been discovered in the cytoplasm of osteoprogenitor cells [11]. Negatively charged glycosaminoglycan that is biocompatible and forms hydrogels when crosslinked In a different study, HA/Gel hydrogels were added to biphasic calcium phosphate (BCP) ceramic to create a distinctive micro- and microporous structure that served as a new bone substitute for BTE. Normally, HA is utilized as an aqueous binder [15].

Benefits

Degradability and ease of chemical functionalization [10].

Aspects

1. Extremely thick
2. Elastic
3. Compatible with biological systems
4. Not immunogenic
5. Compostable

Gelatine

Denaturalized collagen is known as gelatine. Blend forming by cross-linking [10]. Collagen's hydrolyzed by product is gelatine. Since its fundamental structure is comparable to that of collagen, it has the RGD sequence, which stimulates integrin signaling and promotes cell adhesion [13]. Knee osteochondral injuries are still difficult to treat and heal. Damage to the subchondral bone beneath the articular cartilage is also a part of osteochondral injuries. The inherent healing capacity of hyaline cartilage, which makes up articular cartilage, is restricted [19]. A scaffold offers a surface on which cells can adhere, proliferate, and differentiate. Scaffolds can also be utilized to target particular areas for high-loading efficiency medication delivery. As natural polymers, biologic scaffolds that have been used thus far include gelatine, alginate, collagen, fibrin, albumin, hyaluronan, and platelet rich plasma [20].

Synthetic Polymers

A wide range of synthetic polymers, including as polystyrene (PS), poly-l-lactic acid (PLLA), polyglycolic acid (PGA), polycaprolactone (PCL), polyvinylpyrrolidone (PVP), and poly-dl-lactic-co-glycolic acid (PLGA), have utilized in attempts to form scaffolds. These polymers are popular because they can be manufacture in large quantity and which having a long shelf life, and can be constructed with a customized architecture at a lower cost than natural polymers [15].

Benefits

Variable physical and mechanical characteristics

Drawbacks

- Potentially harmful tissue responses brought on by acidic breakdown [10].

Popular Synthetic Polymers

- Polylactic acid (PLA),
- Polyglycolic acid (PGA)
- Polylactic-co-glycolic acid (PLGA)
- Polyurethane (PU)
- Polyvinyl alcohol (PVA)

Ceramics

It has been demonstrated that some ceramics, including hydroxyapatite (HA), tricalcium phosphate (TCP), bioactive glasses, and glass ceramics, have high compressive strengths, excellent bone integration, and osteoconductivity. Unfortunately, owing of their brittleness, difficulties in shape for implantation, and biodegradability, their uses in tissue engineering have been restricted. In order to address these problems, we can combine natural biomolecules with ceramics, synthetic polymers, or biocomposite materials that enhance scaffold characteristics, enhance tissue contact, permit controlled degradation, and enhance application of biocompatible tissue engineering [10].

