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Designing of macroporous polymeric materials for bioengineering applications

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ABSTRACT

Macroporous polymeric materials are three-dimensional porous architectures having enormous utility in biomedical, biotechnological and separation sciences. The ultimate potential for these applications rests upon their chemical and morphological characteristics, which to a great extent are regulated by their fabrication approaches. Many techniques have been investigated in recent years to form highly porous biodegradable scaffolds suitable for use in tissue engineering. Many of these methods are able to form foams with high porosity to encourage cell attachment. These methods exhibit good biocompatibility, making these techniques especially promising for future use in tissue-engineered cell-polymer constructs. Thus realizing the crucial role of macroporous polymeric materials in tissue engineering and allied fields the present review discusses various synthetic routes of macroporous materials and presents a concise but critical analysis of strategies adopted by various workers. This review discusses applications in which fast swelling is needed.

KEYWORDS

Macroporous materials;
Tissue engineering;
Bioseparation;
Freeze-thaw;
Enzyme immobilization.

INTRODUCTION

There are many applications in which a material is needed to swell rapidly in a fluid materials that imbibe large volume of water are very functional in daily use and in specialized applications. Everyday materials such as diapers and sanitary napkins must rapidly contain large volume of biological fluids and retain strength without losing fluids to surrounding dress[1]. Superporous hydrogels (SPHs) are a new generation of hydrogels with pore size in the range of 100μm or larger; mesh size of a conventional hydrogel is below 100nm[2-3]. The swelling kinetics of SPHs is few minutes, much faster than that of conventional hydrogels. It usually takes place hours to days for conventional hydrogels to swell to equilibrium when their dimensions are on the order of centimeters. Rapid swelling of SPHs is due to interconnected pore networks that are formed[4].

A pore defined in general sense as a limited space or spatial confinement is known to form the basis of modern materials science and contributes significantly to a wide spectrum of novel applications ranging from tissue engineering, immunodiagnostics, and chromatographic support materials to combinatorial chemistry[5].

Review

The understanding, design and precise control over pore dimensions have significantly advanced science and technology, and are playing prime roles in exploration and application of new technologies. As per the recent IUPAC conventions, pores are classified into three categories, namely micropore, mesopore and micropore with pore sizes less than 2 nm, between 2 and 50 nm, and larger than 50 nm respectively. No matter the porous materials are non polymeric, polymeric or hybrid in origin, they all are of great scientific and technological significance because of the ability of the pore wall to interact with atoms, ions, molecules and supermolecules. Moreover, the porous materials may be made tailorable to specific function groups for desired application, which also enables these materials highly attractive in frontier research[6]. To increase the response rate of hydrogels, several techniques were proposed:

(i) Submicrometer-sized gel particles[7] since the rate of response is inversely proportional to the square of size of the gel,[8] small gel particles respond to the external stimuli more quickly than bulk gels.
(ii) Gels having dangling chains[9-11] Dangling chains in a gel easily collapse or expand upon an external stimulus because one side of the dangling chain is free.
(iii) Macroporous gels[12]. For a polymer network having an interconnected pore structure, absorption or desorption of water occurs through the pores by convection, which is much faster than the diffusion process that dominates the nonporous gels.

Among porous materials of various chemical and compositional types the macroporous materials owe a prime position in the area of materials science due to ever seen advancements in design and processing of these materials driven by the rapid growth of emerging applications like energy conversion and storage, environment friendly catalysis, sensors, tissue engineering, DNA sequencing, drug delivery, cell markers and photonics[13]. All these technological applications do require a high level of control over the dimension, structure and properties of the pores and porous materials, which can easily be achieved by strategically designing sitemap of the synthesis part of the material.

SYNTHESIS AND MORPHOLOGY OF MACROPOROUS MATERIALS

Designing macroporous materials by various physical and chemical routes is not like conventional synthesis as in the former case an intimate relationship does exist between the method adopted for synthesis and structural and morphological features of the material to be synthesized. Precisely speaking, a high level of accurate control is desired over macro and microstructural properties such as spatial form, mechanical strength, density, porosity, pore size, pore size distribution and pore interconnectivity, respectively. Since both the synthetic methods and internal structures are interdependent the description of various methods need to be accompanied by a parallel discussion on the structure and morphology of the materials. Since the morphology of the material has to meet the demands of the targeted applications the forthcoming portion of the chapter focuses on various methods of preparation of macroporous polymers and morphology of the prepared porous polymers, which is a key parameter to predict the suitability of the material for a specific application.

