Lecture 22-23

BT 636
Tissue Engineering and Regenerative Medicine
(3-0-0-6)

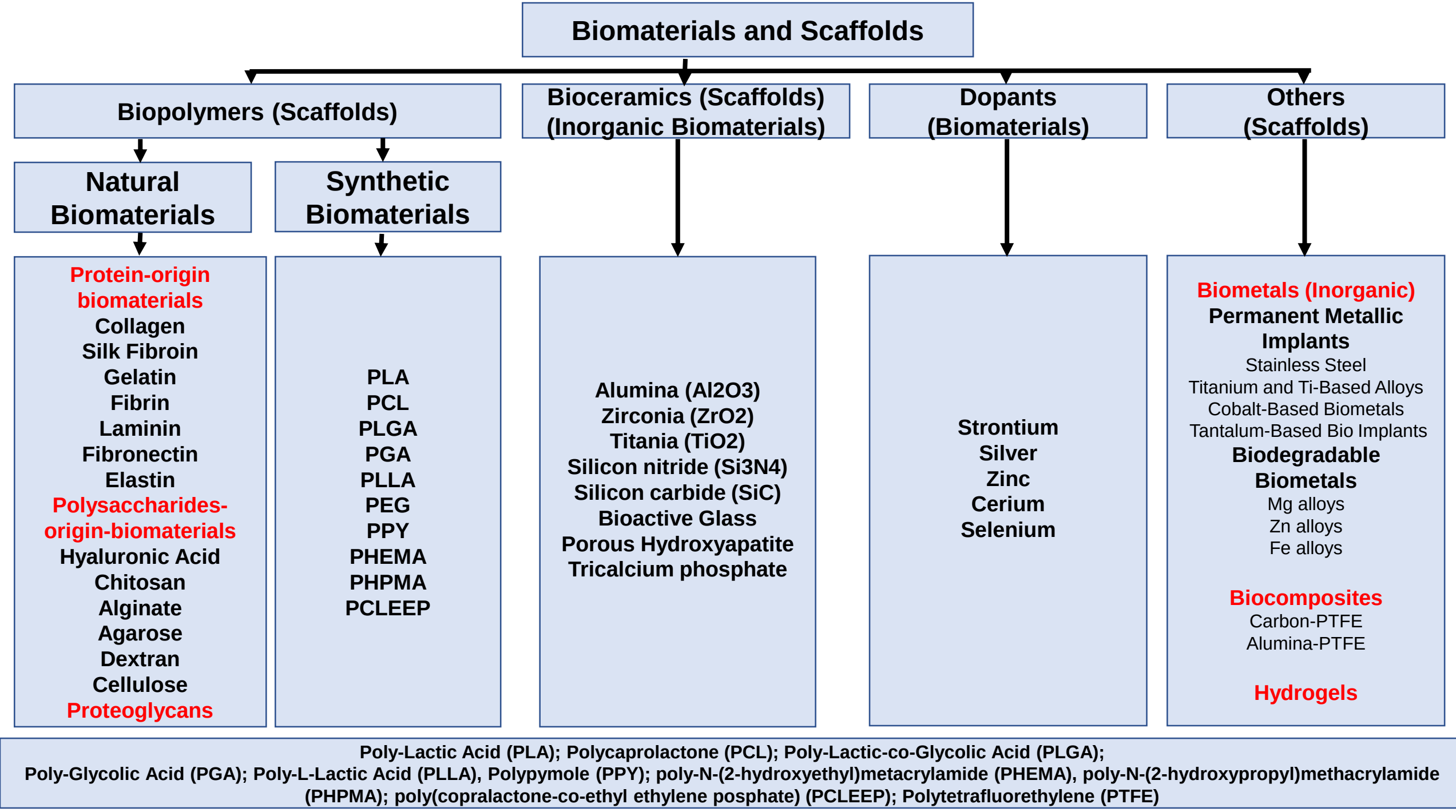
Rajkumar P. Thummer
"O" Block – Room 006; BSBE
Phone: 3208;
Email: rthu@iitg.ac.in

Dr. Rajkumar P Thummer
Assistant Professor
Department of Biosciences and Bioengineering
IIT Guwahati
Guwahati

Scaffolds

- Scaffolds are materials that have been engineered to cause **desirable cellular interactions** to contribute to the formation of new functional tissues for biomedical applications such as tissue engineering and regenerative medicine.

- Cells are often “seeded” into these scaffold structures alongwith growth factors capable of supporting 3D tissue formation.



Poly-Lactic Acid (PLA); Polycaprolactone (PCL); Poly-Lactic-co-Glycolic Acid (PLGA); Poly-Glycolic Acid (PGA); Poly-L-Lactic Acid (PLLA), Polypymole (PPY); poly-N-(2-hydroxyethyl)metacrylamide (PHEMA), poly-N-(2-hydroxypropyl)methacrylamide (PHPMA); poly(copralactone-co-ethyl ethylene posphate) (PCLEEP); Polytetrafluorethylene (PTFE)

Scaffold fabrication techniques

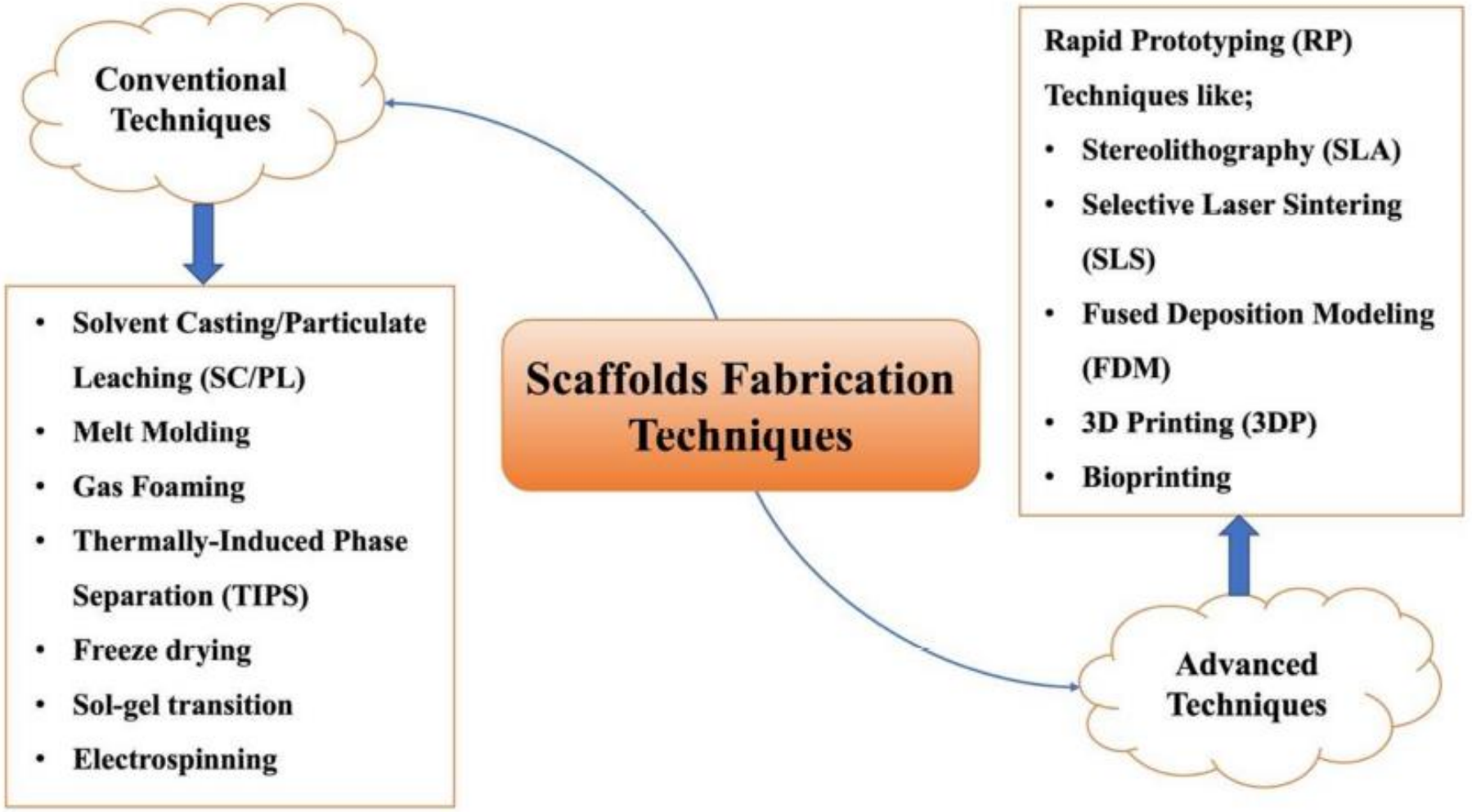


Figure 4. Classification of the numerous techniques that can be used in scaffolds fabrication into conventional and advanced techniques challenges and benefits of any of the mentioned techniques should be addressed prior to the scaffold fabrication to maximize patients' benefits.

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Review

Conventional and Recent Trends of Scaffolds Fabrication: A Superior Mode for Tissue Engineering

Islam M. Adel *, Mohamed F. ElMeligy and Nermeen A. Elkasabgy

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Types of Scaffolds

- Different fabrication techniques produce scaffolds of different characteristics. This mandates that the selection of the technique is made according to the tissue site and the required properties.

- Saying so, there are various types of scaffolds to be highlighted.

  - **Nanofibrous Scaffolds (NFs)** [3D porous matrix or Nanofiber(fibrous) mesh(matrix)]
  - **Hydrogel-Based Scaffolds**
  - **Microsphere-Based Scaffolds**

Nanofibrous Scaffolds (NFs)

- **Elongated, fiber-like scaffolds with very large length (width at the nano range) are referred to as 'nanofibrous scaffolds'.**

- There are many techniques used to prepare NFs, including phase separation, self-assembly, template synthesis, melt molding, etc.

- Each method has its limitations.

- For example, phase separation yields fibers that lack structural stability, template synthesis is a time-consuming process, and self-assembly forces narrow biomaterial choices.

- The most suited technique used in NFs fabrication is electrospinning. Electrospinning allows the production of NFs with high porosity, high interconnectivity, and with large surface area as well as for the precise control over NFs structure and diameter.

- Various biomaterials are suited for NFs manufacturing using the electrospinning technique. These include natural polymers such as collagen and CS, synthetic polymers such as PCL, PLA and PGLA, and biocomposites such as CS/ PCL and CS/SF.

Hydrogel-Based Scaffolds

- Hydrogels, being injectable, 3D printed, or porous, refer to networks formed through chemical or physical crosslinking between hydrophilic polymers, either of natural or synthetic origins.

- Generally, chemically crosslinked hydrogels are more favorable as they offer better mechanical resistance and wider control over scaffold properties than physically crosslinked ones.

- **A key advantage in hydrogel scaffolds over other types of scaffolds lies in their swelling ability when subjected to an aqueous environment, as the case within living tissues, rendering them perfect replicas of normal ECM.** Perhaps the reason for this has to do with the hydrophilic nature imparted by hydrophilic groups (carboxyl, hydroxyl, amine, etc.) present within their structure that facilitates interaction with water molecules in the surrounding environment.

- Hydrogels can act as support networks in tissue engineering, drug carriers, and release mediators, as well as being accelerators of wound healing through retention of nutrients and promotion of angiogenesis.

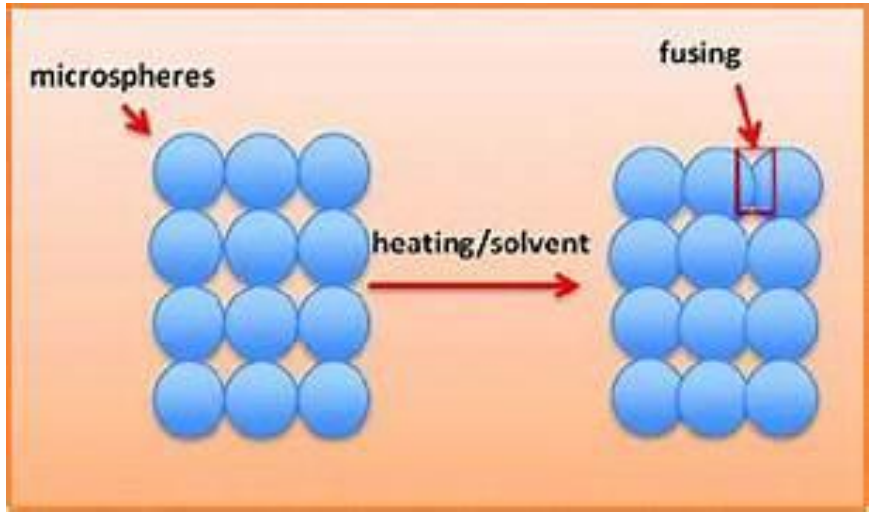
- Hydrogels can be prepared via Solvent Casting (SC), gas foaming, freeze-drying, electrospinning, Three-Dimensional Printing (3DP), etc., and biomaterials used can be natural such as chitosan, gelatin, and Hyaluronic Acid (HA) or synthetic such as PLA, polyvinyl alcohol (PVA), and PEG derivatives.

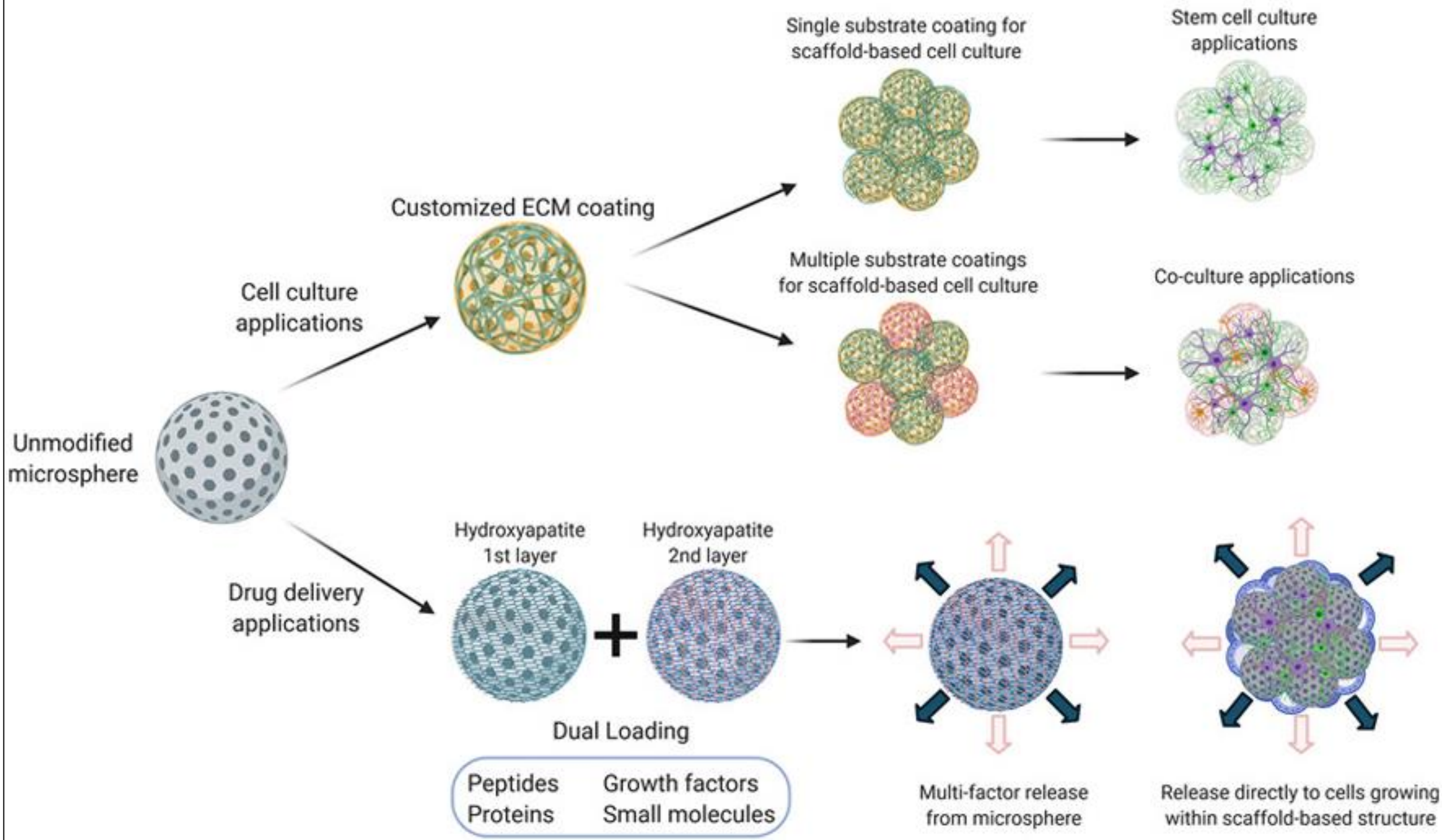
Microsphere-Based Scaffolds

- As the name suggests, **microspheres are free-flowing particles in the micron range (1–1000 μm) capable of encapsulating bioactive molecules and releasing them in a controlled manner**.

- In microsphere-based scaffolds, microspheres serve as the building blocks of the scaffold. This is due to the feasibility by which microspheres can be packed with, either alone or with other biomaterials, to form 3D matrices.

- They can be classified as either injectable or sintered (the process of compacting and forming a solid mass of material by pressure or heat without melting it) scaffolds and are usually prepared by Rapid Prototyping (RP) techniques (such as SLS), emulsion solvent extraction method, TIPS, subcritical CO2 sintering, etc., with biomaterials such as chitosan, collagen, PLGA, alginates, etc., being used in the fabrication process.