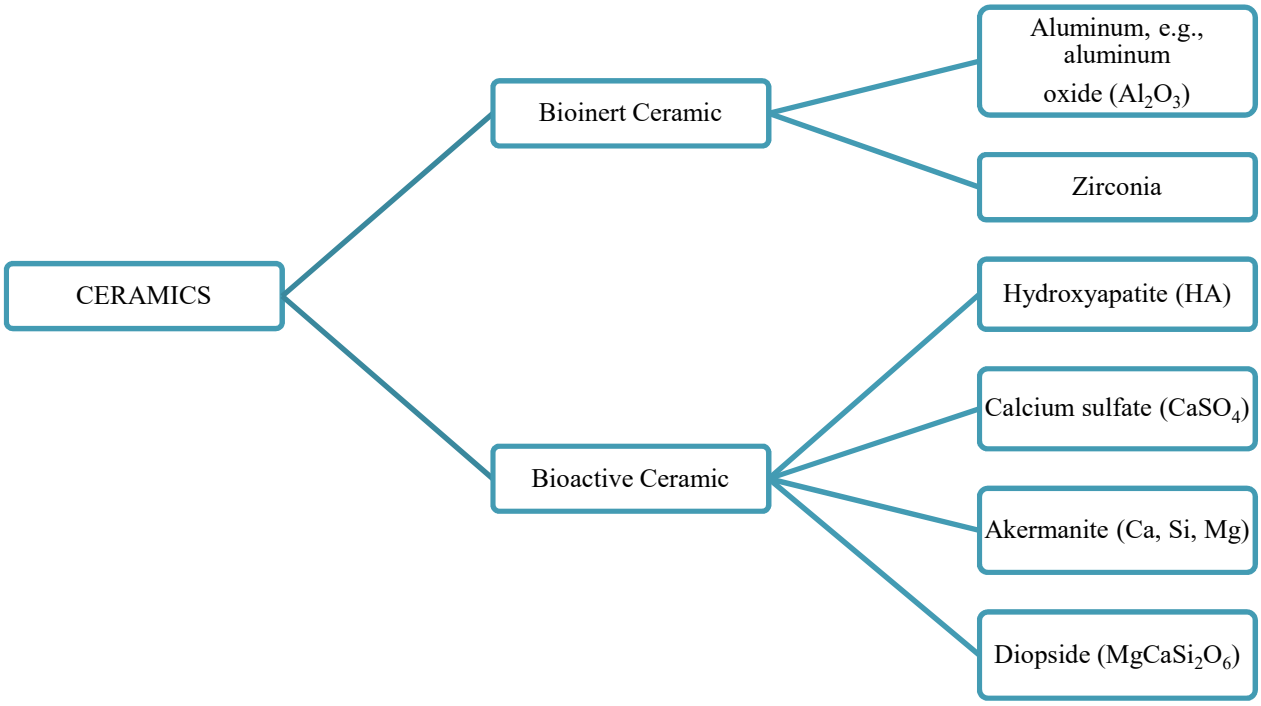

Figure 1: Type of Ceramics

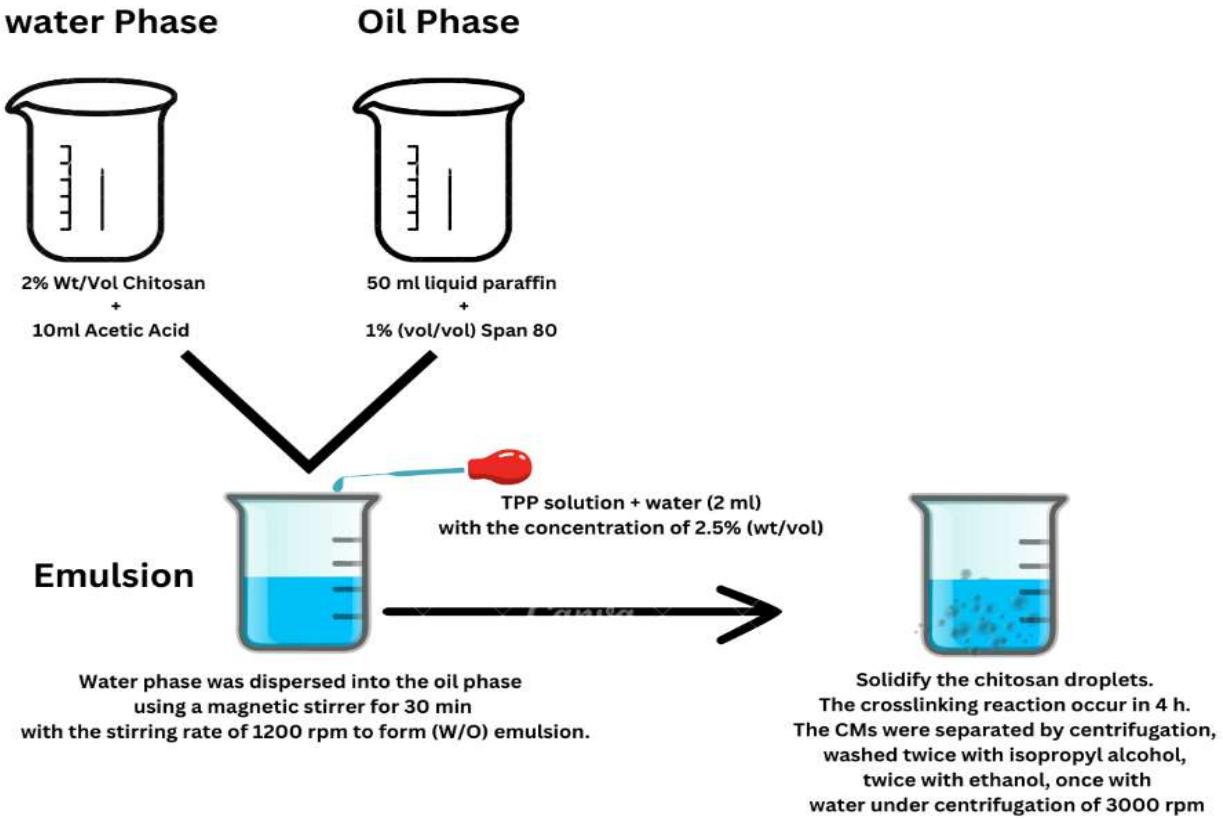

Figure 2: Preparation of non-loaded chitosan microspheres (CMs), TTP- tripolyphosphate

Fabrication Processes

1. Chitosan microspheres
2. Solid-strand scaffold fabrication via the DBRP method
3. Solvent casting and practical leaching
4. Freeze-drying
5. Electrospinning

Chitosan Microspheres

Chitosan was dissolved in a 2% (w/v) acetic acid solution i.e 10 mL in order to prepare non-loaded chitosan microspheres (CMs) or CMs without BSA. The water phase utilized was the chitosan solution, and the oil phase was 50 cc of liquid paraffin with 1% (v/v) Span 80 emulsifier. To create a water-in-oil (W/O) emulsion, the water phase was mixed with the oil phase for 30 minutes at a speed of 1200 rpm using a magnetic stirrer. To solidify the chitosan droplets, the TPP solution in water (2 mL) at a concentration of 2.5% (w/v) was then gradually added to the W/O emulsion. For four hours, the cross-linking process was left to continue.

