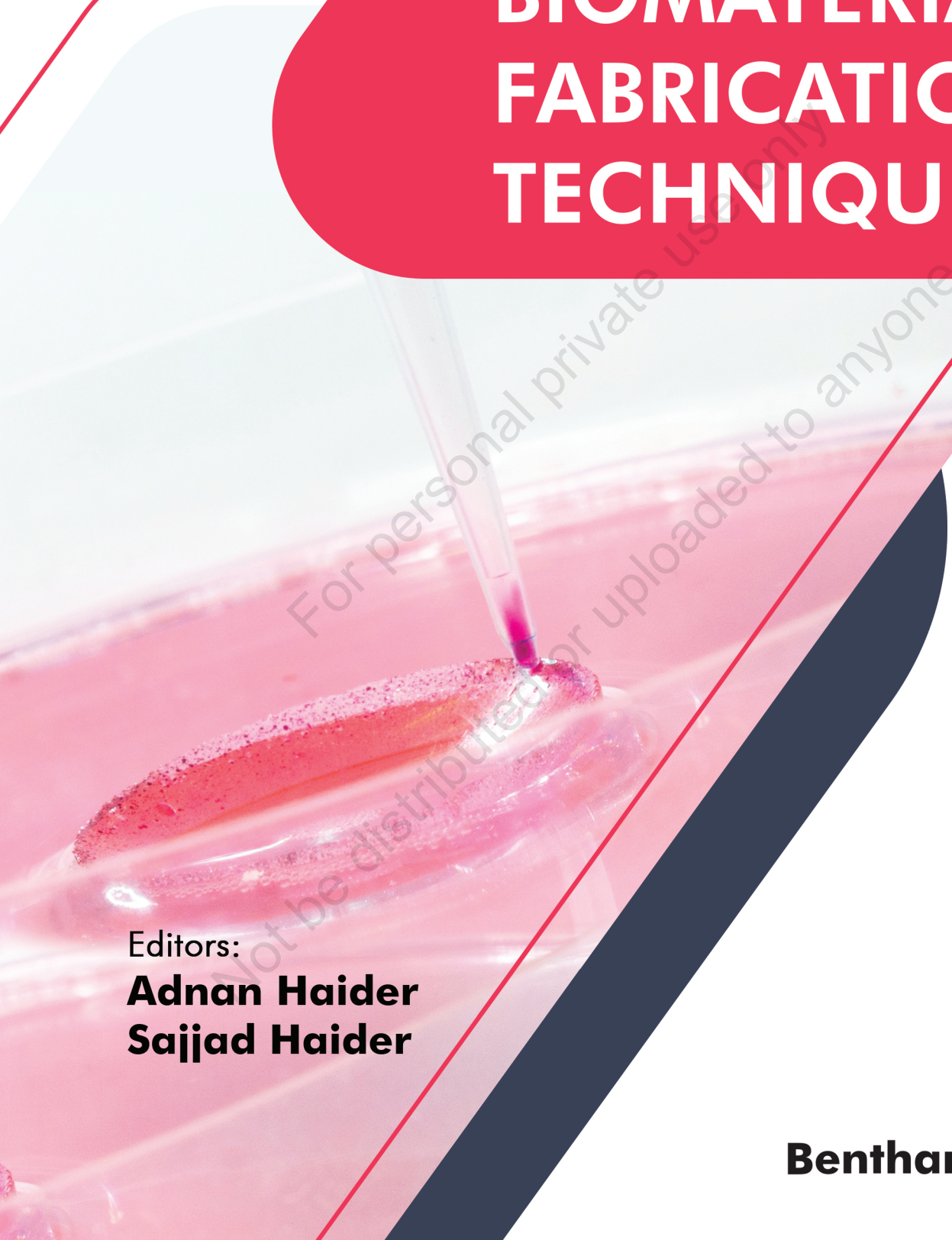
BIOMATERIAL FABRICATION TECHNIQUES



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Biomaterial Fabrication Techniques

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FOREWORD

This book provides an up-to-date, comprehensive, and authoritative overview of advancements in scaffold manufacture and uses in tissue engineering, combining the foundations for a wide understanding of scaffolds for tissue growth and development. The chapters cover a wide range of issues, including innovative materials and methodologies for scaffold preparation, difficulties, and future prospects. The chapters include topics such as novel materials and techniques for scaffold preparation, challenges, future prospects, and much more. The authors have carefully analyzed and summarized recent research findings in the aforementioned areas, providing an in-depth understanding of scaffold that maintains a balance among a variety of topics related to tissue engineering, including biology, chemistry, material science, and engineering, among others, while prioritizing study topics that are likely to be useful in the future.

Professor Inn-Kyu Kang
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PREFACE

This book is a collection of research and review articles from various parts of the world, highlighting the pivotal importance of biomaterials and their potential biomedical application. The articles link new findings and critically review the fundamental concepts and principles that are making the base of innovation. The book comprises ten chapters; the first two chapters deal with vital information about biomaterials and the strategies used for their fabrication. The rest of the chapters highlight the most widely used technique, their principle and their application in detail. The book contains up-to-date knowledge of biomaterials, their fabrication technique and their potential application, which is beneficial both for the experience as well as new researchers.

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CHAPTER 8

Particulate Leaching (Salt Leaching) Technique for Fabrication of Biomaterials

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Abstract: The most important characteristic of a scaffold used in tissue engineering is the possession of appropriate physical and mechanical properties to support or restore the biological function of damaged or degenerated tissue. Pore size, porosity, pore interconnectivity, and mechanical strength are all physical and mechanical properties that must be considered. Various fabrication techniques have been investigated to create a scaffold suitable for tissue engineering. One example is the particulate leaching (salt leaching) technique. The type of polymers and salts used, the particle size of the salt, and the fabrication technique all affect the desired physical and mechanical properties of salt leaching scaffolds. Over the past decade, there have been numerous studies on the fabrication of scaffolds for tissue engineering. This chapter reviews the different types of materials used, the basic salt leaching process, and its new modifications. It also discusses the advantages and disadvantages of the salt leaching technique and its future prospects.

Keywords: Interconnectivity, Mechanical strength, Polymers, Porosity, Salt leaching, Scaffold, Tissue engineering.