Single substrate coating for scaffold-based cell culture
Stem cell culture applications
Customized ECM coating
Cell culture applications
Multiple substrate coatings for scaffold-based cell culture
Co-culture applications
Unmodified microsphere
Hydroxyapatite 1st layer
Hydroxyapatite 2nd layer
Drug delivery applications
Dual Loading
Peptides
Growth factors
Proteins
Small molecules
Multi-factor release from microsphere
Release directly to cells growing within scaffold-based structure

Scaffold fabrication techniques

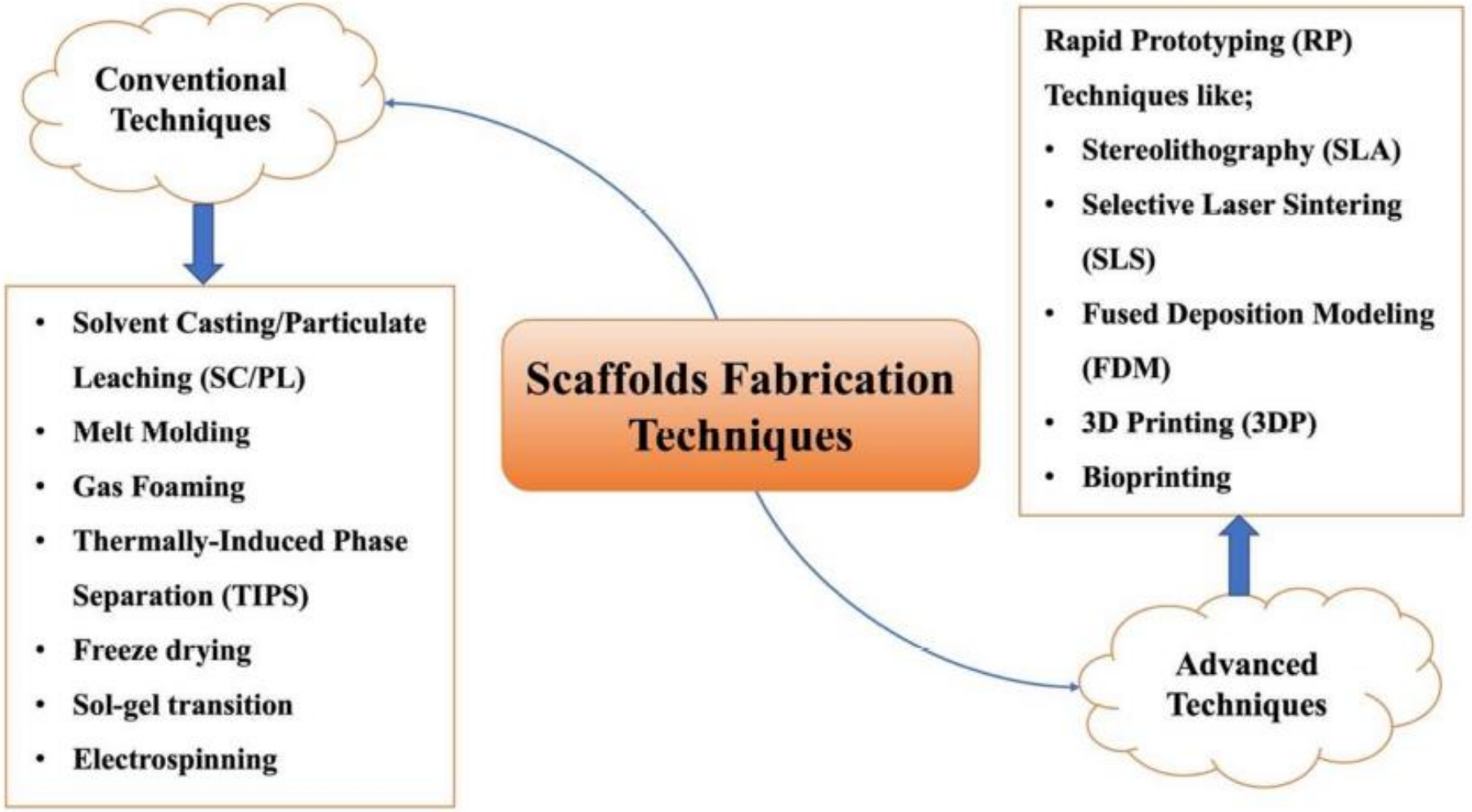


Figure 4. Classification of the numerous techniques that can be used in scaffolds fabrication into conventional and advanced techniques challenges and benefits of any of the mentioned techniques should be addressed prior to the scaffold fabrication to maximize patients' benefits.

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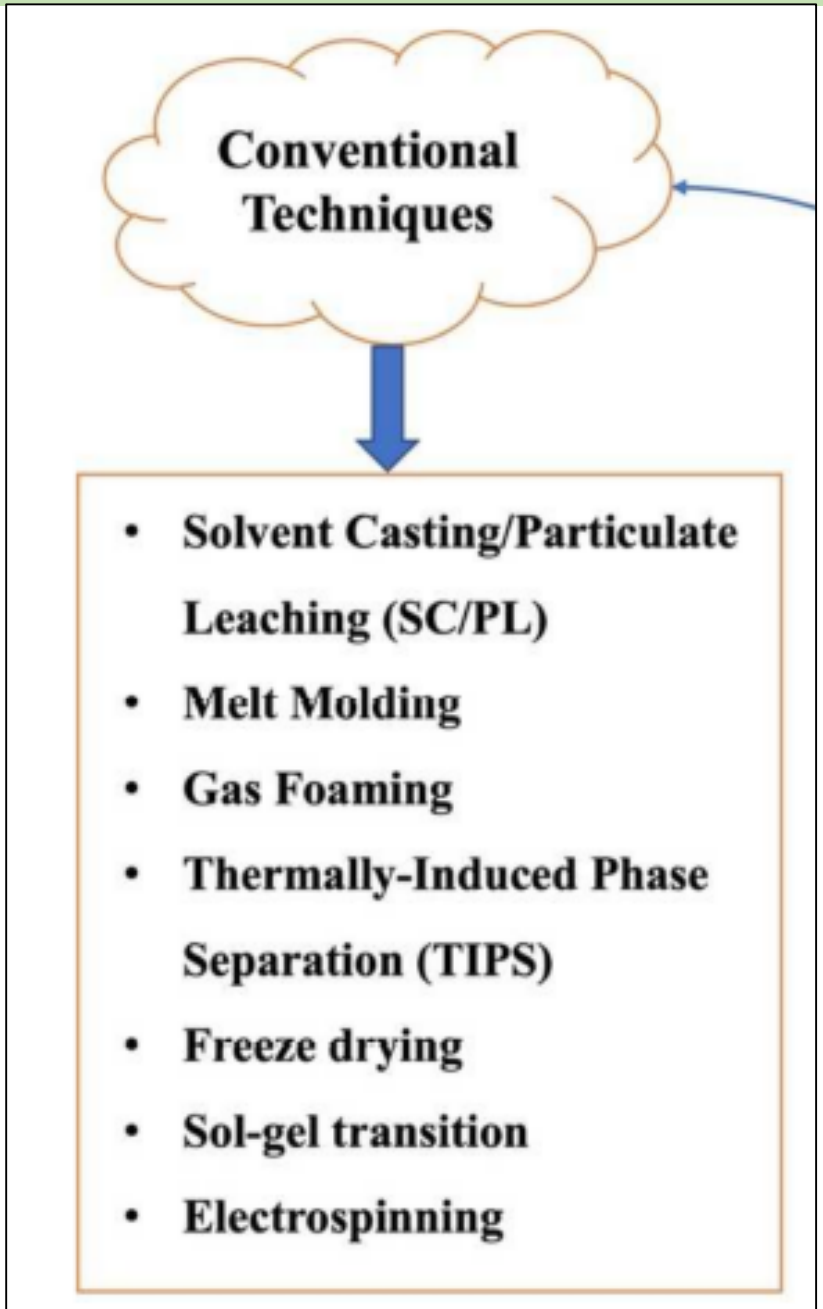
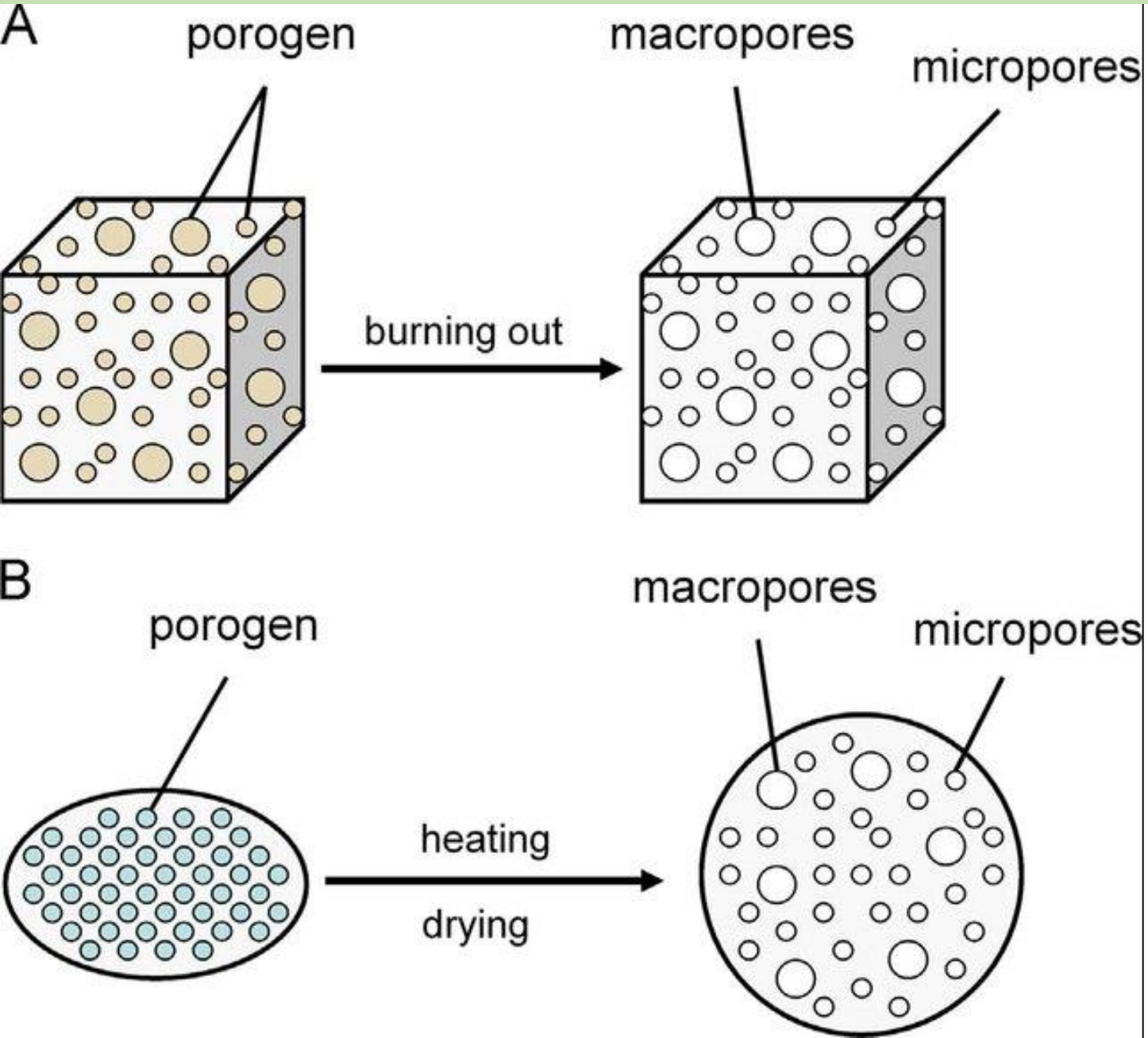
Conventional Scaffold fabrication techniques



Porogen

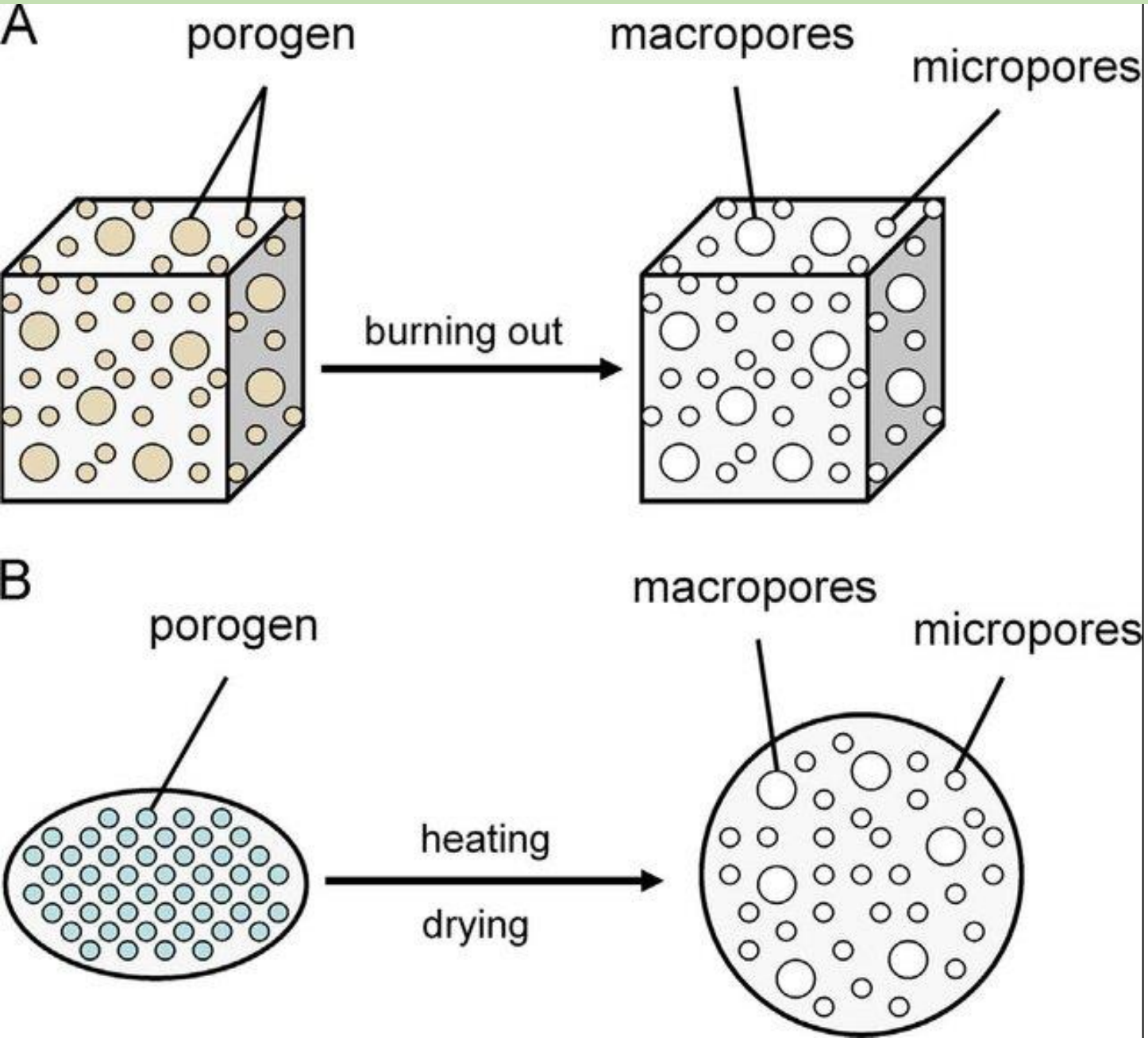


The most common methods of scaffold production involve:

- **Burning out the porogen (pore-forming agent)** – a mixture of Si-HA and porogen is ground in a mill, pressed and sintered at high temperature; the porogen burns out creating pores with sizes dependent on the degree of substrate fineness (see Fig. A)].
- **Addition of a foaming agent** – Si-HA is suspended in a porogen, which decomposes at high temperature to gaseous species; the foamed suspension is then dried (see Fig. B).

- **Rapid prototyping with 3D printing** – enables precise design of various shapes and sizes of scaffolds containing well-interconnected porous systems to enable effective bone ingrowth and angiogenesis.
- **Pores are classified as per IUPAC regulations: micropore (<2 nm), mesopore (2–50 nm) and macropore (>50 nm).**
- **The terminology “porogens” and “pore formers” refers to a material additive that has the ability to disperse in the feed composition and may leach out after the fabrication stage.**

Types and examples of Porogens



Porogens are generally used to prepare the porous support, verified by theory and practice, and the major division is organic and inorganic.

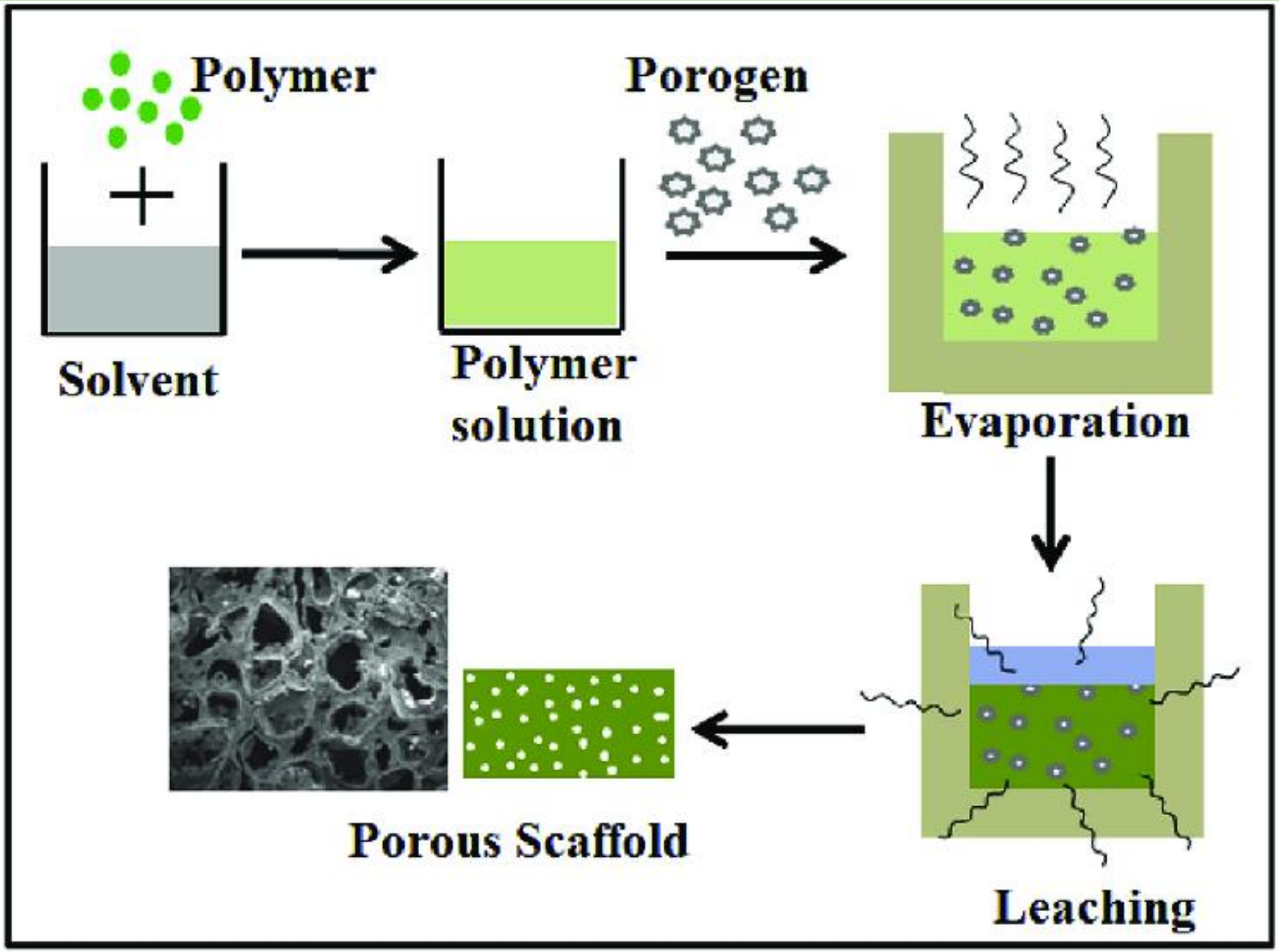
Examples of inorganic porogens are ammonium carbonate, calcium carbonate, ammonium bicarbonate and ammonium chloride, etc.,

Examples of organic porogens are sawdust, shell powder, starch, polystyrene, water-soluble polymers such as Poly ethylene glycol (PEG), Polyvinyl pyrrolidone (PVP), Polyvinylalcohol (PVA), Polymethaacrylate (PMA), Polyacrylicacid (PAA), etc.