Macroporous polymer foams by hydrocarbon templating

Polymer foams are utilized in a range of applications such as mechanical dampeners, thermal, acoustic and electrical insulators etc.[14]. These foams have been produced by dispersion of a gaseous phase in a fluid polymer phase, leaching of a water-soluble inorganic fugitive phase, phase separation methods[15]. However, these processes do not generally offer optimal control over pore structure and bulk characteristics. Recently an attempt has been made to achieve enhanced control over both porosity and bulk properties by combining two distinct foaming processes, (i) leaching of a fugitive phase with (ii) polymer precipitation. This was achieved by using a non-water soluble particulate hydrocarbon fugitive phase derived from waxes, which allowed for the formation of pores with concomitant precipitation of the polymer phase. The macroporosity of the polymer foam was determined by the hydrocarbon fugitive phase (porogen), which also functioned as a template for the rapid precipitation of the polymer. Bulk properties of the foam could be manipulated independently of the macroporosity and pore size by incorporation of inorganic and organic fillers into the highly viscous polymer phase. The whole process may schematically be

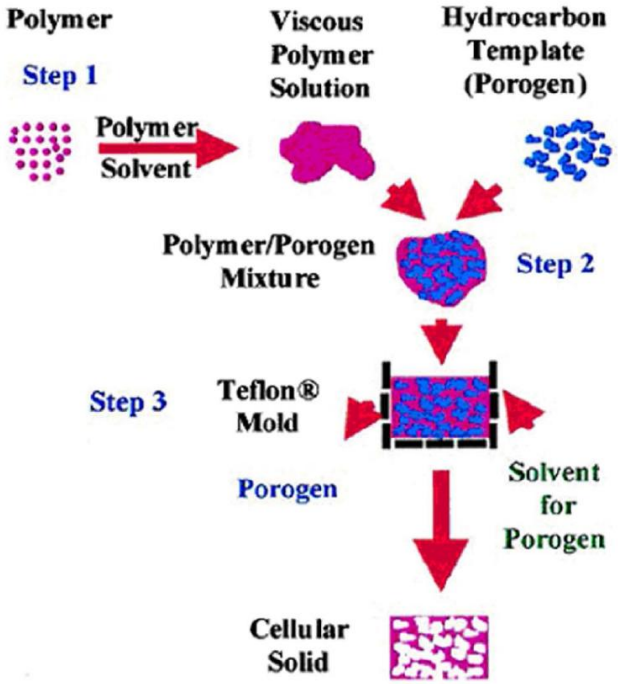

Figure 1 : A schematic presentation of the steps involved in the preparation of macroporous polymer by foaming method[16].

shown as Figure 1.

The process is applicable to a wide range of polymer systems including water-soluble polymers, as long as the following conditions are satisfied: (i) the hydrocarbon porogen is extracted below the melting temperature of the polymer, to ensure isotropy in the properties of the resulting foam; (ii) the polymer has good solubility in a solvent that is a poor solvent for the porogen, to obtain a viscous polymer solution wherein the porogen can be distributed uniformly; and (iii) the polymer has a molecular weight of at lease 40,000, to ensure structural stability of the resulting foam.

In a communication by Shastri and coworkers[16] a series of foams were synthesized of poly (L-lactic acid) (PLLA) and poly (L-glycolic acid) (PLGA) and morphology was studied by SEM technique as shown in Figure 2. It is clear form the SEM images that the foams revealed the presence of a bicontinuous network of the polymer and void with two distinct pore architectures. The geometry and size of larger pores in the foam were nearly identical to those of the particulate hydrocarbon phase. Spherical hydrocarbon particles resulted in pores with spherical morphology(C to E) whereas polyhedral hydrocarbon particles resulted in pores with irregular morphology (A).