INTRODUCTION

Tissue engineering is a discipline of biomedical engineering that aims to facilitate cell ingrowth or replace damaged or diseased tissue with a combination of bioactive molecules, biomaterials, and cells or engineered cells [1]. To achieve these goals, scaffolds are commonly used in tissue engineering. Various biomaterials, from biopolymers to bioceramics to biodegradable metals, have been shown to be useful in the fabrication process [2].

The most important characteristics of a scaffold for tissue engineering are sufficient mechanical strength to support biological function by promoting cell

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adhesion, differentiation, and proliferation [3, 4]. Various techniques have been investigated to fabricate such scaffolds, including particle leaching (salt leaching), freeze-drying, solvent casting, self-assembly, phase separation, electrospinning, rapid prototyping, melt molding, gas foaming, and membrane lamination [5]. This chapter deals exclusively with the fabrication of a framework by particle leaching (salt leaching).

Particulate leaching (salt leaching/porogen leaching) is one of the most common, long-established conventional techniques for preparing porous biomaterials for tissue engineering. It involves dispersion of salts/porogens in a polymeric or monomeric solution, followed by gelation or fixation in the template and removal of salts/porogens to form an interconnecting porous architecture. The method has several advantages and disadvantages, which are also discussed in this chapter.

The main goal of preparing biomaterials for tissue engineering is to create a well-designed three-dimensional (3D) scaffold. The scaffold is an important tool to facilitate tissue formation both *in vitro* and *in vivo*. To regenerate tissue, tissue engineering uses biodegradable or non-biodegradable polymers, with or without the inclusion of molecules or biological cells. Many scaffolds for tissue engineering have been fabricated using the particle leaching technique (salt leaching). However, different tissues require different scaffold properties. For example, scaffolds for bone engineering may have different desirable properties than scaffolds for skin substitutes or retinal neural progenitor cells. Therefore, selecting the right polymers, salts, and salt leaching techniques (simple or modified) is critical, especially if the scaffold is designed to allow the target cells to function in the manner required for tissue regeneration. In this chapter, particle/salt leaching is presented for the preparation of biomaterials for tissue engineering applications. The materials and methods used and their new modifications are compared. Recent studies on scaffold materials fabricated using these techniques are summarized and discussed.

PARTICULATE LEACHING (SALT LEACHING) TECHNIQUE

The technique of particle leaching (salt leaching) involves the use of polymers or a combination of polymers and salt particles of a specific size to produce a suitable scaffold for tissue engineering. The desired physical and mechanical properties of the scaffold depend largely on the choice of the type of polymer and salt, the size of the salt particles, and the fabrication techniques. The types of polymers and salt typically used in the salt leaching technique, as well as the step-by-step approach to the basic salt leaching technique and its modifications, have been discussed in this section.

Polymers

Natural or synthetic biodegradable polymeric materials are widely used for the production of biomaterials because their properties offer greater advantages compared to other materials, such as metal or ceramics. Apart from the fact that biodegradable polymers are naturally absorbed by the human body, some of them are also suitable for tissue regeneration, which is basically helpful in injuries and reconstruction of damaged or aging tissues. Another advantage of polymers as biodegradable drug carriers is their low cost and ability to adapt to target organs or tissues. In laboratory processing, the particle leaching (or salt leaching) technique is often used in the development phase to produce biodegradable or non-biodegradable polymeric scaffolds with sufficient porosity for use in tissue engineering. The fabrication technique of this polymer can be easily extended to a larger quantity through industrial production [6].

Polymers are available with different mechanical and physical properties. Therefore, the basic properties of scaffolds, such as biocompatibility with the human body, sterilizability, and a suitable degradation profile, must be considered before fabrication. The processing of polymers into scaffolds for tissue engineering with specific properties for each application is highly dependent on the type of polymer chosen. The most commonly used biodegradable polymers for salt leaching techniques are aliphatic polyesters, such as poly(lactic acid) (PLA), polyglycolic acid (PGA), polycaprolactone (PCL) and their copolymers. However, there are also some other polymers, such as silk fibroin (SF), nylon and many others that are used to produce biomaterials for tissue engineering. Table **1** summarizes the properties of the polymers used in the production of biomaterials using the salt leaching technique.

Table 1. The properties of the polymer used in the preparation of biomaterial with the salt leaching technique.

Materials		Density (g/cm^3)	*E* (GPa)	σ (MPa)	ε (%)	References
Biodegradable Polymers	Non-biodegradable Polymers or Other Material					
Poly (glycolic acid)	-	1.53	>6.9	>68.9	15-20	[7]
Poly (L-lactic acid)	-	1.210–1.430	2.4-4.2	55.2-82.7	5-10	[8]
Poly (L-lactic-co-glycolic acid)	-	1.3	1.4-2.08	41.4-55.2	3-10	[9]
Polycaprolactone	-	1.14	0.21-0.34	20.7-34.5	300-700	[8, 10]
Chitosan	-	0.15–0.3	-	30	-	[11]
Starch	-	1.5	116.42–294.98	4.48–8.14	35.41–100.34	[12]
Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)	-	1.17–1.2	0.7–3.5	20–60	6–8	[10]
polymethyl methacrylate	-	1.17-1.20	1.8–3.1	48-76	2-10	[10]
Cellulose nanofiber	-	0.96–1.02	138	10	-	[13]
Silk fibroin	-	1.40	9.860	513	23.4	[10, 14]

(Table 1) cont.....

Materials		Density (g/cm^3)	*E* (GPa)	σ (MPa)	ε (%)	References
Biodegradable Polymers	Non-biodegradable Polymers or Other Material					
Gelatin	-	1.3–1.4	1.09–1.57	50.68–128.37	5.86–18.4	[10]
Silk sericin	-	0.00132–0.0014	1.09–1.57	50.68–128.37	5.86–18.4	[15]
PVA	-	1.2–1.3	37–45	67–110 (98-99% hydrolyzed)	225–445	[10]
-	Nylon	1.15	2.7	82.7	10.0–86.0	[16]
-	Polyurethane	0.048–0.961	0.091–0.02	35	-	[17, 18]

E, Young's modulus; σ, tensile strength; ε, elongation at break.