Nowadays, bio/green porogens are attracting the attention of researchers.
Activated carbon from various sources, moringa seed powder and marine-derived polymers such as chitosan, κ- carrageenan, alginate and ulvan have been employed as porogens in recent studies.

In addition, green seaweed-derived sulphated polysaccharides have proven to be superior porogens compared to the others, especially when used in minimum quantities.

Solvent Casting and Particulate Leaching (SC/PL)



Solvent Casting and Particulate Leaching (SC/PL)

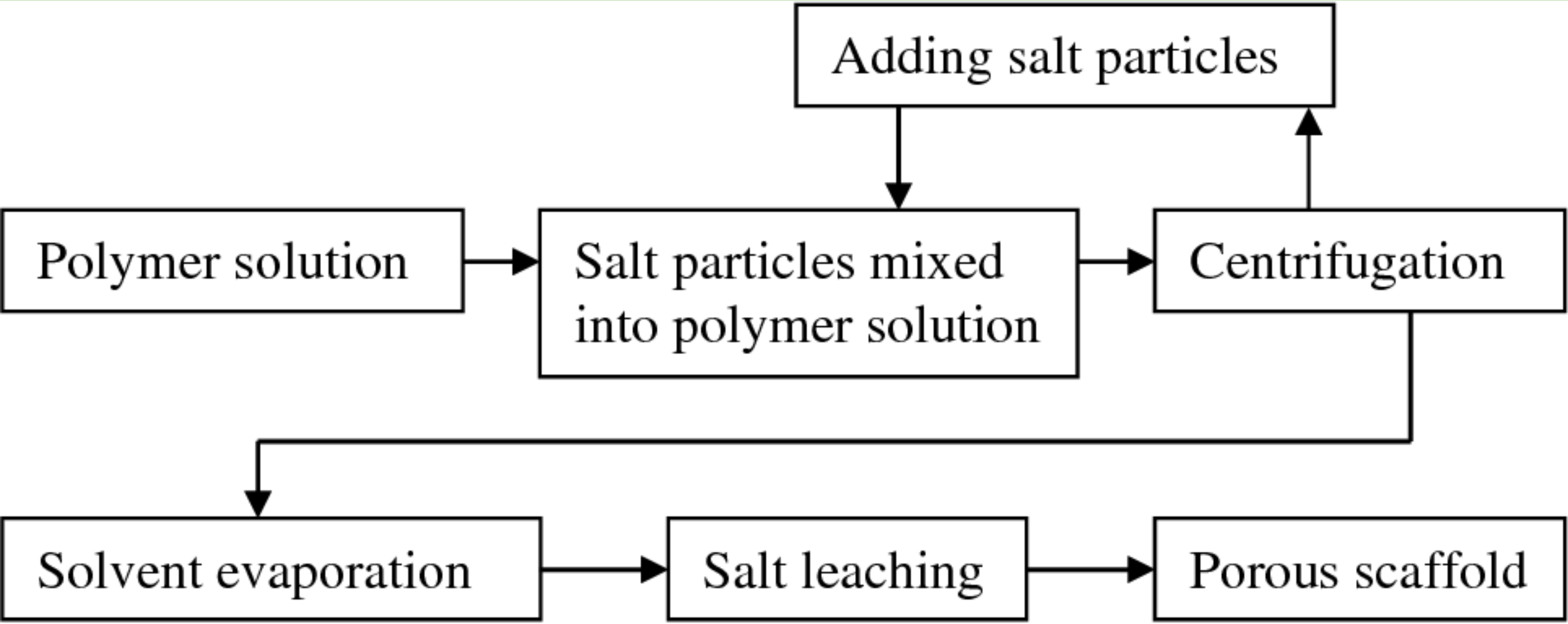
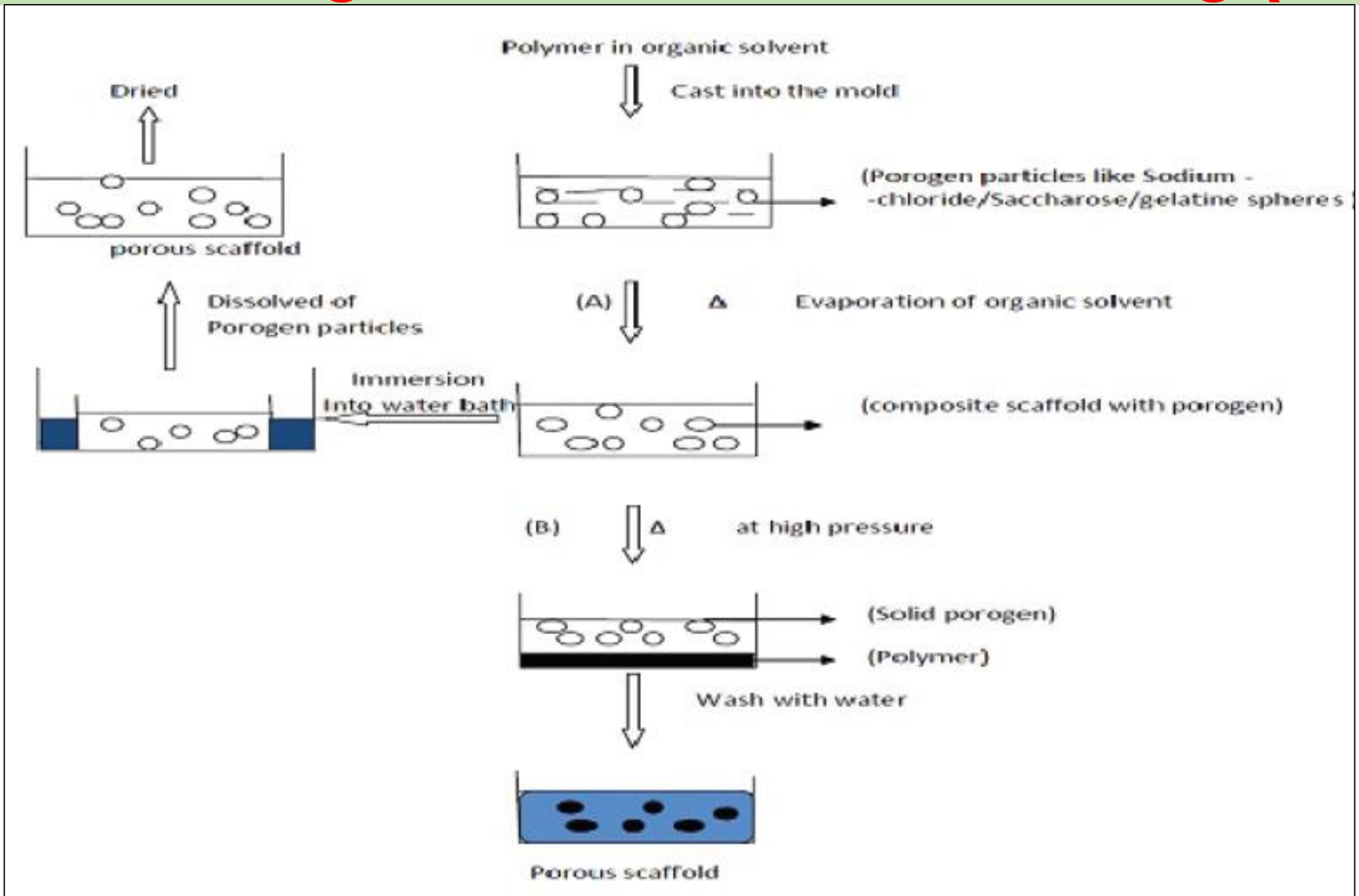


Fig. 1. Steps of the enhanced SCPL method.

Leaching is a process that involves the extraction of soluble substances from a solid by means of a liquid solvent.

Solvent Casting and Particulate Leaching (SC/PL)



Solvent Casting and Particulate Leaching (SC/PL)

- In this technique, the polymer is dissolved in a volatile solvent where a porogen is uniformly distributed. Then, the solvent is evaporated, resulting in the formation of a polymer network in which the porogen is entrapped.
- Finally, the porogen is leached by immersing the scaffolds in a suitable porogen solvent, leaving pores within the matrix. Usually, the used porogens are either organic compounds such as gelatin and collagen or water-soluble inorganic salts such as NH4Cl.

- SC/PL is a simple, reproducible procedure that produces scaffolds with a high degree of porosity as well as allows for fine adjustment of pore size through varying salt/polymer ratio, particle size, or shape of the used porogen.
- However, the residual solvent and/or porogen content should be taken into consideration. The limited mechanical properties, the lack of control over interconnectivity, the long processing time, and the production of thin films are other drawbacks of the method.

- A modified SC/PL was attempted using poly(Ɛ-caprolactone) (PCL) as the sole polymer as well as NaCl and PEG as dual porogen to be used in bone TE. The resulted scaffolds were highly porous with suitable water absorption capacities typical of SC/PL. The added PEG, however, resulted in uniform pore size and high interconnectivity. The optimized scaffolds produced significant bone ingrowth when tested in vitro in mice, calvaria-derived, pre-osteoblastic cells (MC3T3-E1).

Solvent Casting and Particulate Leaching (SC/PL)

- Particulate leaching methods are split into two categories:
  (1) solvent casting–particulate leaching, and (2) melt molding–particulate leaching.

- In solvent casting–particulate solvent is then evaporated to make a polymer monolith embedded with the salt particles, which are then removed by washing the scaffold with water, resulting in the formation of a porous scaffold.
- In melt molding–particulate leaching, the polymer is cast into a mold with the embedded solid porogen. The polymer is set by applying heat and pressure, and again the porogen is leached away by washing the resulting product with water to yield a porous polymer scaffold.

- The solvent casting and particulate leaching method, first developed by Mikos et al. was used primarily to manufacture composite scaffolds later the method found applications in the creation of porous scaffolds for the growth of endothelial cells. PLGA, poly(lactic acid) (PLA), collagen, poly(ortho ester), or small intestine submucosa–impregnated PLGA scaffolds have been fabricated successfully into a biodegradeable sponge structure with more than 93% porosity and a desired pore size of 1000 μm.

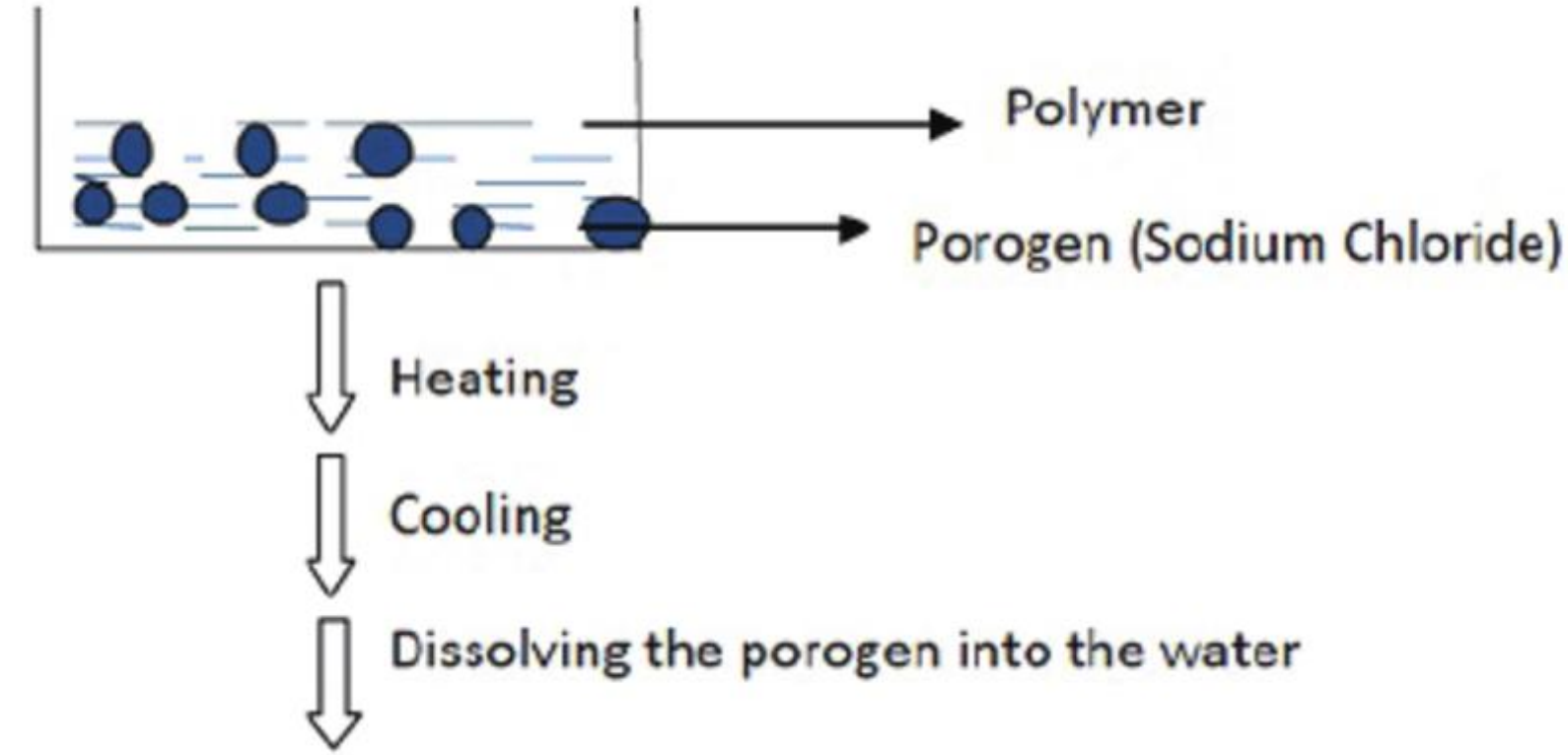
Solvent Casting and Particulate Leaching (SC/PL)

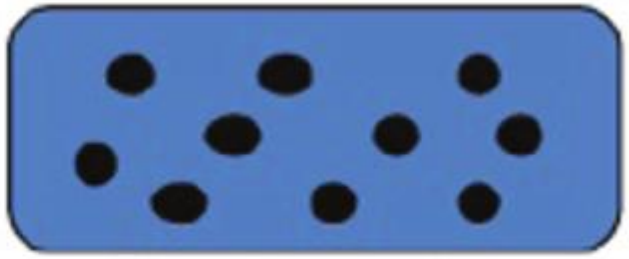
- The advantages of the solvent casting method are that it is a simple and fairly reproducible and does not require sophisticated apparatus.
- A highly porous (up to 93%) scaffold of pore diameters up to 500 μm with ease of control of porosity and geometry can be prepared using this technique.
- The pore size can be controlled by controlling the amount of porogen added and the size and shape of the porogen.
- Complex geometries such as tube, nose, and specific organ types can be fabricated as nanocomposite hybrid scaffolds.
- Advantages include large range of pore sizes, independent control of porosity and pore size, ability to tailor crystallinity, and highly porous structures.

Solvent Casting and Particulate Leaching (SC/PL)

- Nevertheless, this method suffers from major drawbacks because of the long period of soaking in water required to leach all of the salt particles. This leaching period is particularly detrimental to the manufacture of controlled release scaffolds because a high percentage of the drug payload can be lost.
- The method also produces thin membranes with a dense surface skin layer that might contain residual salt particles used during the process.
- Efforts have resulted in the production of thin scaffolds with open-cell morphology with high porosity.
- The other disadvantages include limited membrane thickness (3 mm), limited mechanical properties, the presence of residual porogen and solvents, limited interconnectivity, and poor control over internal architecture. If an organic solvent is used and is not completely removed, it can damage the cells seeded on the scaffold.
- Efforts have been made to overcome the problem of thin membrane formation and to prepare a thick 3D scaffold using PLLA or PLGA porous membranes laminated into multilayer structures with various shapes.

Melt Molding





Porous Scaffold

Melt Molding

- Melt molding is a straightforward conventional technique based on the use of thermoplastic polymers.

- The polymer is first melted then casted in a mold of suitable 3D structure according to the defect tissue. Porosity could be introduced via merging melt molding with other methods such as particulate leaching, gas foaming, phase separation, etc., and finally, the scaffolds are usually freeze-dried.

- The method is simple, allows individual control over morphological characteristics such as pore size and interconnectivity, and avoids the use of organic solvents. However, the elevated temperatures used in the melting might not be suitable for every component such as thermolabile drugs.
- Moreover, there is the problem of residual porogen if used to induce porosity.

- An example of the method can be found in the work carried out by Oh et al.
- PLGA/PVA-based porous scaffolds, cultured with chondrocytes, were fabricated using melt molding then implanted in skull defects of rabbits to be evaluated for their bone ingrowth capabilities.
- The PVA-treated scaffolds showed better bone ingrowth activity as compared to the non-PVA-treated scaffolds, as implied by MTT assay and SEM findings.
- The improvement in bony tissue growth was attributed to the increased hydrophilicity of the PVA-treated scaffolds and hence, better cytocompatibility.