Centrifugation was used to separate the CMs, after which the microspheres were lyophilized after being rinsed twice with ethanol, twice with isopropyl alcohol, and once with water while centrifuging at 3000 rpm. Take a Chitosan which was dissolved in a 2.2% (w/v) acetic acid solution to prepare BSA-loaded CMs. After that take 9 mL of chitosan solution which was then combined with 1 mL of the BSA-containing protein solution at a protein to polymer ratio of 1:50. Using a method identical to the one previously mentioned, the TPP solution (2 mL) at different concentrations (0.5%, 1.25%, and 2.5% w/v) was added to the chitosan solution that was combined with BSA to create the BSA loading microspheres [21-23].

Solid-Strand Scaffold Fabrication via the DBRP Method

40% (w/v) CMs were combined with 20% (w/v) PLLA that had been dissolved in 1,4-dioxane. After being thoroughly combined, the liquid was put into a 5-milliliter syringe using a precision fluid dispensing device (C0720M, A Symtek) for the construction of scaffolds [15, 16]. The mixture was then layer-by-layer extruded onto glass cover slips using a 240µm diameter needle to create scaffolds for three-dimensional structures. After the solvent volatilized, the scaffolds solidified. The scaffolds' micropattern was programmed into the system, and the fabrication procedure was carried out at room temperature [21].