Salt

A variety of salts, including sodium chloride (NaCl), sodium bicarbonate ($NaHCO_3$), sodium acetate (NaOAc), and calcium chloride ($CaCl_2$), have been used in salt leaching techniques to produce biomaterials [19]. They are also known as porogens due to their ability to form porous structures, which is an important property of scaffolds for tissue engineering. The ideal salt that is mostly used in this method is NaCl. Due to its high solubility in aqueous media, it can be easily removed by the leaching process. Since plasma normally contains the ions Na and Cl-, the remaining salt in the scaffold after leaching is not harmful to the human body [20].

Methods

1. Conventional Salt Leaching

The method involves the addition of a water-soluble porogen (leaching agent to create a porous/channel) such as salt. The salt is crushed into small particles or according to the desired size and poured into a mold. The polymers are then dissolved in an organic solvent before being cast into the mold filled with salt. After evaporation of the solvent, the salt is leached in water for two days to form a porous structure [5]. Fig. (**1**) shows an illustration of the basic salt leaching technique. In this method, the size of the salts significantly affects the size and shape of the pores, while the porosity of the fabricated framework depends on the amount of salt added during fabrication. It has also been reported that the pores exhibit high interconnectivity when the salt content is 70% w/w or more [21].

Kwon *et al*. recently reported the preparation of poly(-caprolactone-ran lactic acid) (PCLA-F) scaffolds by the salt leaching method. The PCLA-F polymer was dissolved in methylene chloride and the sieved NaCl particles (200-250 μm diameter) were added to the polymer solution. Then the mixture was poured into a round silicone mold (diameter 10 mm and height 5 mm). The molded slurry was

then dried at room temperature for up to 24 hours, followed by leaching in deionized water at a constant stirring speed of 100 rotations per minute (rpm) for 24 hours. The resulting scaffold has an irregular and well-interconnected pore structure [22]. Fig. (**2**) shows the resulting scaffold as well as scanning electron micrographs of the pores and the interconnectivity between the pores.

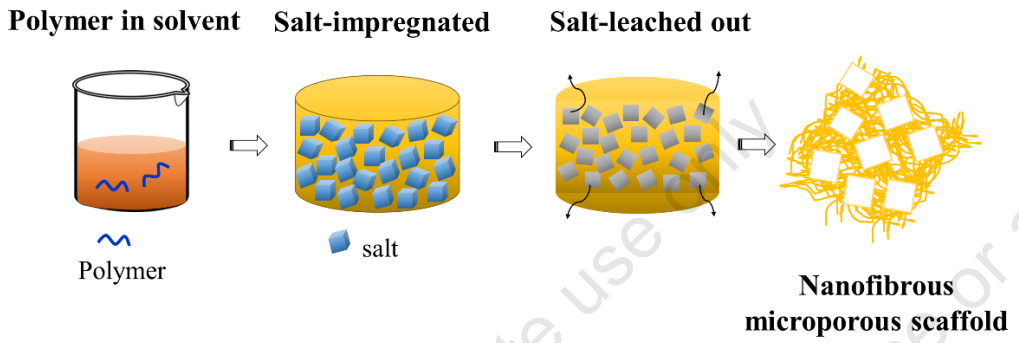

Fig. (1). Schematic illustration of the preparation of scaffold by using conventional salt leaching technique.

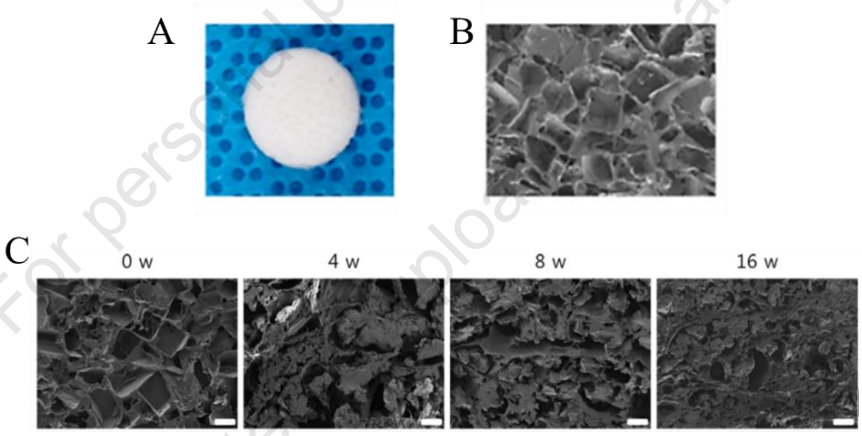

Fig. (2). (A) Macroscopic image of scaffold. **(B)** Pore structure and interconnectivity of scaffold by SEM. **(C)** The cross-sectional SEM image showed the degradation of scaffold after 16 weeks implanted in SD rats. Reprinted with permission from [22].

2. Combination of Melt Mixing and Particulate Leaching

This method is similar to the traditional salt leaching technique. The only difference is that this method uses a batch/measurement mixer. Fig. (**3**) shows the preparation of scaffold by using a combination of melt mixing and salt leaching technique In short, the polymer is mixed with the salt and placed in a batch mixer,

where it is processed after a specified mixing time and temperature. The mixture is then compressed in a compression molding tool (mold diameter 10 mm and height 3 mm) at 180 bar and 190-220 ºC. The salt is then leached in demineralized water under specific pH, time and temperature conditions determined by the experimental design. The porous frameworks are then dried at room temperature for 24 hours. Scaffaro *et al*. reported using this method to produce porous scaffolds with a highly interconnected pore with suitable mechanical properties for tissue engineering [23].

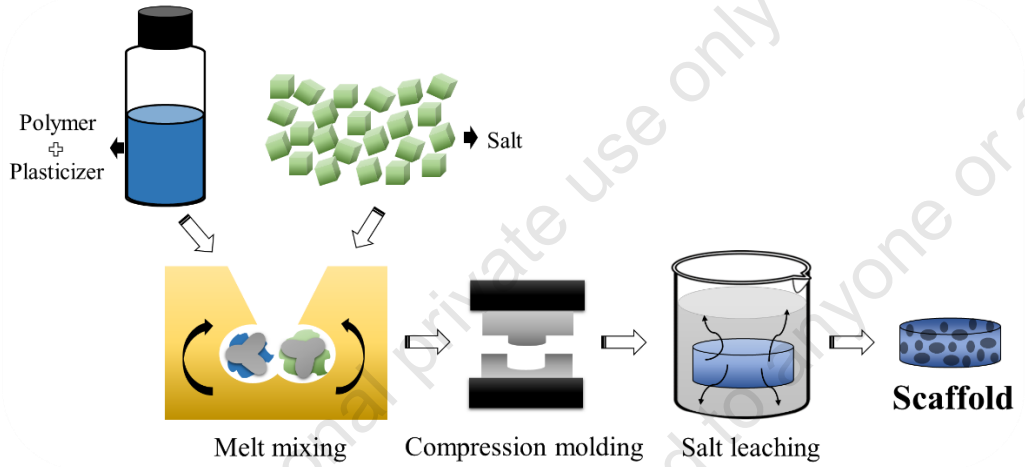

Fig. (3). Schematic illustration of the preparation of scaffold by using a combination of melt mixing and salt leaching technique.