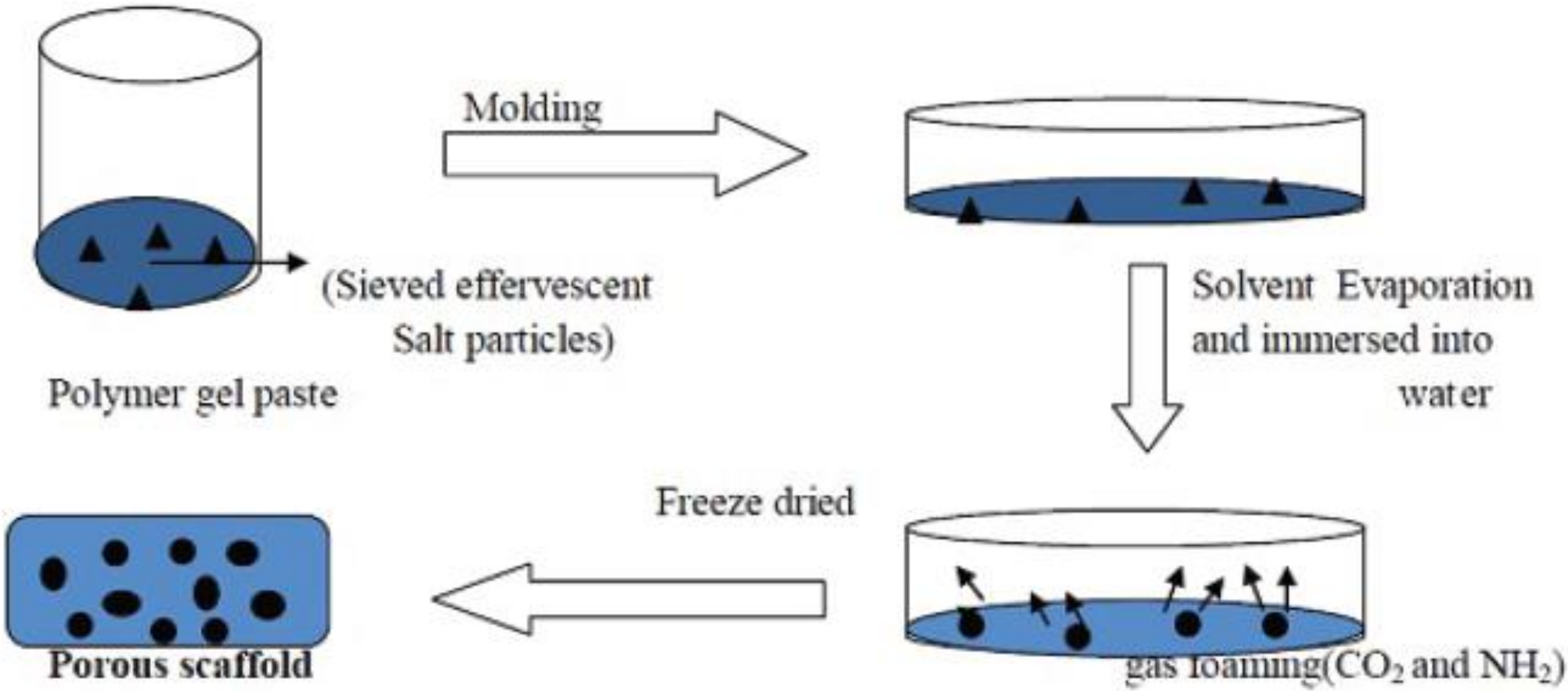
Melt Molding

- Scaffolds are prepared by melting polymers/ceramics in the presence of porogens (such as sodium chloride and sugar crystals); once the mixture has cooled, porosity is achieved by dissolving the porogens in water.
- Finally, the porous scaffolds are usually lyophilized used with PLGA and gelatin microspheres of a specific diameter; the mold was heated above the glass-transition temperature of PLGA while pressure was applied to the mixture. This treatment causes the PLGA particles to bond together.
- Once the mold is removed, the gelatin component is leached out by immersion in water, and the scaffold is then dried.

- The advantage of this technique is independent control of porosity and pore size, macroshape control, and pore interconnectivity and geometry, which is important for the exchange of nutrients/waste from pore to pore.

- The disadvantage is the high temperature required for nonamorphous polymers and residual porogens.

Gas foaming


Molding
(Sieved effervescent
Salt particles)
Polymer gel paste
Solvent Evaporation
and immersed into
water
Freeze dried
Porous scaffold
gas foaming(CO_2 and NH_2)

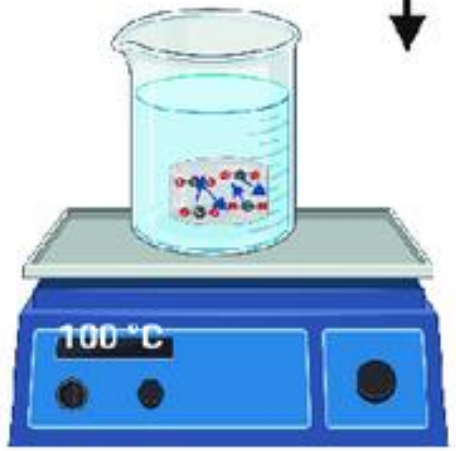
Gas foaming

Polymeric solution

Solvent evaporation

Saturation with gas in water filled tank

Gas foaming in hot water



Freeze drying

Porous scaffold

Processing parameters

1- Temperature
2- Pressure
3- Depressurization rate
4- Polymer concentration

Gas foaming

- A “gas foaming” method was developed by Nam et al using an effervescent salt as a gas foaming agent. Sieved effervescent salt particles (ammonium bicarbonate) in the form of a polymer gel paste was cast in a mold and subsequently immersed in hot water.
- The evolution of ammonia and carbon dioxide gas, along with the leaching out of ammonium bicarbonate particulates from the solidifying polymer matrix, resulted in the formation of pores with high interconnectivity.
- Foaming techniques use gaseous porogens that are produced by chemical reactions during polymerization or are generated by the escape of gases during a temperature increase or drop in pressure; this causes a decrease in solubility of the carbon dioxide within the polymer, and as the carbon dioxide gas tries to escape it causes the nucleation and growth of bubbles, resulting in a porous microstructure.

Gas foaming

- Nam et al. synthesised PLA scaffolds using ammonium bicarbonate, which acted as both a gas foaming agent and a solid salt porogen. The gas foaming/salt leaching method was further improved for preparing porous PLGA scaffolds by adding another salt, citric acid, into the aqueous solution. The porosity and mechanical strength could be controlled by adjusting the extent of an acid–base gas evolving reaction between the two salts. The gas foaming process is used to fabricate highly porous foams without the use of organic solvents. Organic solvents may leave residues behind that can have toxic effects in vitro and may cause inflammation in vivo. Gas foaming yields high porosities (up to 93%) and varying the temperature, pressure, and rates of parameter reductions can modulate pore sizes.
- The significant advantage is no loss of bioactive molecules in the scaffold matrix, given that there is no need for the leaching process and no residual organic solvent. Porosities as high as 90% with pore sizes from 200 to 500 μm are attained using this technique.
- The disadvantage of this technique is the presence of skimming film layers on the scaffold surface, resulting in the process to remove this skin layer, and poor interconnectivity of the porosity.

Gas foaming

- In the conventional gas foaming technique, two approaches can be followed. The difference between both approaches depends on the method of introducing the gas. The blowing gas is either generated in situ (chemical blowing agent) or directly blown (physical blowing agent) in a polymer or a polymer-surfactant solution. On gas evolution, it leaves voids in its place, forming a porous polymeric matrix. Gases as nitrogen (N2) and carbon dioxide (CO2) are used in the first approach, while pressurized gases such as methane/hydrogen mix (CH4/H2) are used in the second one. Surfactants are present to aid in the formation and stabilization of the resultant foam.
- It is apparent that gas foaming avoids the use of organic solvents; hence, hazards related to residual solvents are eliminated. Other advantages of the technique include obtaining highly porous scaffolds, minimal loss of encapsulated bioactive species, mild production conditions, and suitability for both hydrophobic and hydrophilic polymers. However, poor interconnectivity, lack of precise control over pore size, and long operating times are serious limitations to the method. It is worth mentioning that efforts are made to offer precise control over pore sizes and interconnectivity.
- Examples include manipulating temperature and pressure within the apparatus vessel, polymer type/concentration, using gas/organic solvent mixtures as well as applying microfluidics in an advanced gas foaming technique.
- The anticancer gemcitabine was impregnated in foam scaffolds fabricated using PLGA as a polymer and supercritical CO2 as the pressurizing gas where the scaffolds possessed very high impregnation efficiency (>90%).

Thermally Induced Phase Separation (TIPS)

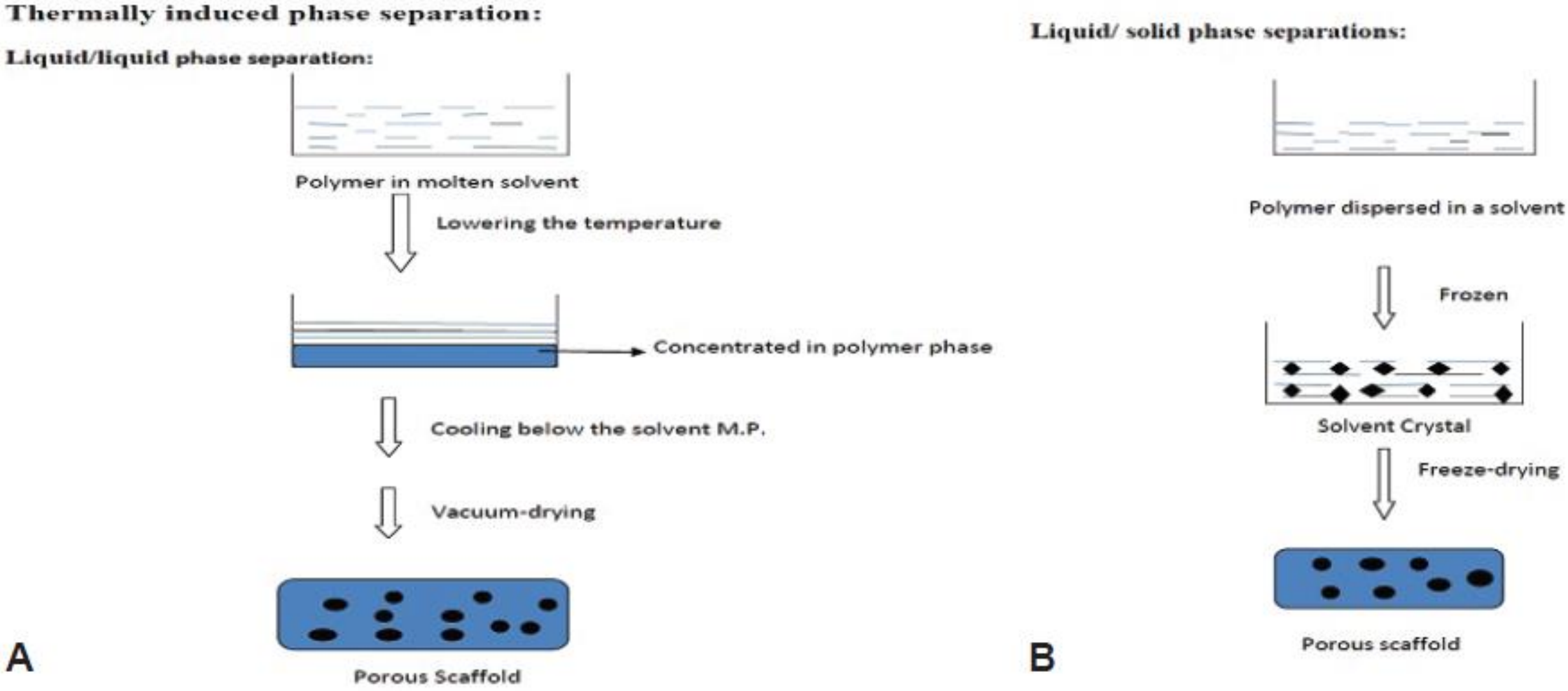


Figure 11. (A) Fabrication of scaffold using liquid–liquid phase separations. (B) Fabrication of scaffold using liquid–solid phase separations.

Thermally Induced Phase Separation (TIPS)

- Thermally induced phase separation (TIPS) was first applied to PLA scaffolds by Schugens et al, and later several other researchers applied this technique to prepare composite scaffolds. It consists of inducing a solid–liquid or liquid–liquid phase separation.
- **This is done by dissolving the polymer in a solvent and quenching the solution at a certain temperature. The quenching induces a phase separation into a polymer-rich phase and a polymer-poor phase. In particular, TIPS uses thermal energy as the latent solvent to induce phase separation. The solvent must then be removed from the phase-separated solutions either by freeze-drying or solvent extraction. The solvent leaves behind microstructural foam.**
- **The main advantage of the phase separation method is that pore morphology and orientation can be tailored by altering the thermodynamic and kinetic parameters of the processing, and it is a highly porous structure and permits incorporations of bioactive agents (hydrophilic or hydrophobic).** It can combine easily with other fabrication technology (particulate leaching) to design 3D structures with controlled pore morphology.
- It can be combined with rapid prototyping to create nanofibrous scaffolds for tissue engineering applications.
- **Its disadvantages include the use of potentially toxic solvents, poor control over internal architecture, and limited range of pore sizes.** The latter disadvantage, however, actually may be beneficial for certain biomedical and industrial applications such as nerve regeneration, filtration membranes, mechanically damping materials, and packaging.
- Nam and Park used the TIPS technique to obtain scaffolds with a macroporous structure with an open cellular morphology. The coarsening process was used to increase the size of phase-separated droplets, thus enlarging the pores (~100 μm). The resultant scaffolds also had pores with a uniform size distribution and porosity of more than 90%.

Thermally Induced Phase Separation (TIPS)

- The phase separation method is divided into freeze drying, freeze thawing, freeze immersion precipitation, and emulsion freeze drying. Phase separation by freeze drying can be induced by a polymer solution with an appropriate concentration by rapid freezing.
- The solvent used is then removed by freeze drying, resulting in a porous structure as a portion of the solvent. Collagen scaffolds with varying pore sizes were developed using a collagen–glycosaminoglycan blend (90–120 μm) and chitosan scaffolds (1–250 μm) by varying the freezing condition. In addition, scaffold structures of synthetic polymers such as PLA and PLGA have been made successfully using this method, with more than 90% porosity and 15- to 250-μm sizes. The freeze thawing technique induces phase separation between a solvent and a hydrophilic monomer upon freezing, followed by the polymerization of the hydrophilic monomer by means of ultraviolet irradiation and removal of the solvent by thawing. This leads to the formation of macroporous hydrogel.
- The freeze immersion precipitation method is quite similar to the freeze thawing technique except the polymer solution is first cooled before being immersed in a nonsolvent and then the solvent is vaporized to form a porous scaffold structure. The emulsion freeze drying method is also useful for the fabrication of porous structures.
- In this case, a mixture of polymer solution and nonsolvent are thoroughly sonicated, quickly frozen in liquid nitrogen at –198°C, and then freeze dried.
- Formation of sponge-structured injectable gel scaffolds also have been reported.
- Injectable, gel forming scaffolds provide several advantages such as the following:
    - 1. They can fill any shape of defect because of their good flow property
    - 2. Loading capacity to various types of bioactive molecules and cells by simple mixing
    - 3. They do not contain residual solvents that may be present in a performed scaffold
    - 4. They do not require surgical procedure for placement

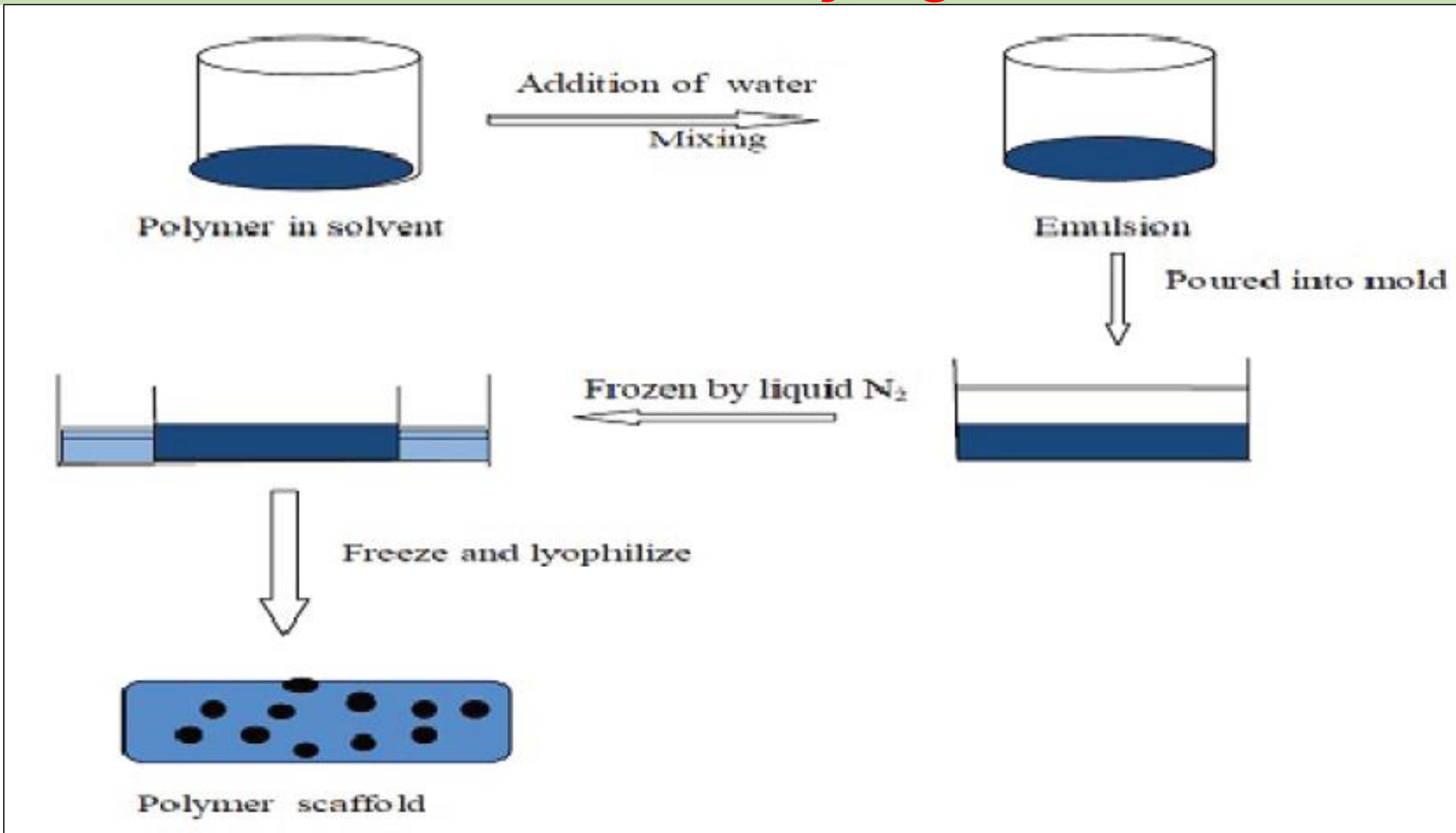
Thermally Induced Phase Separation (TIPS)

- The core principle of TIPS is the conversion of a stable polymer system into a thermodynamically unstable one so that it is separated into two phases: a polymer-rich phase and a polymer-deficit one. Briefly, a pre-heated polymer solution is exposed to low temperatures. Low temperature acts as a trigger that converts the system into the thermodynamically unstable state, inducing phase separation. Later, the solvent is removed by sublimation, extraction, or any other technique where the polymer-rich phase forms the matrix and the polymer-poor one forms the pores within the matrix. Drugs incorporated are added to the polymer solution or with other miscible solvents prior to the solidification step.
- Since it relies on low temperatures and complete removal of solvent after solidification, TIPS is suitable for bioactive drugs, particularly heat-sensitive ones. It is worth mentioning that TIPS could be combined with other techniques such as SC/PL, electrospinning, 3D printing, etc.. The process is inexpensive, allows the fabrication of highly porous scaffolds (>95%) with high interconnectivity, as well as the feasibility by which morphological properties of the produced scaffolds can be tuned. This is possible through manipulation of the process parameters such as polymer type and concentration, solvent/non-solvent ratio, cooling rate, and presence of surfactant.