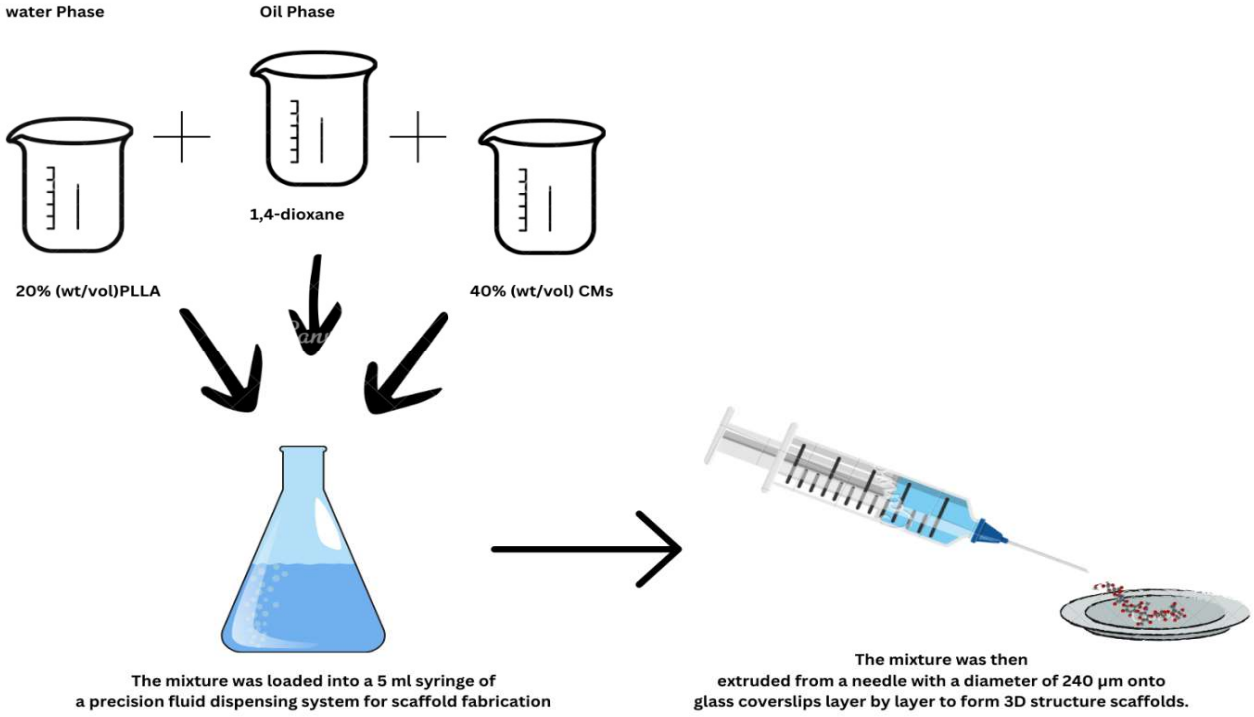

Figure 3: Solid-strand scaffold fabrication via the DBRP method

Solvent Casting and Particle Leaching

This technique's primary disadvantage is the possibility of adding hazardous materials to the scaffold through the use of solvents. Consequently, after scaffold manufacture, careful attention must be made to remove any harmful materials by properly drying the scaffold under vacuum. One of the most basic methods of synthesizing scaffolds is solvent casting. This approach forms the scaffold by dissolving a biopolymer in an organic solvent and letting the solvent evaporate. After dipping a mold of the required shape into a polymer and solvent solution, it is allowed to draw the solution for the appropriate amount of time. As a result, a layer of polymer forms on the mold, which is then treated further to create the scaffold [24]. A new technique known as solvent casting and particulate leaching (SC/PL) was developed to address some of the shortcomings of fiber bonding techniques. This technique gives desired control over the porosity, pore size, surface area-to-volume ratio, and crystallinity of the prepared porous scaffolds [25-27].