3. High Compression Molding-Salt Leaching

This method combines salt leaching with compression molding. Fig. (**4**) shows the preparation of scaffold by using high compression molding-salt leaching. The process essentially consists of using a high-pressure compression molding machine in which temperature and pressure are controlled. This method results in a strict, regular architecture and good mechanical properties of scaffolds due to the effect of the melting process combined with the high pressure. In 2016, Zhang *et al*. developed a home-built high-pressure press molding device for the fabrication of poly(L-lactide)/poly(lactide-co-glycolide)/hydroxyapatite composite scaffolds using the combination technique with salt leaching. The high-pressure cell has a guide column, a mold core and a sample chamber within the main mold, which is heated by an electric heating jacket and tempered by a thermocouple. The scaffold is prepared by dissolving polymers in a suitable organic solvent and mixing them with sieved salt (sodium chloride) of the desired particle size. The mixture is then mechanically mixed in an internal mixer at 50

rpm and 180 °C. It is then molded under high pressure at a predetermined pressure of 640 MPa for 10-15 minutes. The interconnected pore structure is maintained after lowering the temperature to room temperature by leaching the salt with water until a constant weight is achieved. This method results in a framework with a good porous structure and good interconnectivity between pores. The porosity ranges from 81.5 to 82.7%, and the compressive modulus can reach up to 4.64 0.2 MPa, which is comparable to the modulus of human cancellous bone (2-10 MPa) [24].

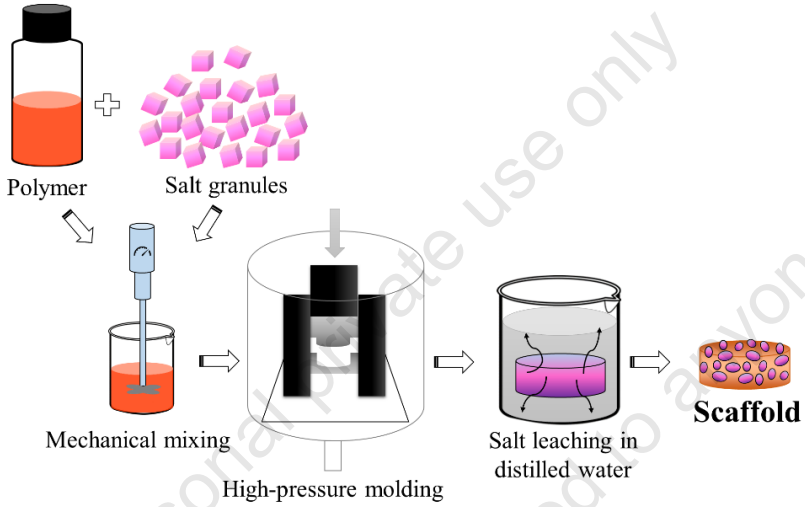

Fig. (4). Schematic illustration of the preparation of scaffold by using high compression molding-salt leaching.

4. Gas Foaming-Salt Leaching

This method offers a fast and solvent-free process. Fig. (**5**) shows the preparation of scaffold by using gas foaming-salt leaching. Gas foam salt leaching technique is divided into three steps: First, the polymer/salt mixture is prepared by melt mixing. Second, the composite is foamed with dense CO_2 gas, and finally, the salt particles are leached out of the scaffold. In 2011, Annabi *et al*. used this method to fabricate a porous PCL/elastin composite scaffold. The scaffold has an average pore size of 540 m and a porosity of 91%. They used a CO_2 pressure of 65 bar, a temperature of 70°C to melt the polymer, a depressurization rate of 15 bar/min, and a processing time of 1 h in a gas foaming reactor [25].

In another study, Bak *et al*. demonstrated the use of supercritical carbon dioxide ($scCO_2$) to produce a porous, solvent-free scaffold. In this method, gas foaming was combined with a salt leaching process. The polymer was melted at the glass transition temperature (Tg) and then mixed with salt at a suitable ratio. The

mixture was then poured into a Teflon mold (diameter 2 cm and height 1 cm). The gas foaming process was carried out in a gas foaming reactor with controlled temperature and pressure. In their study, Bak *et al*. used a temperature of 50°C and a pressure of 8 MPa in a gas foaming reactor, and CO_2 was dissolved in the samples for 6 hours. Then, the pressure was quickly released to allow the scaffold to form a bimodal pore structure. After that, the salt was leached with distilled water for one day. The prepared scaffold has an average pore size of 427.89 m and a high degree of pore interconnectivity. The scaffold also promotes proliferation and adhesion of MC3T3-E1, indicating a potential application in bone tissue engineering [26].

It is known that the amount of gas generated and expanded in the internal structure of a scaffold affects the percentage of porosity and the pore size of the scaffold. The higher the percentage of porosity and the larger the pore size of the scaffolds, the more gas is generated and expanded. However, in the fabrication of silver nanocomposite scaffolds by El-Kady *et al*. using a combination of gas foams and salt leaching, the addition of silver nanoparticles hindered the expansion of the generated gas throughout the scaffold matrix. The percent porosity and pore size of the framework were reduced by this process [27].