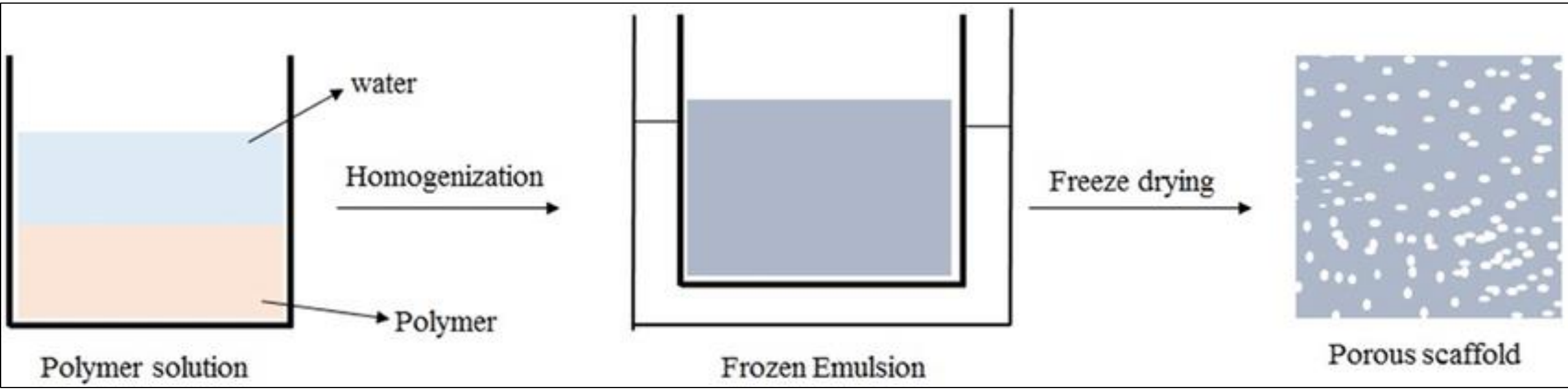
- A major setback of TIPS is the use of organic solvents that needs to be completely removed to avoid their hazardous outcomes.

- In a study conducted by Si et al., they prepared chitosan/collagen scaffolds using TIPS to be assessed in peripheral nerve regeneration. The prepared composites showed superior mechanical properties and reduced degradation rate when compared to the collagen-only scaffolds, while porosity and water uptake, despite being lower, remained within acceptable values. When the composite scaffolds were tested against Schwann cells, they showed enhanced cellular adhesion and proliferation while lacking any cytotoxicity.
- Their in vivo findings confirmed modulation of neural degradation behavior with the absence of inflammatory reaction suggesting their potential in nerve regeneration.

Freeze drying



Freeze drying



Freeze drying

- Freeze-drying can be used to produce scaffolds or to dry preformed constructs.
- The method involves four successive steps.
- Firstly, a polymer solution/dispersion, along with drugs and other additives, is first prepared. The used polymer could be dissolved in either organic solvent alone (non-emulsion-based freeze-drying) or in organic solvent-water emulsion (emulsion-based freeze-drying). Furthermore, water insoluble polymer could be instead suspended in water. Depending on the nature of the drug, it can be dissolved/suspended in either phase.
- Secondly, the solution/dispersion is mold-casted then frozen below its triple point (the temperature at which the three states of matter co-exist) by liquid nitrogen, refrigeration, etc.
- In the third stage, primary drying takes place to remove most of the formed ice crystals via sublimation.
- Finally, the remaining ice crystals are removed during the secondary drying phase.
- On sublimation, pores are formed in place of the previously formed crystals.

Freeze drying

- Freeze-drying has several advantages. It is a common method that produces completely or nearly dry, highly porous scaffolds with high interconnectivity. Pore sizes in the formed scaffolds could be controlled by altering the process parameters such as temperature, rate of drying, and polymer concentration. Restrictions to largescale scaffolding using freeze-drying are related to the high cost of operation and lengthy preparation times.

- Several studies succeeded in preparing freeze-dried scaffolds. Composite scaffolds, made up of keratin, fibrin, and gelatin, were fabricated using freeze-drying and used for the drug delivery of the antibiotic mupirocin. The fabricated scaffolds were evaluated as wound healing dressing. SEM micrographs confirmed high porosity of the resultant scaffolds (77%) while maintaining suitable mechanical properties, as evident by tensile strength testing. Cell line studies on NIH 3T3 fibroblasts and human keratinocytes (HaCaT) showed enhanced cellular adhesion and proliferation as compared to the control (untreated cells). Similarly, testing of antimicrobial activities against S. aureus and E. coli showed prominent zones of inhibition in the case of the medicated scaffolds.

Freeze drying

- The freeze-drying technique is use for fabrication of porous scaffolds. This technique is based upon the principle of sublimation (transition of a substance from the solid phase to the gaseous phase without changing into the liquid phase).

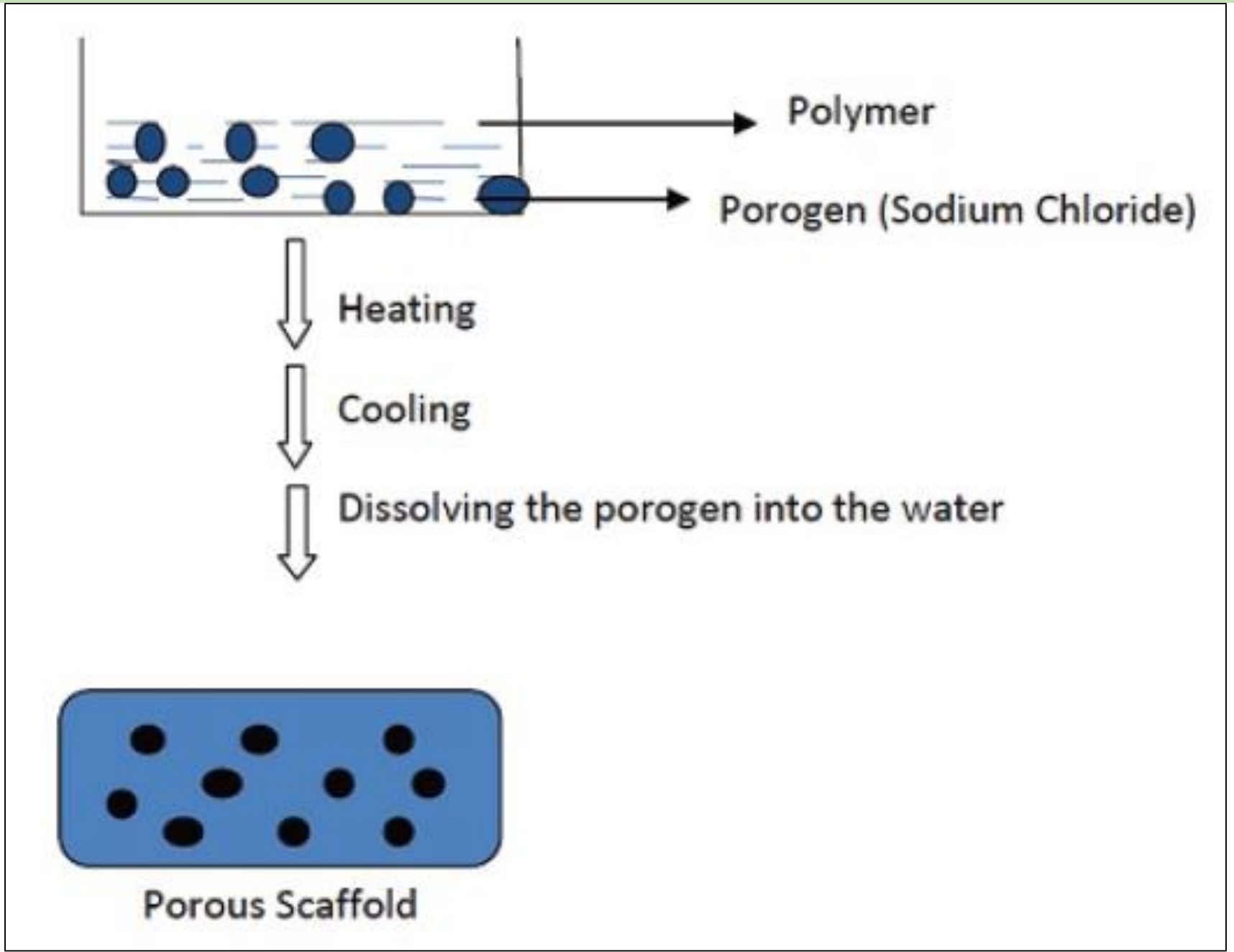
- Scaffolds are generally prepared by dissolving/suspending polymers/ceramics in water or in an organic solvent followed by emulsification with a water phase. After pouring this mixture into a mold, solvents are removed by freeze-drying and porous structures are obtained.

- Freeze-drying is conducted by freezing the material and then reducing the surrounding pressure by applying a vacuum and adding enough heat to allow the frozen water in the material to sublime directly from the solid phase to the gas phase. This technique is applied to a number of different polymers including silk proteins, PEG, poly-l-lactic acid (PLLA), PLGA/poly(propylene fumarate) blends.

- Emulsification/freeze-drying allows for faster preparation of highly porous structures with high pore interconnectivity. **The main advantage of this technique is that it requires neither high temperature nor a separate leaching step. Its disadvantages, however, are limited to small pore size (porosity is often irregular) and a long processing time. The pore size can be controlled by optimizing the freezing rate and pH; a fast freezing rate produces smaller pores.**

- Yannas et al. prepared collagen scaffolds by freezing a dispersion or solution of collagen and then freeze drying. Preparation of PLGA scaffolds of pore sizes of up to 200 μm with 95% porosity was reported by Whang et. al.

Sol-gel transition



Sol-gel transition

- This method is particularly useful in the fabrication of bioceramics and bioactive glasses.
- It consists of many steps, as follows.
- Inorganic or organic metal compounds are dispersed in water where hydrolysis and polycondensation take place, turning the system into a colloidal state. The formed system can be easily casted into the defined molds where 3D network formation and gelation start through various interactions between the components. The casted dispersions are allowed to dry in the mold. Gentle heating is applied to solidify the preformed matrix. Chemical stabilization or dehydration could be carried out to produce ultra-stable bioceramics.
- Attractions of the technique are summed in the low temperatures used and the chemical homogeneity of the ceramics. Moreover, it is an effective method for delayed drug delivery.
- However, the high cost of the raw materials and the long processing times are the main limitations to the process.
- β-TCP scaffolds were prepared using the sol-gel technique, where Pluronic F127 (a non-ionic surfactant) was used as a template. The microporosity increased with a fall in the sintering temperature, while a decline in Pluronic F127 concentration caused the increase in macropores sizes as well as the evolution of nanoscale grooves. Overall, immersion studies in simulated biological fluids showed the bioactivity of the scaffolds suggesting their potential use in bone tissue engineering.

Sol-gel transition

- Scaffolds are prepared by dissolving inorganic metal salts or metal organic compounds in a solvent, where a series of hydrolysis and polymerization reactions allow the formation of a colloidal suspension (sol); after casting the sol into a mold a wet gel is formed, and with further drying and heat treatment the gel is converted into dense ceramic or glass articles.

- **Sol-gel techniques have become popular recently because of their high chemical homogeneity, low processing temperatures, and the possibility of controlling the size and morphology of particles. The sol-gel–derived materials provide excellent matrices for a variety of organic and inorganic compounds.**

- One of the most important features of doped sol-gel materials is their ability to preserve chemical and physical properties of the dopants.

- The advantages of sol-gel technology can be used for construction of biomedical sensors, laser materials, or for delayed drug delivery.

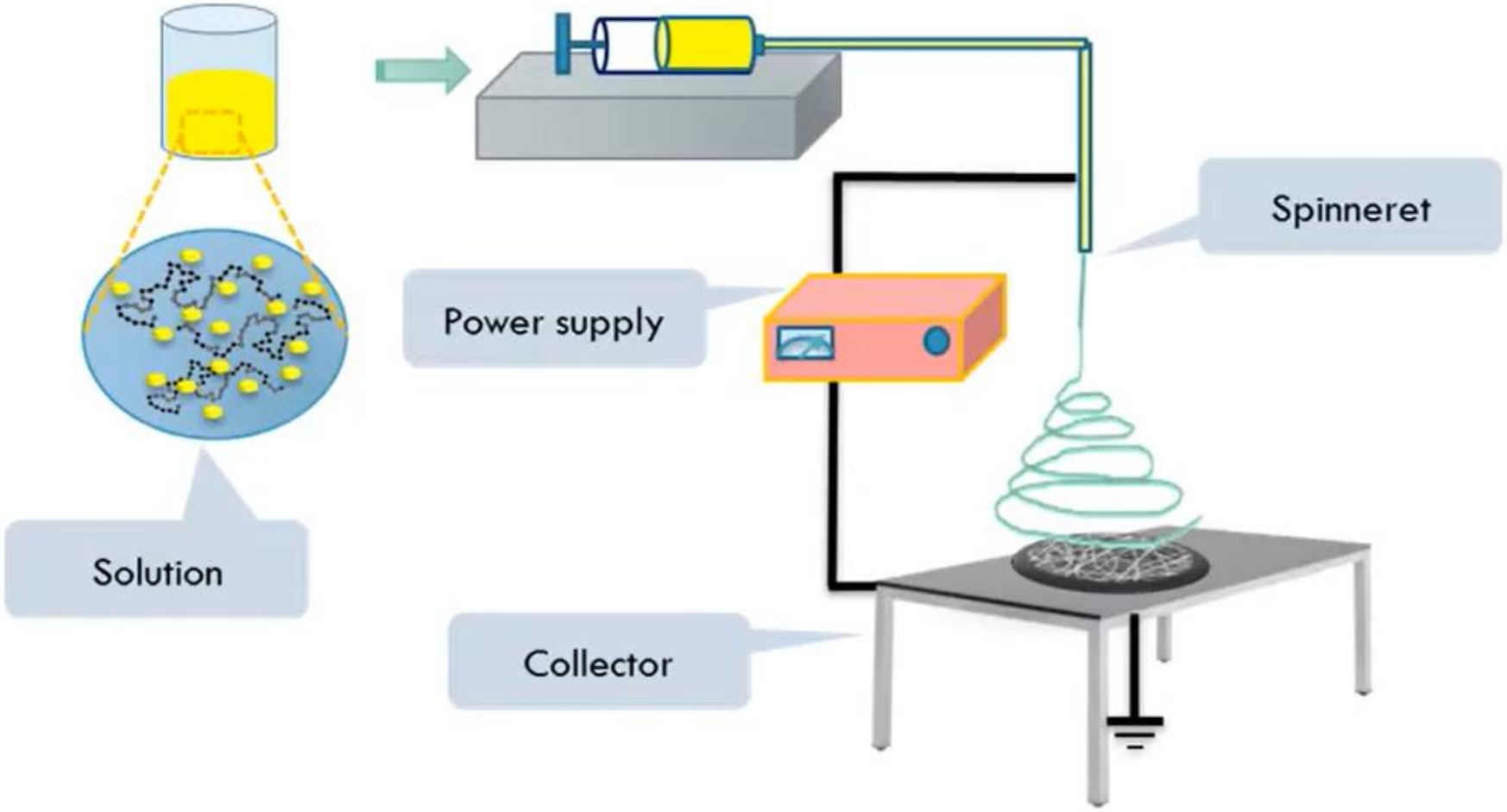
- The disadvantages are the high cost of raw materials; large shrinkage during processing; residual fine pores, hydroxyl, and carbon; and a health hazard from the long processing time of the organic solution.

Electrospinning

- 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.
- Nanofiber scaffolds have adequate porosity throughout which allows for the infiltration of new cells while also possessing sufficient mechanical strength.
- The most promising and widely studied technique for producing nanofiber scaffolds is through **electrospinning**. For example, the electrospinning technique is best suited for cases for implantation in tissues where high flexibility is required, such as in soft tissues.
- This technique is able to produce nanofiber scaffolds from a variety of materials, including polymers and biomaterials.

- Researchers are also able to finetune certain characteristics of the resulting scaffold such as the fiber diameter and the surface morphology depending on the requirements of the tissue.
- In addition to this, nanofibers are able to closely mimic the size of extracellular matrix proteins which are sized between 50 to 500 nanometers in diameter.