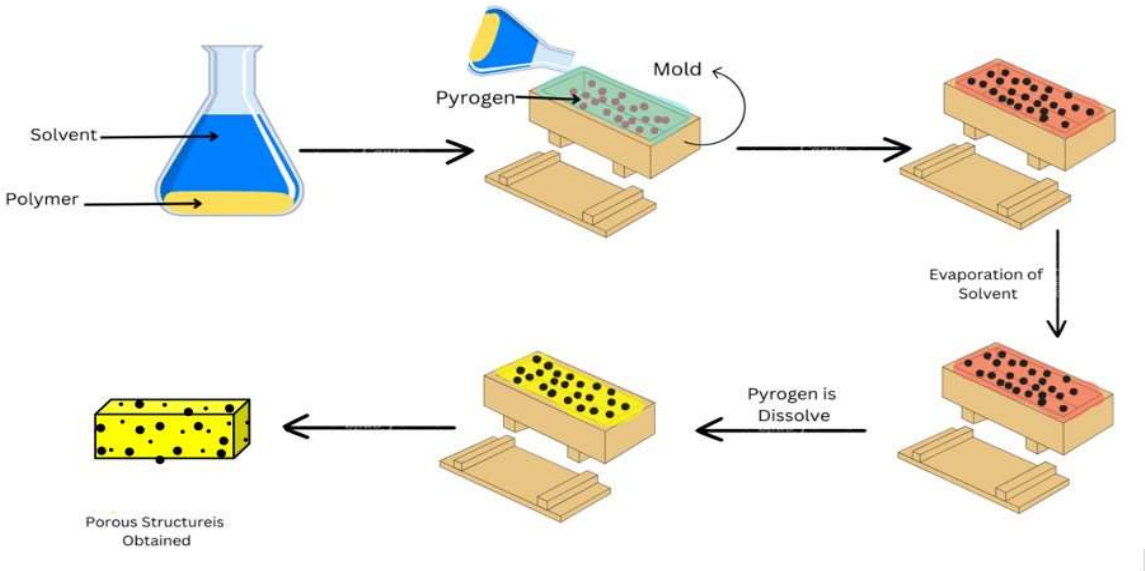

Figure 4: Preparation of Scaffold by using solvent casting and practical leaching process.

Benefits

- Fits thin wall three-dimensional specimens' thin membranes
- 50–90% high porosity
- Low-cost method.

Drawbacks

- Time-consuming due to the limited utilization of thin membranes.
- The extensive use of extremely hazardous solvents [28].

Freez Drying Technique

The freeze-drying procedure does not introduce undesirable residues that could harm tissues or interfere with cellular functioning; it is a better option for use in biomedical applications [24].

The gas foaming process was created to get rid of cytotoxic, organic solvents. In order to pressurize molded biodegradable polymers with water or flour form until they are saturated and full of gas bubbles, the technique uses relatively inert gas-foaming agents (nitrogen, CO_2). Sponge like structures having average pore sizes between 30 and 700µm and porosity up to 85% are usually produced by this approach [29].

High-pressure carbon dioxide gas with 800 psi pressure is allowed to saturate the polymeric solution in the gas foaming process. As a result, the CO_2 becomes unstable and clusters inside the solution.

The nucleation caused by the CO_2 cluster causes the structure to become porous [21]. Sometimes the particle leaching approach is used with the gas foaming technique for open pore geometry. Porogens, such as sugar and salt, are introduced for this reason. This method may produce pore diameters of 100µm and porosities of about 90%. The interconnectedness of the pores in this synthesis process is a significant problem [24]. The use of high heat during compression molding, closed, non-interconnected pore architectures, and a nonporous skin layer at the scaffold surface are some of the disadvantages of gas foaming technique [29].

Benefits

- Up to 85% porosit

Drawbacks

- The final product may have a solid polymeric skin or a closed pore structure if the fabrication procedure remained unchanged [28].

Electrospinning

Another name for this method of employing electricity to create fibers from a solution is electrospinning. In tissue engineering, this method is essential for creating nanofibrous scaffolds. The highly complex process of electrospinning involves charging a liquid at a high voltage, which interacts with electrostatic repulsion to induce droplets on the spinneret to erupt and stretch [28].