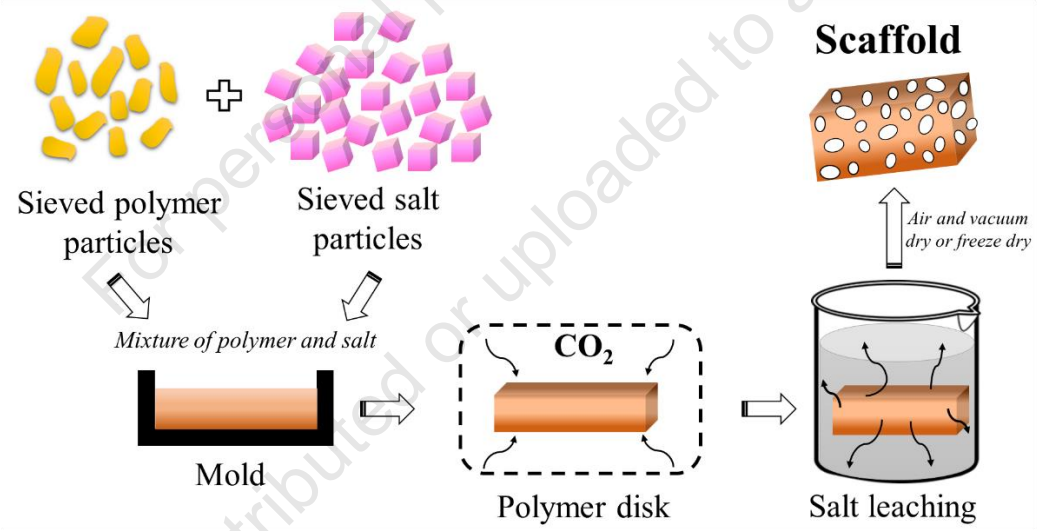

Fig. (5). Schematic illustration of the preparation of scaffold by using gas foaming-salt leaching.

5. Salt Leaching Electrospinning (SLE)

In 2016, Park *et al*. fabricated a scaffold for skin tissue engineering using a combination of salt leaching and 3D electrospinning techniques. Since the

structure and physical properties of the skin scaffold fabricated by this method are similar to those of an extracellular matrix (ECM), the scaffold can mimic the ECM. The preparation of scaffold by using salt leaching electrospinning can be seen in the Fig. (**6**). To obtain stable nozzles, the polymer solution was placed in a syringe with the proper needle size, and the electrospinning device was set at an appropriate voltage and distance between the needle tip and the collector. The salt with the appropriate particle size was then released from the rotating cylinder above the drum collector at the desired rate. After the fabrication process, the salt from the nanofiber scaffold was leached in distilled water for one day and then freeze-dried. The scaffolds have advantageous properties such as high interconnectivity between pores, porosity and water uptake capacity. Therefore, the scaffold can support cell infiltration and proliferation, which could be useful for skin tissue engineering [28].

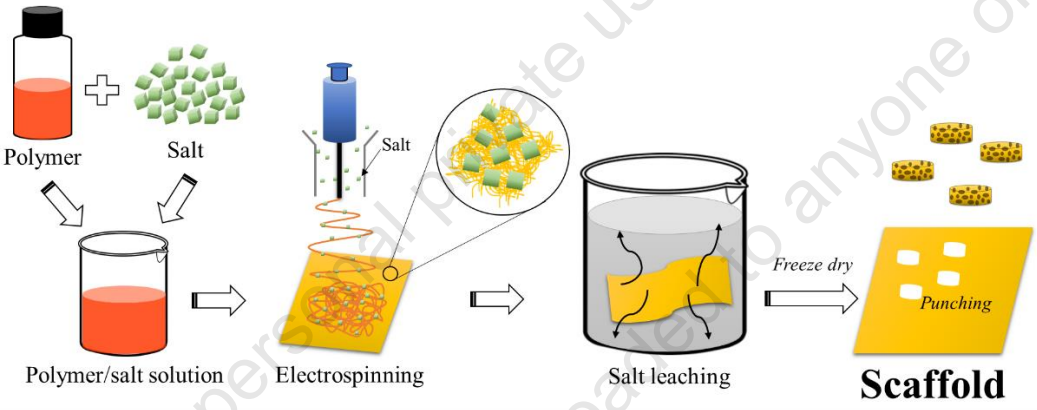

Fig. (6). Schematic illustration of the preparation of scaffold by using salt leaching electrospinning.

6. Salt Leaching Using Powder (SLUP)

Fig. (**7**) shows the preparation of scaffold by using salt leaching using powder. The heat treatment used in several previously described methods to alter salt leaching can be avoided in this method. In the SLUP method, the sieved powder (*e.g.*, Bioglass 46S6) was mixed with a polymer. The salt was then applied homogeneously and stirred continuously for 10-15 minutes. The mixture was then poured into a mold and dried. The salt was then leached from the framework with distilled water for at least two days. The finished scaffold was then allowed to dry for 24 hours. Recently, Refifi *et al*. reported the application of this method to fabricate hybrid biomaterials from bioglass (46S6) and chitosan scaffolds. The fabricated scaffold exhibited high porosity (90%) and well-interconnected pores in the internal networks of the scaffold [29].

Advantage and Disadvantage of Scaffolds Produced by Salt Leaching Technique

Scaffolds produced by the salt leaching technique have several merits and demerits that can be seen in Table **2**.

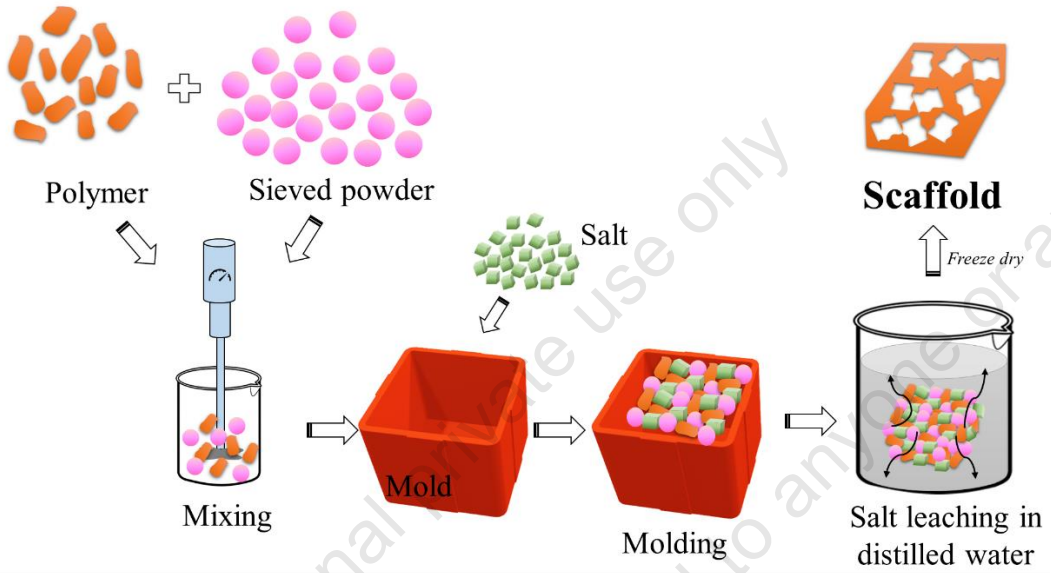

Fig. (7). Schematic illustration of the preparation of scaffold by using salt leaching using powder.