Electrospinning

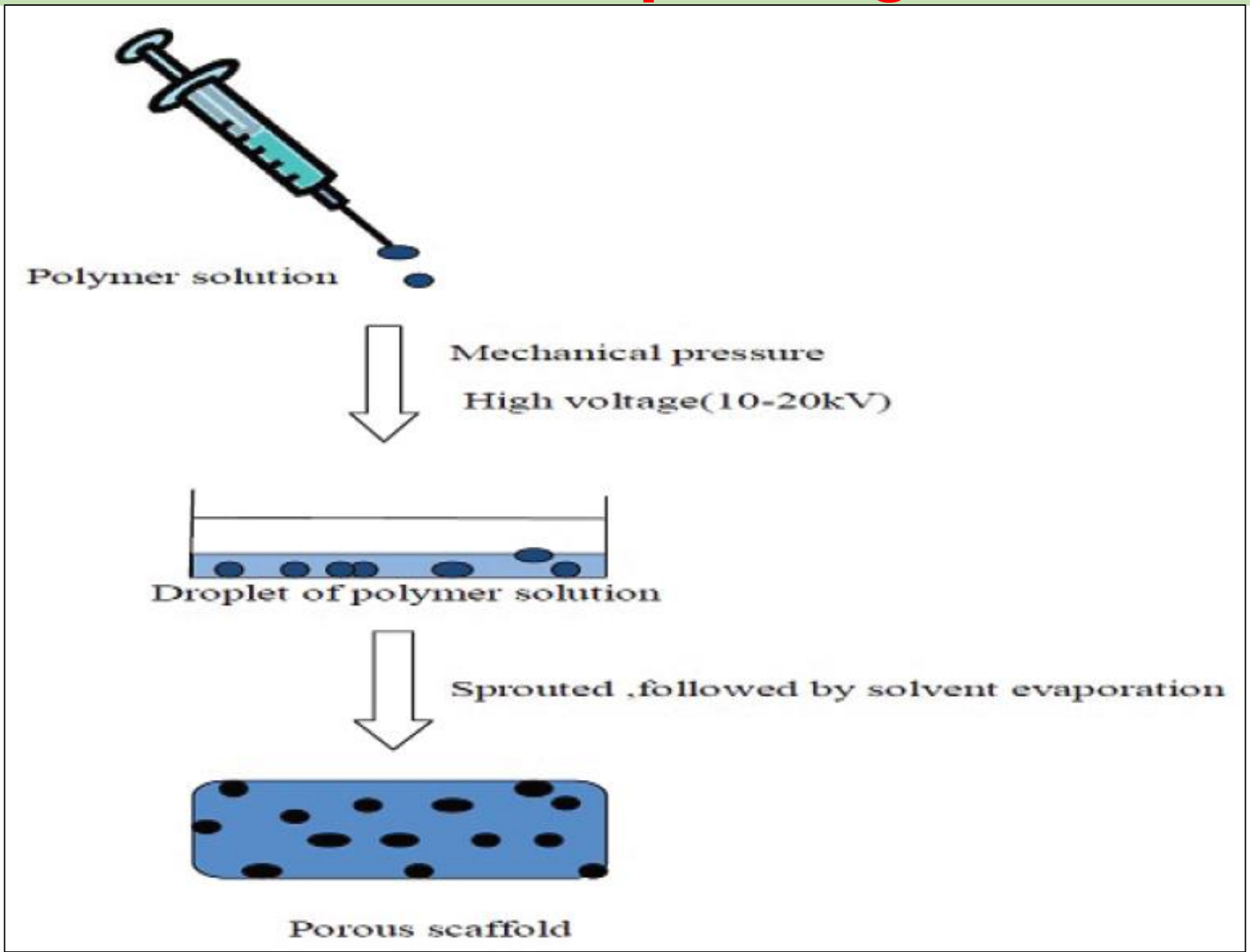


1. A high voltage **power supply** (normally working in a range between 10 and 30kV);
2. A **polymer reservoir** that can maintain a constant flow rate of solution, commonly a syringe connected to either a mechanical or a pneumatic syringe pump;
3. A conductive dispensing **needle** as polymer source connected to the high voltage power supply;
4. A conductive substrate, normally grounded, which serves as a **collector** for the electrospun fibers.

Components of Electrospinning Technique

- Polymer source (Injection/Syringe Pump)
- A metallic needle (spinneret)
- High voltage power supply
- Collector plate (drum)

Electrospinning



Electrospinning

- Electrospinning is one of the most used conventional techniques used to produce nanofibers (NFs). The device is composed of a syringe pump, a metallic needle (spinneret), a high-voltage power supply, and a collector.
- **An electric charge is imparted to the polymer solution by means of the attached power supply, as it is being pumped via the syringe pump through the spinneret's nozzle. Due to the potential difference between the charged polymer solution and the oppositely charged collector, the solution heads from the syringe toward the collector, where it is collected in the form of NFs. The exact mechanism of NFs formation can be illustrated as follows. Above a certain voltage, the accumulated charge on the surface of the polymer solution exceeds its surface tension. This causes the elongation of polymer solution as it escapes the spinneret and, due to the volatility of the used solvent, it evaporates, causing the deposition of the spindle-shaped NFs at the collector.**
- **In this technique, the polymer solution is passed under mechanical pressure through a high voltage (10–20 kV). The droplet of polymer solution obtained sprouts followed by solvent evaporation, leading to the formation of fine fibers that mat in to porous scaffold.**
- Processing parameters such as the flight time (time during which the solution passes from the spinneret to the collector, controllable via distance control), volatility of the solvent, needle tip size, voltage applied, and geometry of the collector could be manipulated to produce NFs of the desired porosities and morphological characteristics.
- Combining various biomaterials to overcome their individual limitations, as well as incorporation of bioactive materials, are both feasible with electrospinning.
- Electrospinning yields NFs of high surface area/volume ratio, which, when combined with the tunability of scaffold morphological properties such as pore size and mechanical strength, demonstrates the benefits of the process in tissue engineering. However, electrospinning is not applicable to all polymers, and residual solvent may remain and affect the biological attributes of the scaffolds.
- In one study, gelatin/PCL fibers were prepared using electrospinning and investigated for the delivery of various drugs such as simvastatin and vancomycin as well as for fluorescein isothiocyanate-bovine serum albumin. While gelatin improved the osteoblast cellular adhesion and proliferation as visualized by increased ALP activity and bone mineralization, PCL enhanced the mechanical strength of the fibers and imposed a controlled release pattern for hydrophilic and hydrophobic drug molecules.

Electrospinning

- Electrospinning is a process whereby electrical charge is used to form a mat of fine fibers. The apparatus using this method was patented by J.F.Cooly as early as 1902.
- **In this technique the polymer solution is passed under mechanical pressure through a high voltage (10–20 kV). The droplet of polymer solution obtained sprouts followed by solvent evaporation, leading to the formation of fine fibers that mat in to porous scaffold.**
- Electrospinning is the most widely used method for fabrication of nonwoven nanofiber matrices.
- Various materials can be electrospun into nanofiber-like biodegradable polymers such as PLGA and polycaprolactone, poly(ethylene oxide), polyvinyl alcohol, collagen, silk protein, and other peptides.
- Li et al. developed a novel PLGA nanofibrous mesh with fiber diameter ranging from 500 to 800 nm and pores that were well interconnected.
- The main advantages of this technique are that it can produce the scaffold with a main structural feature suitable for growth of the cell and subsequent tissue organization. It can produce ultrafine fibers with special orientation, high surface area, and high aspect ratio that have control over pore geometry, all of which are favorable for better cellular growth in vitro and in vivo.
- The other advantage of this technique is its simplicity, high efficiency, and sheets; cylindrical shapes can be fabricated with this technique but the electrospinning method primarily results in a two-dimensional mesh structure with a nanoscale pore size, which is not sufficient for cell seeding and infiltration.
- Cell seeding is the main problem of the electrospinning method. This is overcome by sacrificial biopolymer or cryospinning, which allows creation of a hole of a desired size in electrospun matrices.

Electrospinning

- When a sufficiently high voltage is applied to a liquid droplet, the body of the liquid becomes charged, and electrostatic repulsion counteracts the surface tension and the droplet is stretched; at a critical point a stream of liquid erupts from the surface. This point of eruption is known as the Taylor cone. If the molecular cohesion of the liquid is sufficiently high, stream breakup does not occur (if it does, droplets are electrosprayed) and a charged liquid jet is formed.

- As the jet dries in flight, the mode of current flow changes from ohmic to convective as the charge migrates to the surface of the fiber. The jet is then elongated by a whipping process caused by electrostatic repulsion initiated at small bends in the fiber, until it is finally deposited on the grounded collector. The elongation and thinning of the fiber resulting from this bending instability leads to the formation of uniform fibers with nanometer-scale diameters.

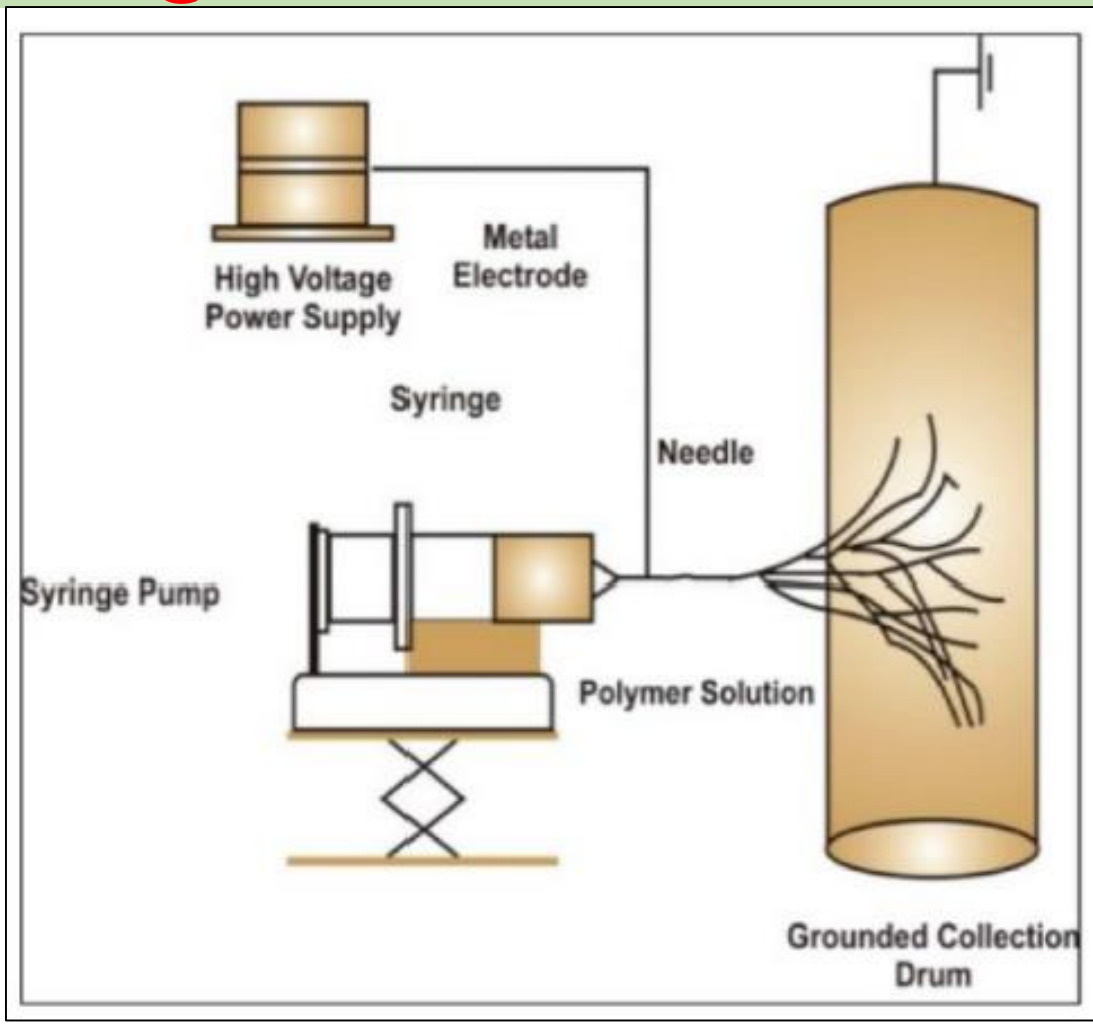
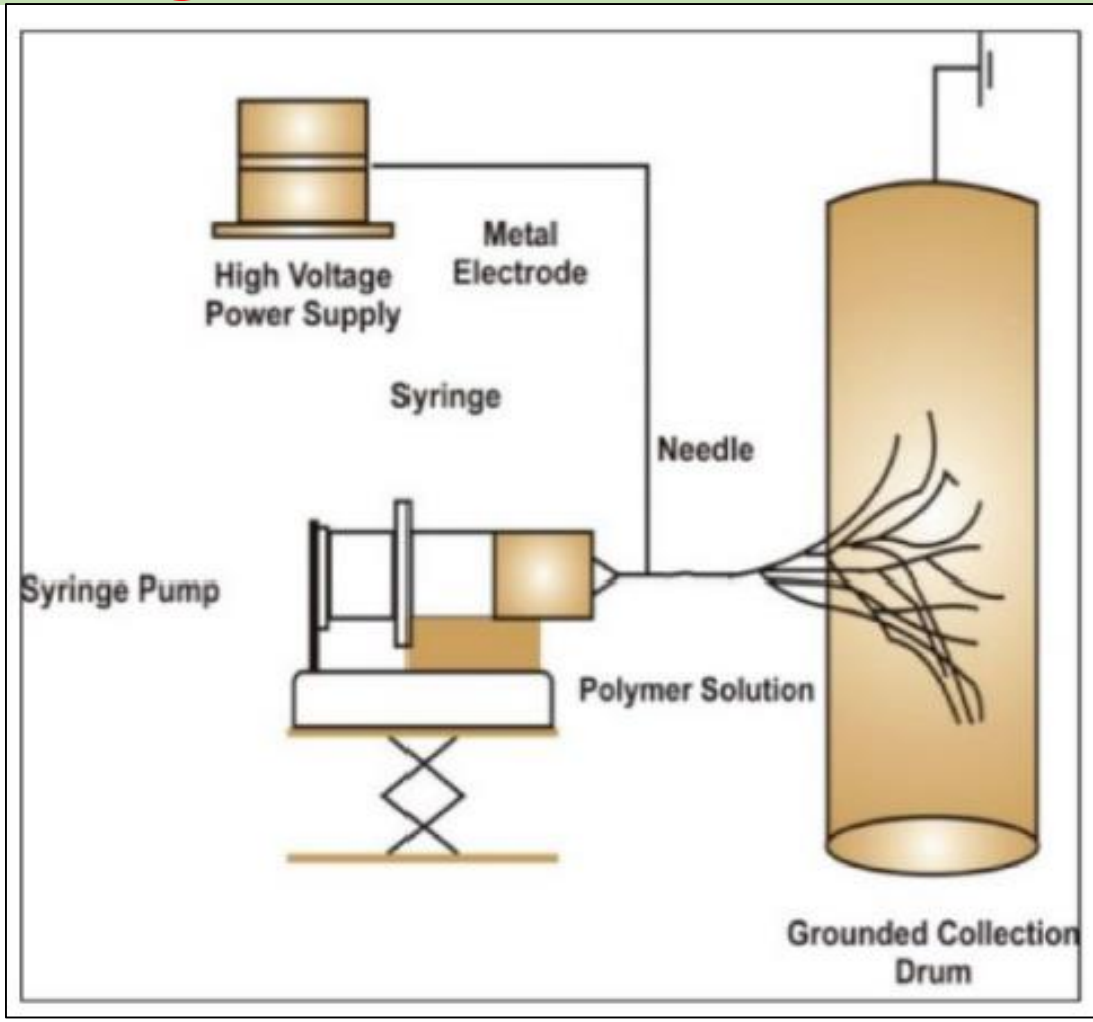


Figure: The setup of the process of electrospinning is depicted here.
An electrical current is applied as the polymer solution exits the syringe at a steady rate set by the syringe pump. The resultant nanofibers are collected on the rotating collection drum.

Electrospinning

- The polymer solution is dispensed through a needle at a specific rate.
- When a high voltage is applied to the resulting droplet, the resulting electrical field overcomes the cohesive forces which are present in the solution, primarily in the form of surface tension. This then leads to a constant stream of polymer solution which is attracted to the collector.
- In the space between the needle and the collector, the solvent evaporates which causes the polymer to solidify in long fibers on the collector.



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Electrospinning

- The exact mechanism of NFs formation can be illustrated as follows.
- Above a certain voltage, the accumulated charge on the surface of the polymer solution exceeds its surface tension. This causes the elongation of polymer solution as it escapes the spinneret and, due to the volatility of the used solvent, it evaporates, causing the deposition of the spindle-shaped NFs at the collector.
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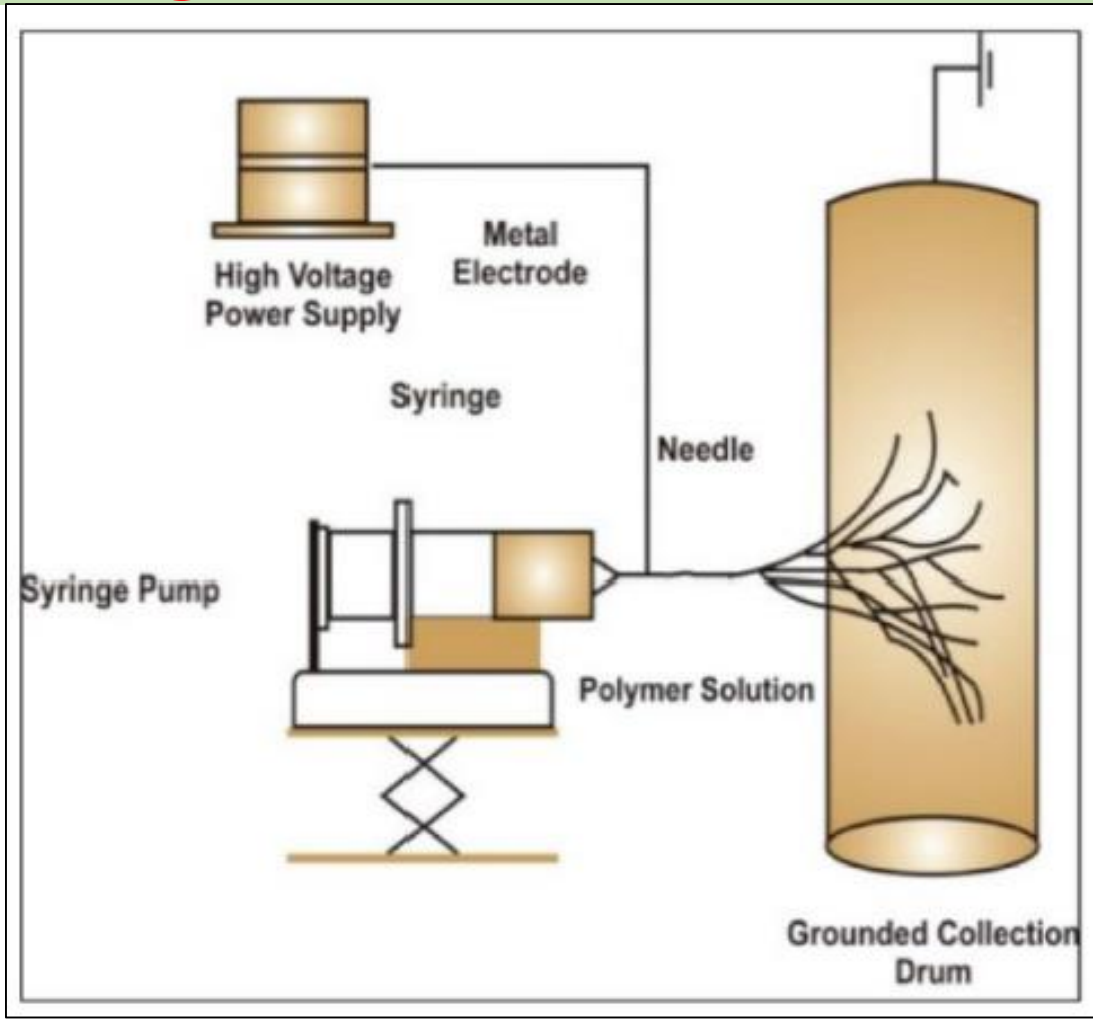