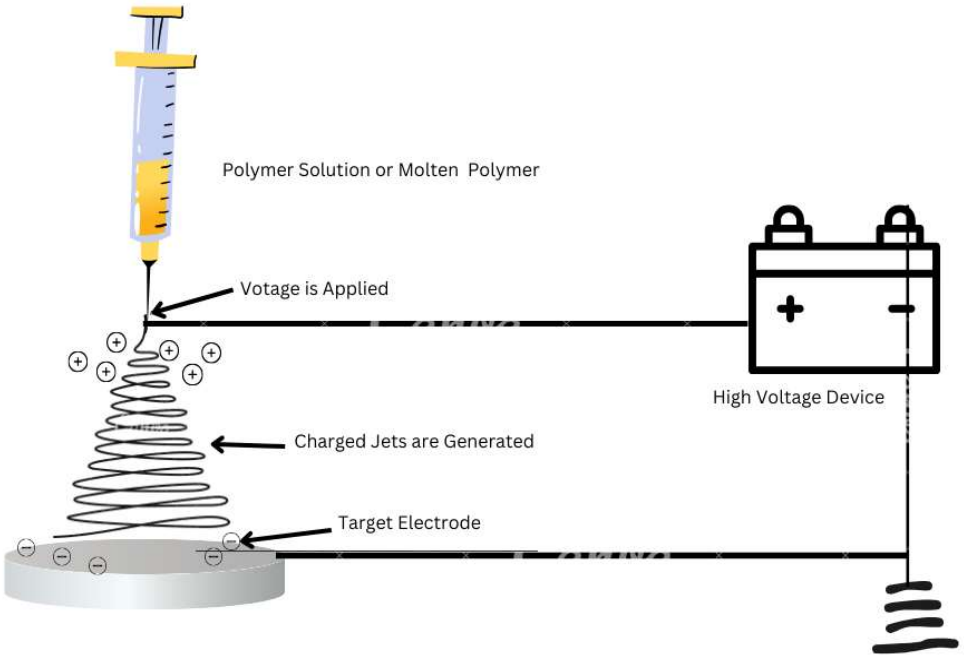

Figure 5: Preparation of Scaffold by using Electrospinning Method

It has been demonstrated that an isotropically distributed polymer fibers, which can be created with a rotating mandrel or patterned electrodes, affect the polarity of the cells [25]. Four main parts are needed for a conventional system: a grounded collector, a high-voltage power source, a syringe pump, and a spinner with a metallic needle. The intensity of the electric field overcomes the droplet's surface tension, creating a charged liquid jet that is continually whipped and extended by electrostatic repulsion until it lands on the grounded collector. During this process, the solvent was evaporating and the jet hardens to create a fibrous nonwoven membrane [29].

In this method the dissolved polymer solution is pushed through a needle at a regulated pace in a basic setup. The surface of the polymer droplet retained at the needle tip is charged by an electric field generated using a high voltage source across the needle and a grounded collector. A thin polymer jet forms when surface tension is overcome by the forces of electrostatic repulsion within the solution. As the jet moves from the tip of needle to the collector, where polymer fibers are created and deposited, solvent evaporation takes place [25].

Benefits

- A crucial method for creating Nanofibrous Tissue Engineering Scaffolds.
- A homogeneous blend consisting of high-tensile strength fibers.

Drawbacks

- Solvents used may be harmful

Characterisation of Scaffold

Mechanical Test

The challenge of accurately cutting and securely grasping specimens is a significant issue in the mechanical evaluation of porous ceramic scaffolds. Because of this, porous materials are typically not amenable to the traditional mechanical characterisation techniques including tensile, biaxial, and impact testing [30]. In a compression test, utilizing Zwick material prüfung 1446, the ASTM-recommended porous scaffolds D3410/D3410M [31] (d = 8mm, h = 10mm) were employed. The load was applied using a 10kN load cell at a crosshead speed of 2mm/min under ambient circumstances. The mechanical properties were ascertained using the obtained stress-strain curves. The maximum load that was recorded was used to calculate the compressive strength. For every condition, three specimens underwent testing [32].

Mechanical Characterization of Scaffolds

It is obvious that knowledge of the relationship among porosity, pore structure, and mechanical properties of scaffolds is essential to scaffold architectural optimization. Compressive mechanical testing is typically performed to gauge a scaffold's mechanical strength. Uniaxial testing is typically used in studies to determine compression strength. Generally, specimens are squeezed at a speed of 1.0mm/min between two stationary steel plates. The load carried at the 0.2% offset point divided by the initial scaffold cross-sectional area yields the compressive strength. As a result, the most often used technique on hydroxyapatite, bioactive glass, and composite scaffolds are the compression strength test, which is extensively and effectively utilized to assess the mechanical properties of porous ceramics [33].

It is the technique most frequently used on composite scaffolds, bioactive glass, and hydroxyapatite. As a gauge of the scaffold's toughness, the area under the stress displacement curve acquired during a compression strength test is typically regarded as approximating the work of fracture [34].

Measurement of Porosity

The porosity of the scaffolds was determined by using a specific gravity bottle using a recognized method based on Archimedes' Principle [35]. The porosity of a scaffold was computed by given formula:

$$\text{Porosity } (\%) = \frac{(W_2 - W_3 - W_s)/\rho_e}{(W_1 - W_3)/\rho_e} \times 100$$

where W_1 is the weight of specific gravity bottle filled with ethanol, W_2, the weight of specific gravity bottle containing ethanol and scaffold, W_3, the weight of specific gravity bottle taken out of ethanol saturated scaffold, W_s, is the weight of the scaffold, and ρ_e is, the density of ethanol [32].