Table 2. Advantage and disadvantage of particulate leaching (salt leaching) technique [5, 30, 31].

Advantage	Disadvantage
Relatively easy technique	Result in Thin Membranes (up to 3 mm Thick)
Porous 3D structure	A brief period of use
Low cost	Limited porous size
High porosity (50%–90%)	Toxicity due to the use of solvent (solvent residue)
Capable of tailoring 3D cell growth by tuning pore size	Time consuming (evaporation of solvent takes days to weeks)
A variety of polymers can be us	Particle entrapment in the polymer matrix prevents the formation of open cell structures
Possibility of controlling porosity and crystallinity	Irregular shaped pores
-	Inter-pore openings are difficult to control

PHYSICAL AND MECHANICAL CHARACTERISTICS

1. Pore Size

A scaffold produced by particulate leaching (salt leaching) was reported to have a thickness of less than 3 mm. The average pore diameter has been reported to be up to 500 μm [32]. The larger porous matrix can be used, for example, to fabricate complex geometric tissue scaffolds such as bone models and ear models [33]. The pores in tissue engineering scaffolds serve several important functions, including facilitating the transfer of metabolites and nutrients for cells during cell development, improving cell visibility and adhesion, and accelerating scaffold degradation. The preferred pore size of scaffolds that provide a suitable structure for tissue engineering is on the microscale [34]. Scaffolds with a pore diameter of 50-300 μm are generally well suited for cell penetration and neovascularization, while smaller pore diameters of 0.5-10 μm are required to facilitate the transport of nutrients and physical stimuli that can stimulate cell function and accelerate healing [35 - 37]. Based on this fact, several studies have proposed the development of a scaffold with a hierarchical porous structure that is more advantageous, especially for tissue engineering. Lao *et al*. fabricated hierarchically porous PLLA/-TCP nanocomposites that promoted MG -63 osteoblast proliferation, penetration, and deposition of ECM, suggesting the possibility of bone tissue engineering [38]. Zhang *et al*. proposed a hierarchical 3D poly(-caprolactone) scaffold (pore size 3-250 μm), which showed potential for the neo-tissue formation and uniform cell distribution in another study [39].

2. Porosity

One of the most important properties of scaffolds for tissue engineering, especially for tissue engineering of cartilage, is their porosity. Porosity serves a number of functions, including ensuring uniform distribution of physiological cells, facilitating the organization of the new extracellular matrix (ECM) formed by cells, and effectively facilitating nutrient supply and waste removal during *in vitro* cell culture [40].

The salt leaching method has been used to produce scaffolds with porosity ranging from 50% to over 90% [41]. It has been reported that porosity greater than 70% is highly recommended for the regeneration of bone tissue, especially cartilage. This allows ECM distribution similar to that in physiological tissue [42]. In 2014, Nasri-Nasrabadi *et al*. discovered that increasing the sodium chloride content leads to increased interconnectivity of the pores. A scaffold containing 90% salt had a higher percentage of porosity than a scaffold containing 70% salt

[21]. However, it has also been reported that higher porosity can lead to poor mechanical properties and is therefore unsuitable for bone tissue engineering [43].

3. Mechanical Properties

The mechanical properties of a prepared scaffold are determined by the host tissue to be regenerated. Therefore, the more similar the mechanical properties are, the more suitable the scaffold is for tissue engineering. It is known that the mechanical strength of a scaffold has a major impact on inter/intracellular signaling, motility, cell ingrowth and response to stimulation or inhibition [44].

4. Interconnected 3D Structures

The interconnectivity of pores in the internal structures of the scaffold is another important feature of tissue engineering. A good or well-connected pore can facilitate cell ingrowth or cell proliferation. If a scaffold has pore structures but they are not interconnected, it is useless and unsuitable for tissue engineering. A scaffold with 100 percent contiguous pore volume can support cell migration and proliferation while enhancing the diffusion and exchange of nutrients within the scaffold's pore networks. ECM infiltration of the target tissue is also supported by the highly interconnected pores [45].

BIOMATERIALS PREPARED WITH PARTICULATE LEACHING TECHNIQUE

A modification of the conventional salt leaching technique was developed to improve the current scaffold design by controlling the parameters affecting pore size, porosity, pore interconnectivity, and pore properties for better and appropriate cell attachment and nutrient diffusion. Table **3** summarizes the latest scaffold-based salt leaching techniques that can be considered for tissue engineering applications. Based on the scaffolds listed in the table, readers can learn about the types of polymers and salts used, potential applications, scaffold properties, and toxicological experiments and results.

Table 3. Biomaterials-based salt leaching techniques and toxicological evaluations.