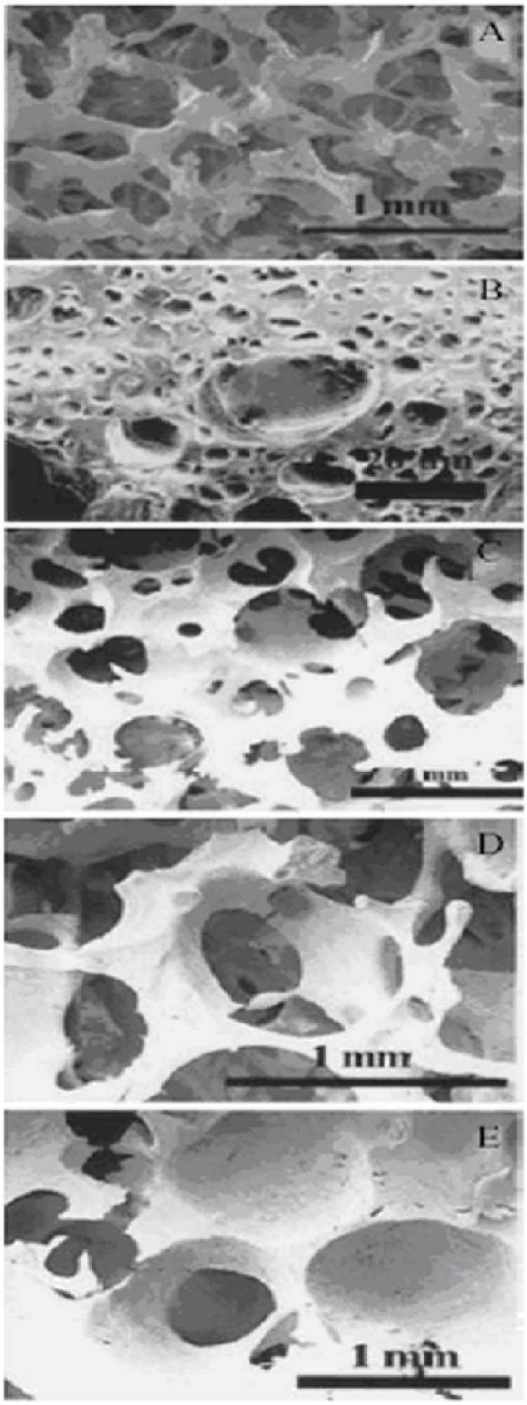

Figure 2 : Scanning electron microscopic characterization of the foams (PLGA and PLLA) using particulate hydrocarbon porogen[16].

Macroporous polymers by radiation initiated polymerization

Synthesis of macroporous polymer materials by free radical polymerization is rather simple technique and provides opportunities to regulate morphology of the end polymer. The variables that control pore size

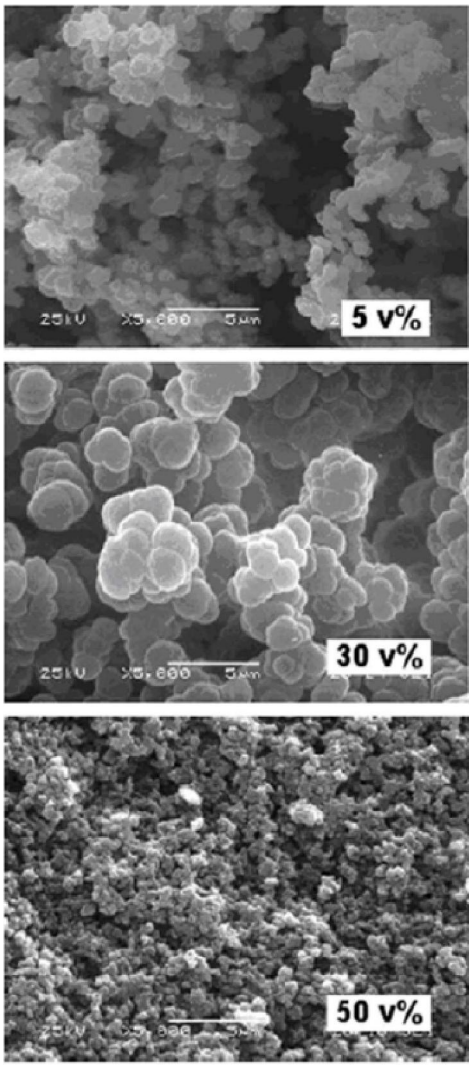

Figure 3 : SEM images of macroporous polymers from methanol solutions with different monomer content. The incubation was done at 25 0C, with dose rate of 16 kGy/h, with total dose of 30 kG[20].