Permeability

An effective bone Permeability is a crucial design factor for scaffolds because tissue engineering relies on the scaffold's capacity to permit waste removal from and nutrient diffusion to the regeneration site. The degree of pore interconnectivity and permeability are directly

correlated. Systems that create gravity have been implemented with compressed air or water or oil as the fluid, accordingly. While Maxwell and Wei employed dry air as the fluid medium to enable quick measurement operations, Swider et al. used a high-resolution MRI approach to characterize the permeability and fluid velocity [36-38].

Applying basic mathematical relations makes determining scaffold permeability simple. The intrinsic permeability k [m^2] can be found using Darcy's law, which provides the relationship between the pressure Gradient and Flow Rate by the given equation [39].

$$Q = \frac{-KA}{\mu}\frac{(P_b - P_a)}{L}$$

Where, A is the cross-sectional area (m^2), Q is the flow rate (m^3/s), μ is the dynamic fluid viscosity (Pa s), L is the scaffold thickness, and P_b - P_a is the drop pressure between two sites spaced L [36].

Morphology Study

SEM (Phillips XL 30: Eindhoven, The Netherlands) was used to assess the morphologies of porous structures [32].

Analyses of Phase Structures

PCL-FHA100 scaffolds with varying FHA100 contents (10, 20, 30, and 40 weight percent FHA100) were subjected to phase structure studies using Ni-filtered CuKa (kCuKa = 0.154186nm, radiation at 40kV and 30mA) during a two-hour period on an X-ray diffractometer (XRD, Philips X pert) [32].

Applications

Advanced Materials Based on Scaffolds for Biological Sensing

Many attempts have been made to create sophisticated materials that can quantify a wide range of biomolecules and recognize a molecular target in real time. Scaffold materials can hold cells and macromolecules because of their intrinsic nanocavities and porous structure, which have a greater surface area. In addition to providing a fibrous structure for cell adhesion and growth in tissue engineering, scaffolds' three-dimensional (3D) structure can also be used to release medications and other substances under controlled conditions for use in biomedical applications [39].

Polymeric Scaffolds for Use in Tissue Engineering

Scaffold design and production are important areas of biomaterial research and are also essential topics for tissue engineering and regenerative medicine research [1].

A special function of scaffold is seen in tissue regeneration and repair. To restore function or regenerate tissue, a scaffold must act as a transient matrix for cell proliferation and extracellular matrix deposition, with additional in growth necessary until the tissues are fully recovered and regenerate. In order to create cartilage, bone, skin, ligament, neural tissues, vascular tissues, and skeletal muscle, scaffolds have been utilized in tissue engineering. They have also been used to transport medications, proteins, and DNA under precise control [40].

Synthetic and Natural Scaffolds for Bone Regeneration

Clinical factors that impair bone regeneration capacity, such as critical-sized defects caused by high-energy trauma, tumor excision, infection, and skeletal anomalies, make managing bone defects more difficult. A bone scaffold is a three-dimensional matrix of structural elements that is inserted into defects as a template to encourage vascularization, the recruitment of growth factors, osteogenesis, osteoconduction, and mechanical support. A naturally produced bone scaffold with outstanding bioactivity, biocompatibility, and osteogenic qualities provides a microenvironment that is more akin to *in vivo* circumstances following decellularization and demineralization. In the meanwhile, there is less chance of disease transfer thanks to an artificially created bone scaffold that offers uniformity and scalability [41].

In Cartilage Tissue Engineering, Synthetic and Hybrid Scaffolding

Due to its limited capacity for regeneration, numerous studies in tissue engineering and regenerative medicine are being conducted on cartilage tissue. A temporary cellular environment that allows cells to function as they would in native tissue is essential for the appropriate application of scaffolding. The relevant specifications, such as suitable architecture and physicochemical and biological qualities, should be met by these scaffolds. This is required for healthy cell proliferation, which is linked to sufficient cartilage regeneration [42].

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