Biomaterials	Material Component		Porous 3D Scaffold Applications	Outcomes	Toxicological Experiment	Toxicology Result	References
	Polymer/Other Materials	Salt Particle					
Bioglass®45S5-Polycaprolactone (PCL) composite scaffolds	PCL	Sodium chloride (NaCl) + Sodium bicarbonate ($NaHCO_3$)	Bone regeneration and vascularization	-a high degree of interconnected porosity -excellent mechanical properties -the addition of bio glass had no effect on mechanical properties -simple to use -good cell proliferation -good cytoplasmic continuity	-	-	[19]
Porous HA/nylon 6,6 scaffold	Nylon	NaCl	Bone regeneration	-a well-developed interconnected porosity -pore size is around 200-500μm -ideal for bone regeneration and vascularization -the mechanical strength is comparable to cortical bone	-	-	[20]
Porous starch/cellulose nanofibers composite	Starch + cellulose nanofiber	NaCl	Tissue engineering	-the diameter of nanofibers is between 40-90 nm -interconnected porous morphology -good hydrophilicity -uniform porous structure -good mechanical properties -good biodegradability	MTT assay	No toxicity	[21]
Polylactic acid/hydroxyapatite porous nanocomposites (PLA/HAp)	PLA	Ammonium bicarbonate (NH_4HCO_3)	Bone engineering (bone implant)	-↑porosity by 39% -↑hydrophilicity of nanocomposite -↑surface area capable of improving mechanical properties -after immersing the nanocomposite in simulated body fluid, hydroxyapatite shaped on its surfaces	-	-	[46]
Poros Poly (Lactide-c--glycolic acid) (PLGA) scaffolds	PLGA	Sodium chloride (NaCl)	Pluripotent stem cells and neural retinal precursor cells	-Pore size is less than 10 μm -Scaffolds have amorphous smooth and irregularly shaped pores -cells can attach to the scaffolds and proliferate well -successful early retinal development -beneficial to neural phenotypes	-	-	[47]
Porous Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) scaffolds	PHBV	Sieved sodium chloride (NaCl)	Tissue engineering (particularly bone engineering)	-high porosity (88.8%) -good mechanical properties for 3D cell culture -elevate the proliferation of MG-63 human osteoblast-like cells and MC3T3-E1 pre-osteoblast cells -up-regulated transcription of extracellular matrix and growth factors genes	-	-	[48]

(Table 3) cont.....

Biomaterials	Material Component		Porous 3D Scaffold Applications	Outcomes	Toxicological Experiment	Toxicology Result	References
	Polymer/Other Materials	Salt Particle					
Solvent-casting particulate leaching (SCPL) polymer scaffolds	polymethyl methacrylate (PMMA), Polyurethane (PU)	NaCl	Bone marrow	-well-interconnected and high porosity (82.1vol%–91.3vol%) -easy control of pore size -mechanical properties are determined by the polymer matrix and the architecture of the scaffold -improved 3D stromal cell support -capable of stimulating the bone marrow microenvironment	-	-	[49]
Porous PLGA scaffolds-impregnated small intestinal submucosa (SIS)	PLGA	NaCl particles	Tissue-engineered bio discs	-good mechanical properties -seeding of cells uniformly -good attachment of cells -↑cell growth and ECM synthesis -easy adjustment of pore size and scaffold area for nucleus pulposus regeneration.	-	-	[50]
3D electrospun silk-fibroin nanofiber	Silk fibroin	NaCl crystal	Skin tissue engineering (Skin substitutes)	-larger pores -high interconnectivity between pores -high porosity and water uptake -↑Proliferation of fibroblast in the deep layer -↑keratinocytes differentiation in the superficial layer	-	-	[28]
Porous polycaprolactone (PCL)/elastin composite scaffold	PCL	NaCl	Tissue engineering (bone regeneration)	-high porosity (91%) -large pores size (average 540μm) -high degree of interconnectivity and homogenous 3D scaffolds	-	-	[25]
PCL scaffolds with dual leaching method	PCL	NaCl	Bone tissue engineering	-high interconnectivity of the pores -3D structure -high porosity -high water uptake -uniform pore size -support bone cell proliferation and differentiation in culture media	MTT assay	No toxicity	[51]
Porous poly (lactic acid) (PLA) scaffolds	PLA	Stable salt stack	Tissue engineering	-PLA foams are less crystallizable than the bulk materials -the pores and caves have a diameter of about 250 μm -polymer foam can be crystallized without damaging the structure of scaffolds	-	-	[52]
Poly (ε-caprolactone-r-n-lactic acid) (PCLA-F) scaffolds	PCLA-F	Sieved NaCl	Tissue regeneration	-irregular structure -rapid bulk erosion -the resulting scaffold degrades quickly *in vivo*	-	-	[52]

(Table 3) cont.....

Biomaterials	Material Component		Porous 3D Scaffold Applications	Outcomes	Toxicological Experiment	Toxicology Result	References
	Polymer/Other Materials	Salt Particle					
Composite glass/chitosan (BG-CH) scaffold	CH	NaCl	Tissue engineering	-high porosity -no solvent residue due to SLUP method -↑pore size interconnectivity - three-layer scaffolds with varying porosity and pore size in each layer	-	-	[29]
Porous PCL scaffolds	PCL	NaCl	Bone tissue engineering	-the mean pore size is ~420 µm -high degree of pore interconnectivity -↑adhesion and proliferation of MC3T3-E1 cells	MTT assay	No toxicity	[26]
PLA nanofibrous microporous scaffolds with bioactive glass nanoparticles	PLA	NaCl	Bone tissue engineering	-high porosity with size of ~90 nm -nanofibrous structure with 90-95% porosities -↑hydrophilicity -rapid hydrolytic degradation -↑formation of apatite throughout the scaffolds	-	-	[53]
PCL/starch nanocomposite scaffold	PCL, starch	NaCl	Bone tissue engineering	-uniform pore morphology and structure -good comprehensive-loa- -resisting capabilities in human cancellous bone -the porosity ranges from 50 to 90% -↑porosity results in decreased mechanical properties -appropriate pore size and pore interconnectivity -adequate mechanical properties	-	-	[54]
Silk fibroin/hyaluronic acid (SF/HA) 3D matrices	SF, HA	NaCl	Cartilage tissue engineering	-sponge-like structure -high porosity -the porosity is in the range of 61–72% -decrease pore volume due to the presence of HA -high crystallinity -scaffold porosity, micro porosity of the pore wall, and material inhomogeneity all had an effect on water absorption and mechanical strength	-	-	[40]
Biomimetic hybrid porous scaffold of chitosan/polyvinyl alcohol/carboxymethyl cellulose (CH/PVA/CMC)	CH, PVA, CMC	NaCl	Soft tissue engineering	-a pore structure that is uniformly distributed and interconnected -the pore size is 13.6 µm to 15.5 µm -the porosity is 90% -addition of CMC increased hydrophilicity -good mechanical strength -facilitate cell migration and proliferation	-	-	[55]
Polylactic acid porous scaffolds	PLA	NaCl, $CaCl_2$	Tissue engineering	-highly interconnected pores -appropriate mechanical properties	MTT assay	No toxicity	[23]

(Table 3) cont.....