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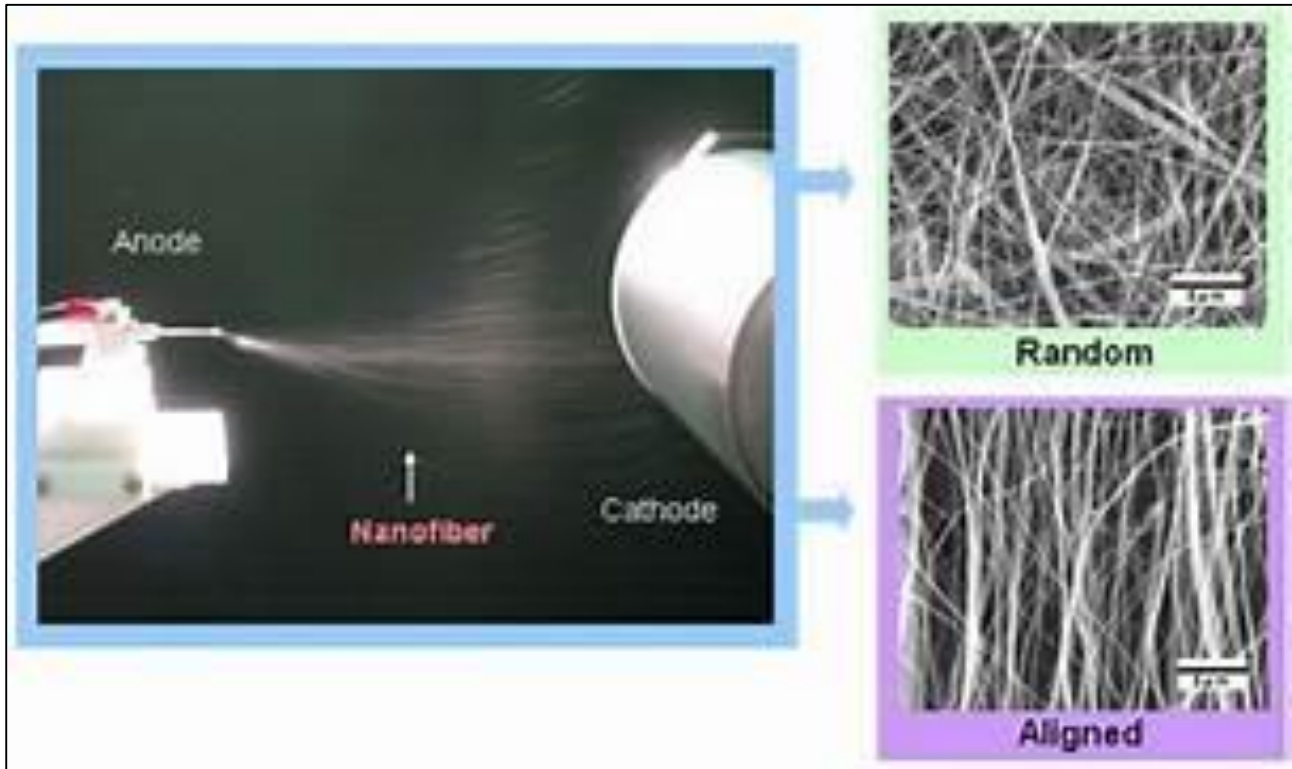
An electrical current is applied as the polymer solution exits the syringe at a steady rate set by the syringe pump. The resultant nanofibers are collected on the rotating collection drum.

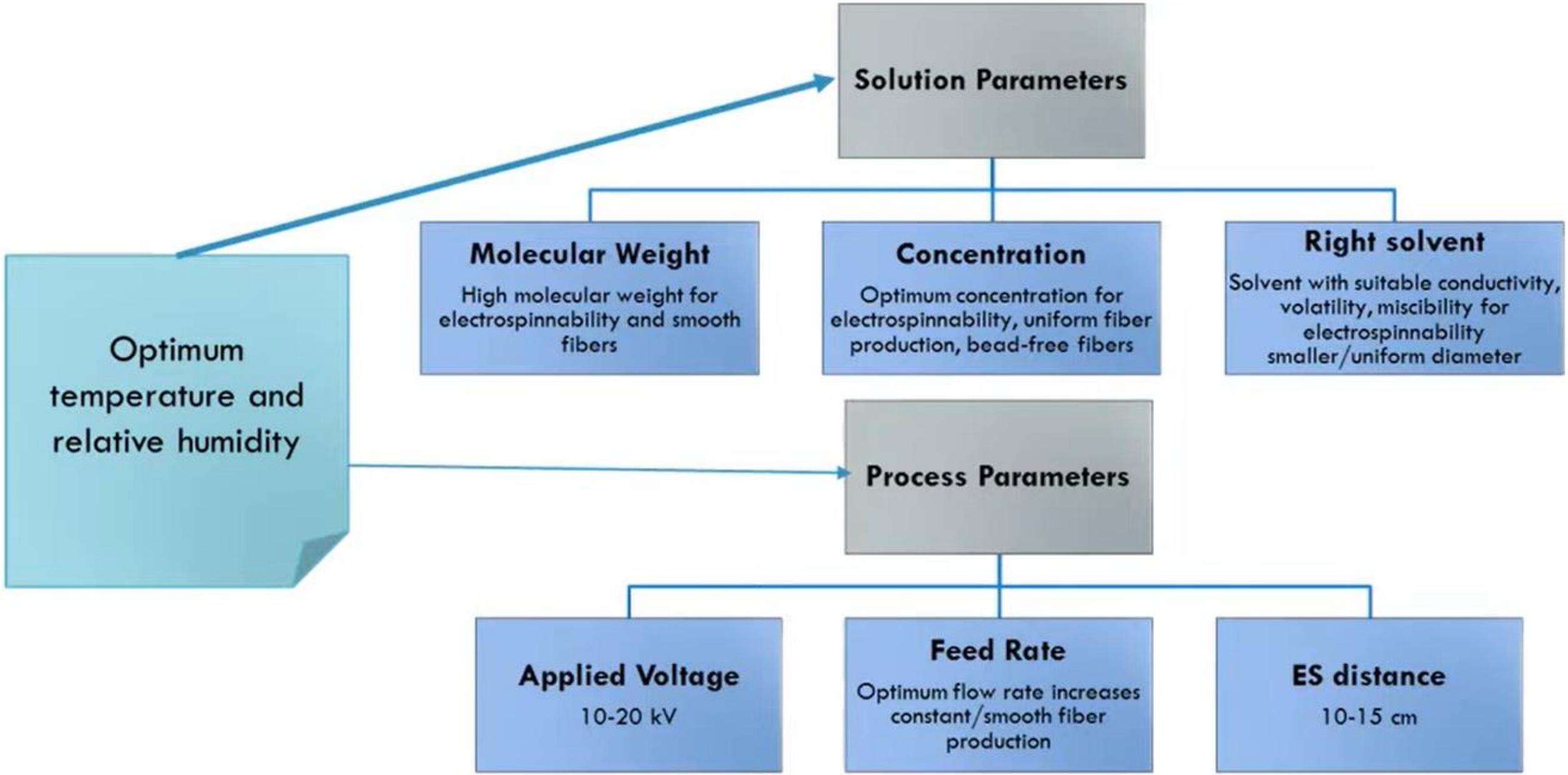
Electrospinning Parameters

To optimize material properties, fiber thickness, homogeneity, density, distribution, etc.

- Polymer material
- Solvent and solution additives
- Polymer concentration
- Needle to collector distance
- Voltage
- Flow rate



Electrospinning Parameters



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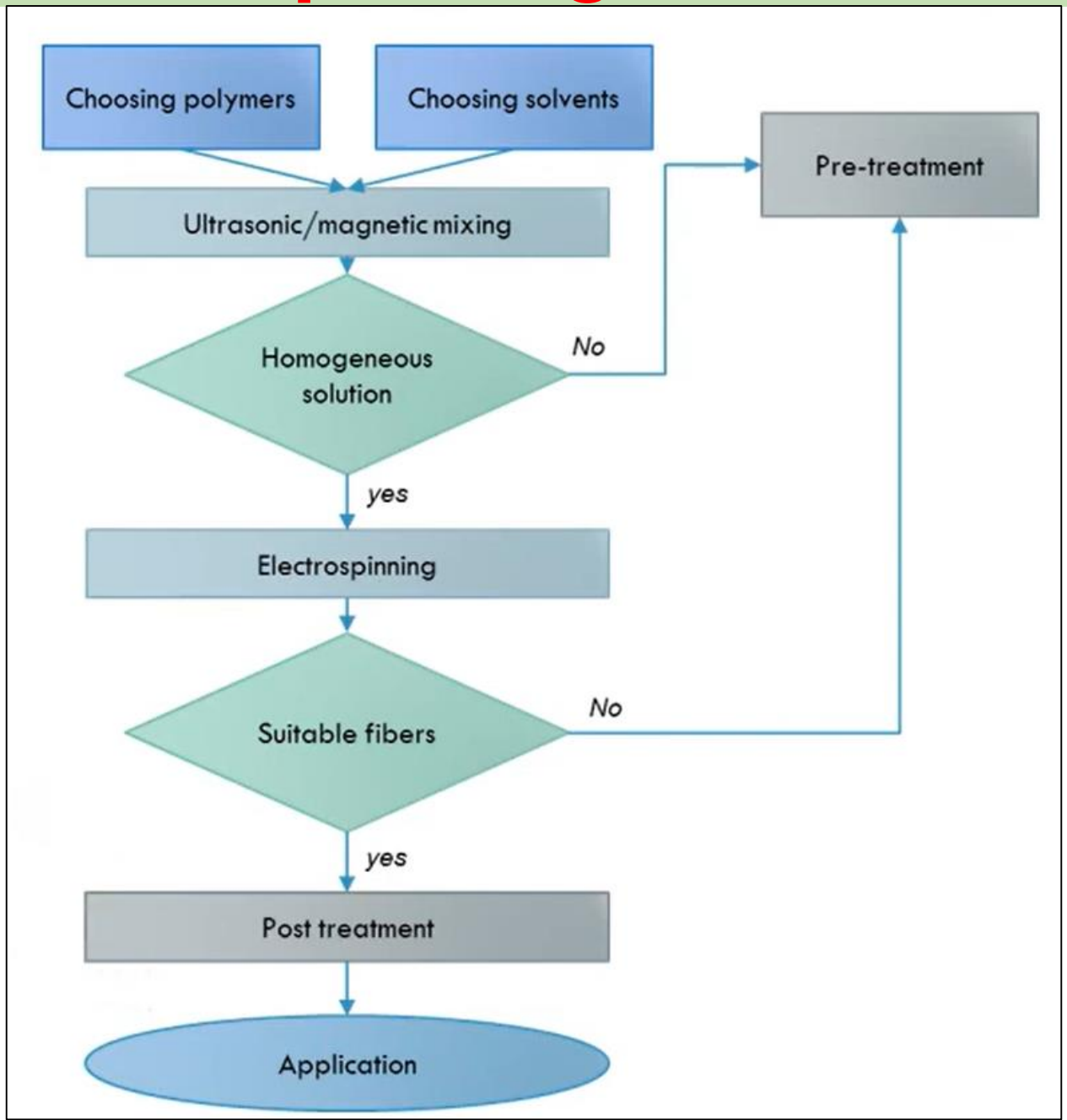
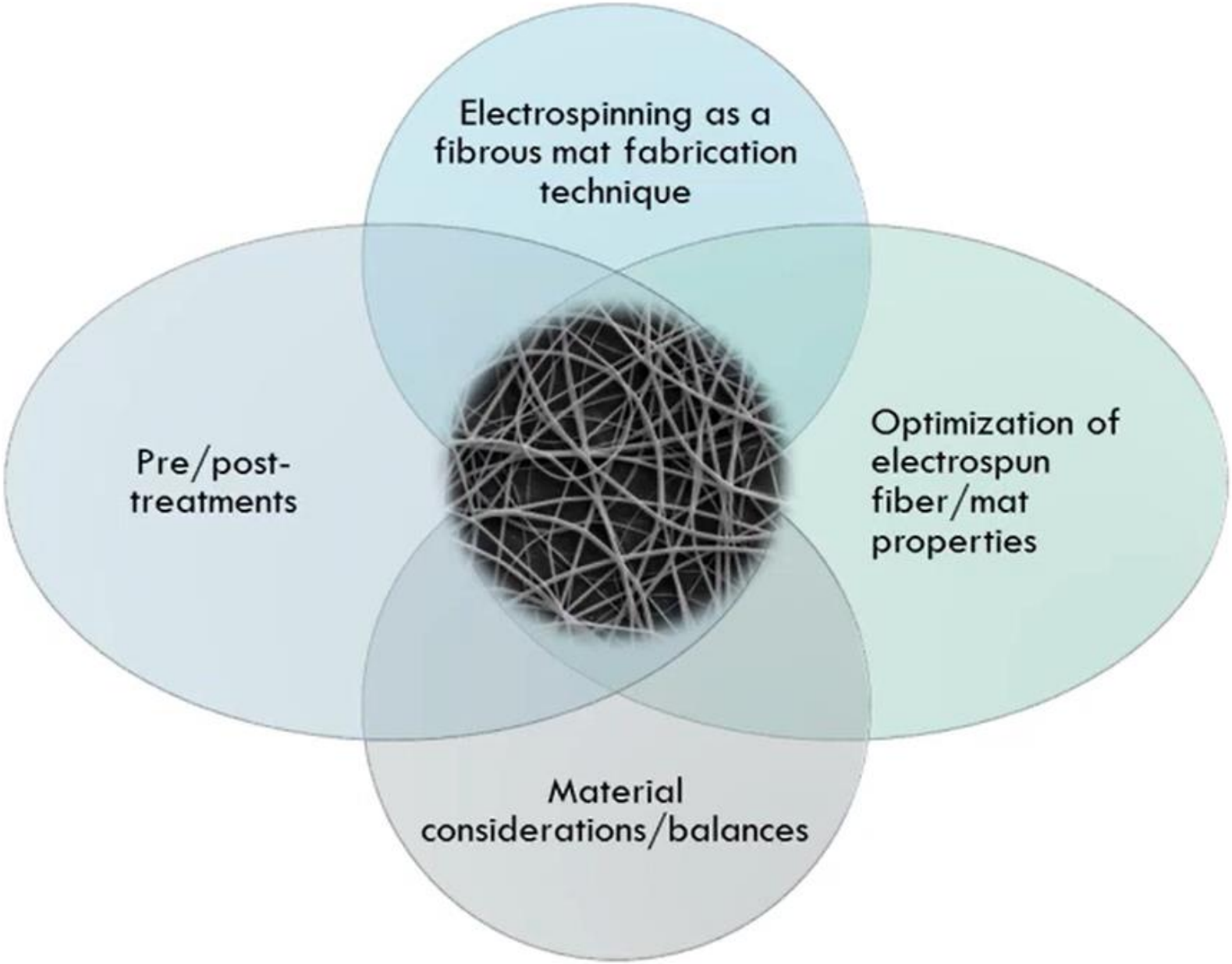


Electrospinning Parameters



Electrospinning

- Electrospinning yields NFs of high surface area/volume ratio, which, when combined with the tunability of scaffold morphological properties such as pore size and mechanical strength, demonstrates the benefits of the process in Tissue Engineering.
- However, electrospinning is not applicable to all polymers, and residual solvent may remain and affect the biological attributes of the scaffolds.