Biomaterials	Material Component		Porous 3D Scaffold Applications	Outcomes	Toxicological Experiment	Toxicology Result	References
	Polymer/Other Materials	Salt Particle					
Cell-loaded gelatin/chitosan scaffolds	Gelatin/CH	NaCl	Skin tissue engineering	-high porosity -uniform pores morphology inside and on the surface of the scaffold -highly interconnected pores -support cell proliferation -appropriate mechanical properties -great potential for skin tissue engineering	MTT assay	No toxicity	[56]
Sericin/PVA/glycerin scaffolds	Silk Sericin, PVA, Glycerin	NaCl	Wound dressing	-large porous structure -good interconnectivity structure -fast biodegradation rate -less adhesive to the wound site -support fibroblast proliferation	MTT assay	No toxicity	[57]
Nanocomposite scaffold-releasing silver (Ag)	Neat polymer (Poly (L-lactide) + Ag	Sodium bicarbonate ($NaHCO_3$)	Bone treatment	-good-interconnected structures -pore size is in the range of 100-250 μm -high porosity -the scaffold induced an apatite layer on its surface	-	-	[27]
PLLA/β-TCP nanocomposite scaffolds	PLLA	NaCl	Bone tissue engineering	-hierarchical porosity -500 nm to 300 μm pore diameter -PLLA nanofibrous matrix with fiber diameters ranging from 70-300 nm -good mechanical properties -↑bioactivity of the PLLA matrix -support cells proliferation and penetration, as well as ECM deposition	-	-	[38]

POROUS 3D SCAFFOLD APPLICATIONS WITH SALT LEACHING TECHNIQUE

Bone Engineering

The new gold standard for the treatment of bone defects (*e.g.*, autologous bone grafts) is limited by the lack of donor sources, the body's response to the graft, and, most importantly, the patient's resources [58]. The advancement of tissue engineering technology can overcome this limitation. In the tissue engineering concept, the scaffold of biodegradable polymers combined with autologous cells is a promising strategy to obtain an ideal bone graft. The patient's cells were isolated and grown *in vitro* before being seeded into 3D scaffolds. After successfully culturing the cells to a specific cell number *in vitro*, the cells were transplanted to the patients. The development of native tissue is then followed by biodegradation of the scaffold [59]. In this method, newly formed tissue from patients would be used to replace the implanted scaffold and eventually treat the

bone defects.

Scaffolds for bone implants must have a variety of properties, including compatibility, mechanical strength and stability, and a highly open porous structure that allows tissue regeneration. Careful consideration must also be given to the size and distribution of pores for new cell growth [60 - 62].

Neuronal Retinal Precursor Cells

Stem cells are a viable treatment option for patients suffering from neurodegenerative diseases or age-related macular degeneration, which is characterized by the death of light-sensitive photoreceptor cells in the outer neuronal retina. However, there are still limitations with this approach, such as the minimal number of surviving and integrated cells after subretinal bolus injection in patients, especially in patients with end-stage disease. The main reason is that the donor cells do not receive physical support during bolus injection [63, 64]. In this case, making a scaffold to support the donor cells during transplantation could solve the stem cell problem. Several porous PLGA scaffolds have been reported to improve the survival rates of retinal progenitor cells (RPCs) after transplantation and even promote the integration of the cells into the host retina [65, 66]. The salt leaching scaffold has also been used in the treatment of retinal degeneration as a cell replacement therapy. In 2016, Worthington *et al*. developed PLGA scaffolds using a salt leaching/solvent evaporation process. The resulting scaffold product was shown to have the ability to support and facilitate the proliferation of induced pluripotent stem cells (iPSCs) within the amorphous smooth pore network of the scaffold. Therefore, this scaffold represents a promising approach for the treatment of retinal degeneration prior to transplantation [47].

Skin Substitutes

Skin tissue engineering has emerged as a new and promising strategy for wound healing because autograft implantation is associated with limitations, such as the occurrence of infection, pain, slow healing at the donor site, scarring, and major skin loss. It must have appropriate physical, biological, and mechanical properties for nutrient and gas exchange, just like scaffolds for another tissue engineering [67]. An example of skin tissue engineering is the regeneration of the skin layer by culturing fibroblast cells on polymeric scaffolds that mimic ECM to accelerate the healing of skin wounds [68]. Park *et al*. developed a skin substitute using 3D electrospun silk fibroin nanofiber scaffolds. NIH 3T3 fibroblast cells were successfully cultured in silk fibroin scaffolds, and the metabolic activity of the cells within the pore structure of the scaffold increased significantly from day 1 to day 14. The salt leaching technique was also used in combination with electrospinning to artificially create larger pores for better cell adhesion and

nutrient and gas exchange [28]. Thus, the scaffold produced by the salt leaching technique has great potential for tissue engineering of the skin.

FUTURE PROSPECT

Future research should focus on modifying the salt leaching technique to improve the physical properties of the scaffold for better application or efficiency in tissue engineering. As mentioned earlier, mechanical strength, pore size, porosity, and interconnectivity are important properties of the scaffold to provide a desirable platform for tissue engineering. According to Cheng *et al*., the incorporation of two-dimensional nanomaterials into silk fibroin-based scaffolds enables the delivery of chondrogenic growth factors to the target site of cartilage regeneration [69].

In addition, further studies should be conducted to completely remove the organic solvent from the scaffold without affecting the pore architecture of the scaffold. Reportedly, one of the alternative methods to remove residual solvent from biodegradable polymers is carbon dioxide extraction. However, this method had an impact on the pore architecture of the scaffold matrix [70]. Future research needs to address the potential toxicity of the remaining organic solvent without affecting the physical and mechanical properties of the scaffold by broadening the choice of polymers and solvents.

CONCLUDING REMARK

The purpose of this chapter was to describe how particulate leaching (salt leaching) is used to prepare biomaterials for tissue engineering, as well as new discoveries in the methods and polymers used. The salt leaching method is undoubtedly the most widely used and relatively simple method for preparing scaffolds for tissue engineering applications such as bone regeneration, skin replacement, and neural retinal progenitor cells. However, because this method uses organic solvents, the risk of these scaffolds being toxic due to solvent residues must be carefully considered. For example, the solvents (hexafluoroacetone and hexafluoroisopropanol) used to dissolve PGA are both extremely toxic to cells. Many of the examples discussed previously, which were from recent studies, lacked an assessment of toxicity. Therefore, before further recommendations can be made regarding their potential uses and applications in tissue engineering, scaffolds prepared using salt leaching techniques need to be evaluated for toxicology, safety risks, and potential effects on damaged tissue.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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