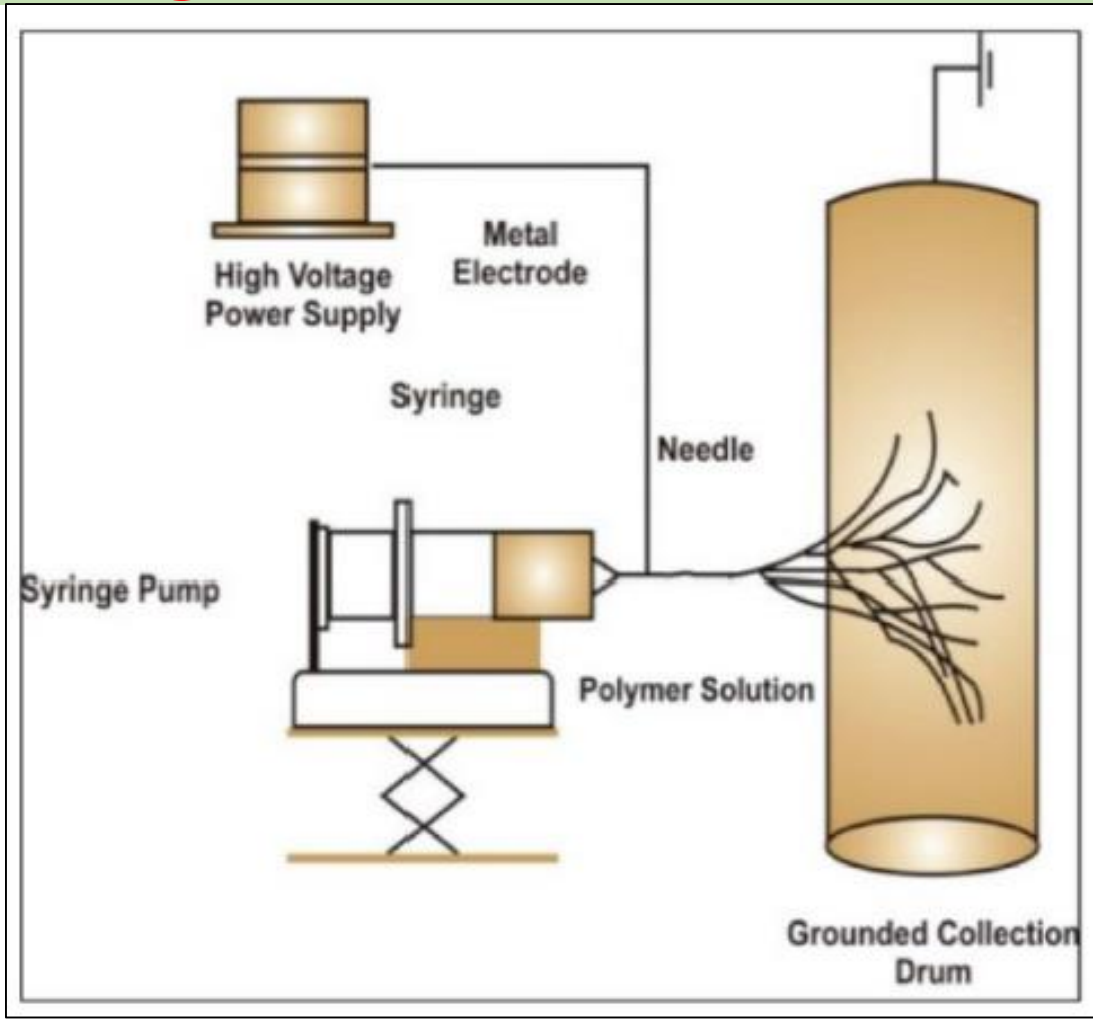
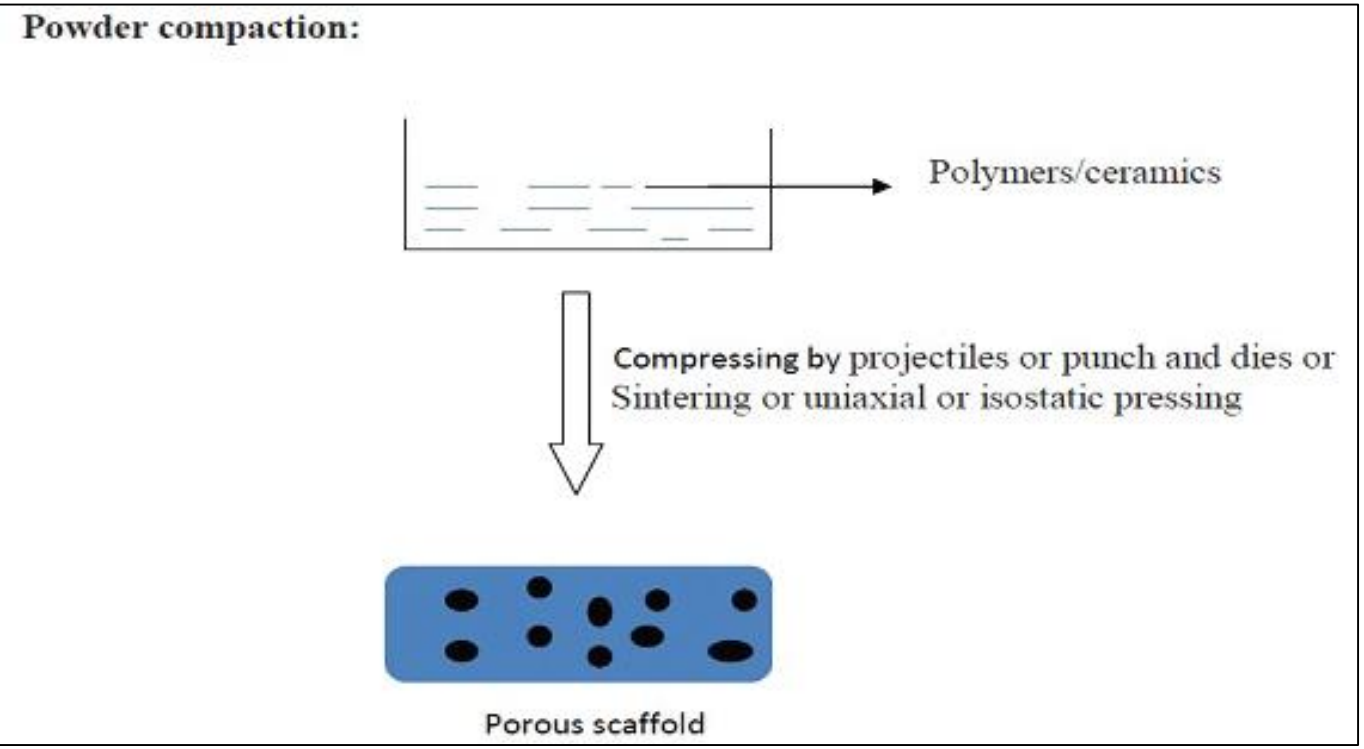
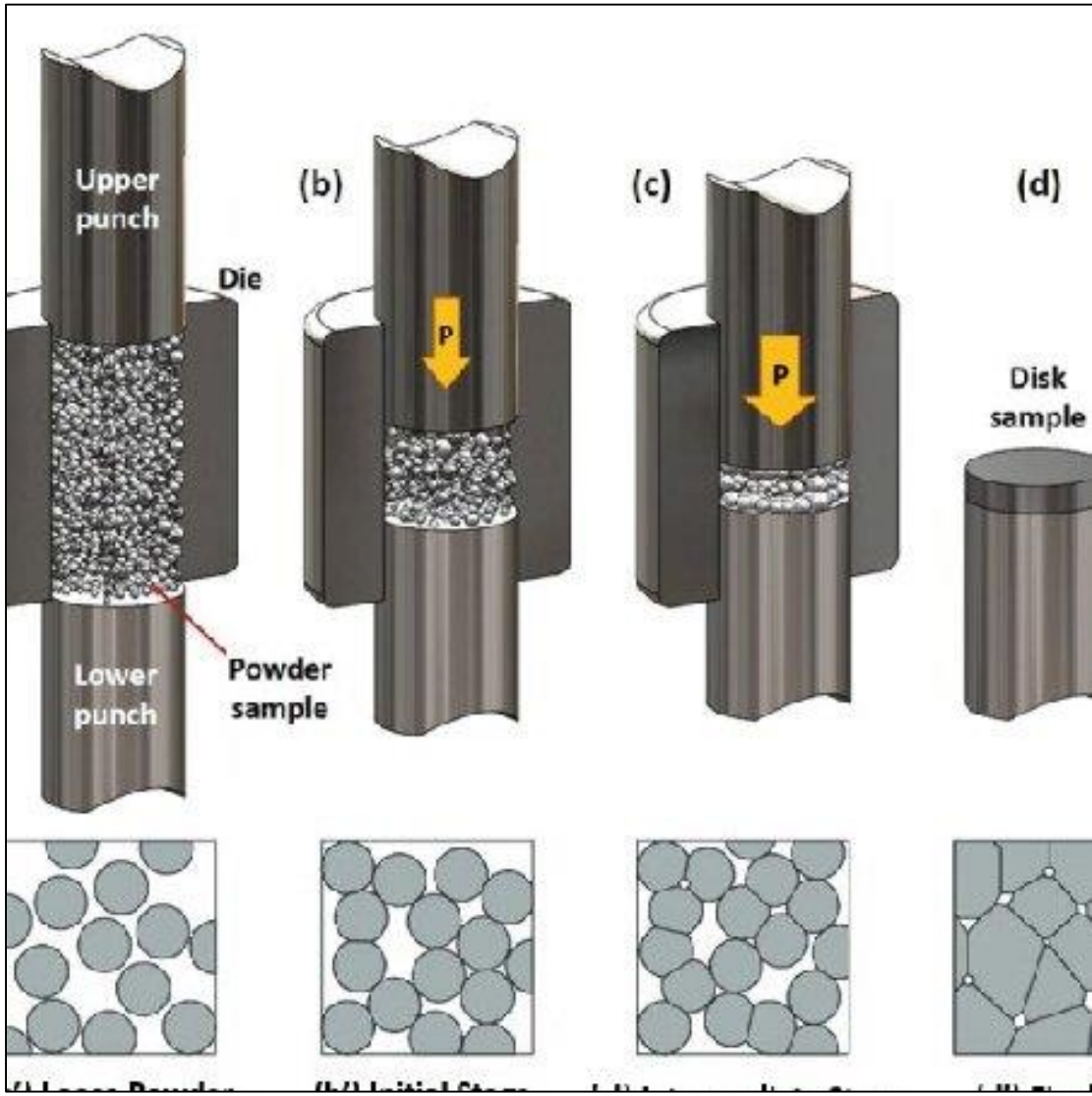


Figure: The setup of the process of electrospinning is depicted here.
An electrical current is applied as the polymer solution exits the syringe at a steady rate set by the syringe pump. The resultant nanofibers are collected on the rotating collection drum.

Powder compaction

- **Scaffolds are prepared by compressing the polymers/ceramics using projectiles or punch and dies; the velocity of compaction of the projectile or punch and dies is adjusted to achieve powder consolidation with the desired porosity.** The process can include sintering as an alternative to use uniaxial or isostatic pressing.





Fiber Bonding

- The fiber bonding method was first developed by Cima et al, who produced scaffolds made of polyglycolic acid (PGA) polymer. They took advantage of the fact that PGA was available as sutures and thus in the shape of long fibers.
- Mikos et al improved the structural stability of the constructs developing a fiber-bonding technique in which the PGA **fibers are joined at their cross-linking points by "sintering" above their melting point temperature**.

- For example, PGA fibers have been bonded by embedding in PLLA solution, cooling, and subsequent removal of PLLA. The scaffolds were fabricated by bonding a collagen matrix to PGA polymers with threaded collagen fiber stitches.

- The main advantage of the fiber-bonding technique is the high surface area:volume ratio, which makes them ideal for tissue engineering applications and high porosity, which provides more surface area for cell attachment and sufficient space for the regeneration of ECM.

- Disadvantages are poor mechanical integrity, residual organic solvents, lack of structural stability, that they can be used only to make small membranes, all the materials cannot be used for all the processes, membrane porosity is difficult to control, and morphology.

Sintering is a process of heating a material below its melting point to form a solid mass by bonding the particles together.

Fiber mesh

- **The fiber mesh technique for scaffold fabrication consists of individual fibers either woven or interwoven into a 3D pattern of variable pore size. It is prepared by the deposition of a polymer solution over a nonwoven mesh of another polymer followed by subsequent evaporation**.

- PGA is the first biocompatible and biodegradable polymer to be spun into fiber and used as a synthetic suture thread.

- The main advantage of this technique is the rapid diffusion of nutrient that is favorable for cell survival and growth and for high cell attachment because of the large surface area.

- One of the main drawbacks of this technique is a lack of structural stability. This problem can be overcome by hot drying of PLLA fibers to improve crystallinity and structure orientation.

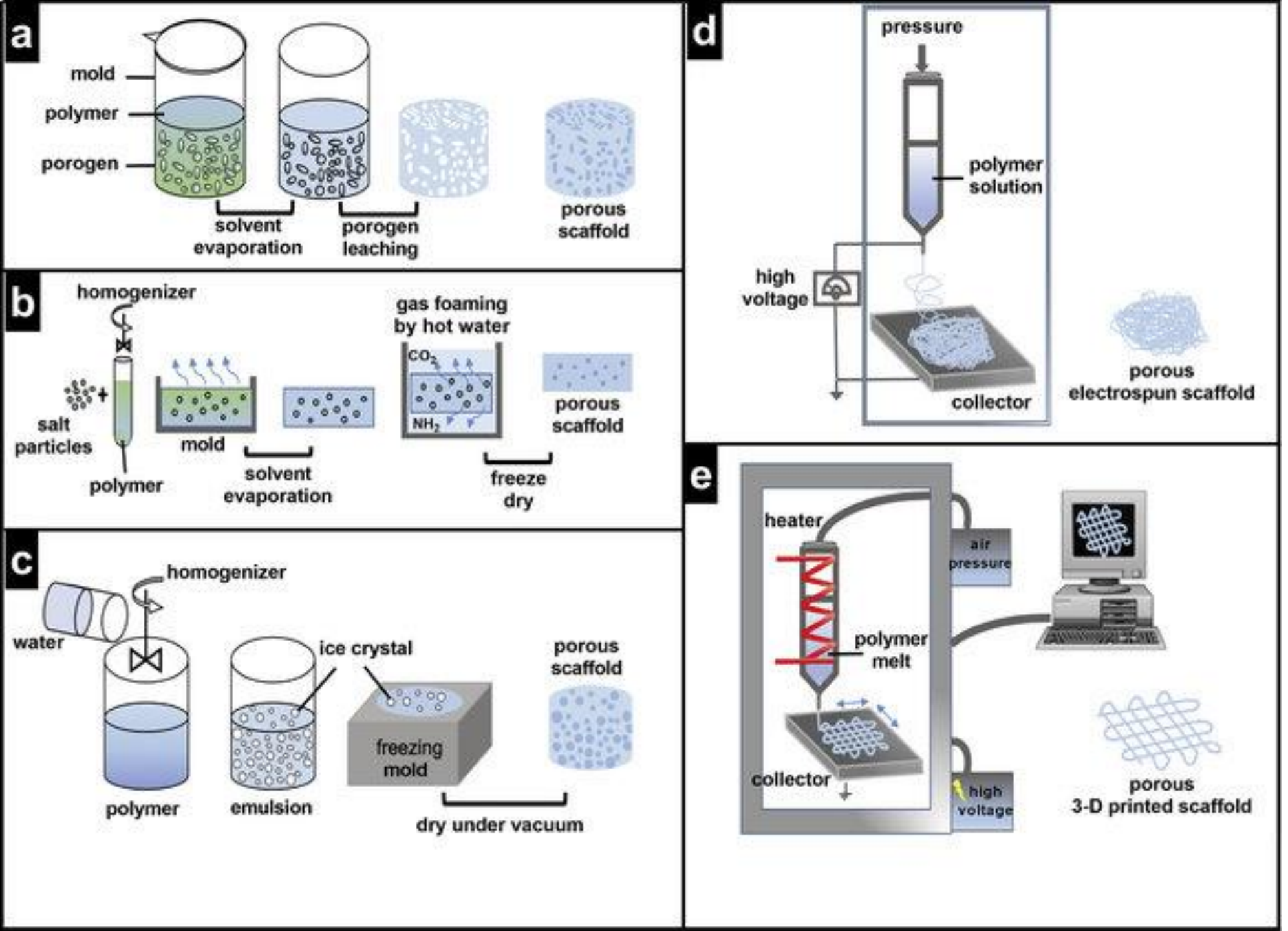


Fig. Various porous scaffold fabrication techniques.

(a) Porogen leaching
(b) Gas foaming
(c) Freeze-drying
(d) Solution electrospinning
(e) Melt electrowriting and 3-D printing

Scaffold fabrication techniques

Table 1 List of techniques used in scaffold fabrication

Techniques	Properties	Pore size/pore density	References
Solvent casting/particulate leaching	• Only the porogens and polymer remain after evaporation • Properties can be tuned accordingly by changing the salt concentrations/size and polymer • Low cost and easy	30–300 µm 75–88%	[195–197]
Thermally induced phase separation	• Pores are formed when the solvent is evaporated Materials/agents (depending on the target tissue) to the primary polymer solution can be easily incorporated • Varying some TIPS processing parameters like polymer concentration (from 2.5 to 15 wt%) and freezing temperature (from 4 to − 60 °C) structural and mechanical properties could be controlled	1–100 µm 95%	[196, 198]
Self assembly	• Material very similar to ECM can be developed • Components are organized into patterns/structures autonomously • Used for nerve and cartilage tissue engineering	5–200 nm	[199]
Freeze drying	• The solution is cast and frozen at − 70 to − 80 °C and dried in a low-pressure chamber • Extraction of material takes place inside the chamber by sublimation and the final drying occurs due to desorption • Porosity can be controlled by maintaining the freeze-drying pressure accordingly	20–200 µm 90%	[197, 199]
Gas foaming	• A foaming agent, e.g., sodium bicarbonate, is used • High pressure is required to initiate nucleation and development of gas and then lyophilized • Liberation of gas forms the pores	40–800 µm 85%	[16, 197]
Electrospinning	• Electrostatic force is used to produce fibres • When a liquid is charged at a high voltage, the surface tension and electrostatic repulsion interact, causing droplets on the spinneret to erupt and stretch • Variables: material used, collector setup, post-processing	10 nm–6 µm (fibre diameter) 80–95%	[6, 197, 200]
Rapid prototyping	• Stereolithography, selective laser sintering, solvent-based extrusion free forming, bioprinting, fused deposition modelling • Various types of 3D structures could be fabricated • Accurate cell–cell interactions in a 3D environment by controlling geometry at the micro and nano cellular levels • Layer by layer arrangement with interconnected pores	25–150 µm	[199, 201]

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Review

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Advancing strategies towards the development of tissue engineering scaffolds: a review

Ipsita Pattanayak[1], Y. Alex[1], and Smita Mohanty[1,*]

[1] *Laboratory for Advanced Research in Polymeric Materials (LARPM), School for Advanced Research in Polymers (SARP), Central Institute of Petrochemicals Engineering & Technology (CIPET), Patia, Bhubaneswar 751024, Odisha, India*

Scaffold fabrication techniques

Table 1. Classification of scaffold fabrication techniques used in skin tissue engineering.

Fabrication Techniques		Advantages	Disadvantages
Conventional fabrication techniques	Electrospinning	Essential for developing nanofibrous scaffolds, homogenous mixtures made of fibres with high tensile strength [24]	Process depends on many variables, problematic to obtain 3D structures with the required pore size needed for biomedical application [26,27]
	Freeze drying	Used in a variety of purposes, capability of obtaining high temperature, manageable pore size by changing freezing method [24]	High energy consumption, long term timescale, generation of irregular size pores [28]
	Gas foaming	Porosity up to 56.71% [29]	Temperature dependent, product obtained from decreased temperature might have closed pore structure or a solid polymeric skin [30]
	Thermal induced phase separation	Porosity up to 80% [31], can use low temperature to integrate bioactive molecules [24]	Only used for polymers amenable to phase separation [31]
Rapid prototyping (RP)	Bioprinting	Low cost, higher accuracy, and greater shape complexity [24]	Depends on the cells/biomaterials used [32]
	Fused deposition modelling (FDM)	High tensile strength [24]	Has limited application to biodegradable polymers [33]
	Solvent based extrusion free forming (SEF)	Used to make ceramic, metal, and metal/ceramic composite part; used for precise control of scaffold structure at the micron level [24]	Variation in temperature affects extrusion pressure, including nozzle length-to-diameter ratio, and the extrusion velocity [34]
	Stereolithography	High resolution, uniformity in pore connectivity [24]	Requires a massive number of monomers and post-polymerization treatment to improve monomer conversion [35,36]

polymers

MDPI

Review

Synergistic Effect of Biomaterial and Stem Cell for Skin Tissue Engineering in Cutaneous Wound Healing: A Concise Review

Shaima Maliha Riha, Manira Maarof and Mh Busra Fauzi *

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Thank you for your attention