are the percentage of the crosslinker, the type and volume of the porogen, the concentration of the free radical initiator in the reaction mixture, and the reaction temperature. Photopolymerization initiation can also be used in which case the synthesis is much faster and can be achieved even at low temperature[17]. The subject has been nicely reviewed in recent past[18].

Another more efficient and advantageous method of polymerization may be the use of ionizing radiations[19]. The method is beneficial from the viewpoint that the radicals can be directly generated on the monomer molecules thus avoiding use of initiator. More advantageous is that simultaneously it also causes crosslinking of growing macroradical chains thus keeping away the use of toxic crosslinking agents from the reaction scheme. The polymerization may be carried out at any temperature and within quite short time periods. Owing to the larger penetration depths of ionizing radiations compared to Photoinitiated polymerization, the shape and size of porous polymers can be readily optimized for specific applications.

Safrany and coworkers[20] polymerized diethyleneglycol dimethacrylate (DEGDMA) with gamma radiation of varying doses using various aliphatic alcohols as porogen. The authors studied morphology of the prepared porous materials and found that the size of the pores decreased with increasing concentration of monomer. It was also noticed that the nature of organic porogen had a pronounced effect on the pore size as evident from the scanning electron micrographs shown in Figure 3.

The effect of dose rate on morphology of the materials was also investigated.

Surfactant reverse micelles swelling method

A variety of methods have been developed for producing large pore sizes in a porous polymer. In one of the most common techniques a soluble polymer is used as porogen[21]. The method, however, suffers from the problem that it is not always easy to wash the polymer porogen out of the polymeric particles. In another method, called nano-particles agglomeration method[22], it was very difficult to control the pore sizes and even reproducibility was not good. Several workers employed inorganic particles as porogen to produce macroporous particles. For instance, Sun et al.[23] prepared poly (glycidyl methacrylate-ethylene dimethacrylate) P (GMA-EDMA) microspheres with calcium carbonate granules and organic diluents as mixed porogen. The authors obtained porous materials of different sizes depending on the nature of porogen.

Ma and coworkers[23] developed a simple method of surfactant reverse micelles swelling method to prepare poly (styrene-divinylbenzene) [P (ST-DVB)] microspheres with pore size of 500 nm[23]. he advantage of this method is that the preparation process is very easy. The oil phase contained monomer (ST), crosslinking agent (DVB), surfactant, diluent and initiator (benzoyl peroxide). Due to the high surfactant concentration, a lot of reverse micelles were formed in

the oil phase. After the oil phase is dispersed in the aqueous phase, the reverse micelles in the oil droplets could absorb water from the aqueous phase and formed bicontinuous structure. The water phase in the oil droplets formed pores after polymerization. The authors observed that whereas the surfactant was important for the formation of larger pores, the smaller pores were related to the nature and amount of diluents.

Freeze-thaw technique

Cryogels are gel matrices that are formed in moderately frozen solutions of monomeric or polymeric precursors. Cryogels typically have interconnected macropores (or supermacropores), allowing unhindered diffusion of solutes of practically any size, as well as mass transport of nano- and even microparticles. One of the new types of polymer gels with considerable potential in biotechnology is 'cryogels' (from the Greek krios (kryos) meaning frost or ice. Cryogels are formed as a result of cryogenic treatment (freezing, storage in the frozen state for a deûnite time and defrosting) of low- or high- molecular-weight precursors, as well as colloid systems all capable of gelling[24]. Figure 3 shows SEM images of cryogels and hydrogels. The main characteristic features of the cryotropic gelation processes[24]

(i) The reaction mixture containing gel-forming agents is frozen at temperatures a few degrees centigrade below the solvent crystallization point. The frozen system, despite looking as a single solid block, remains essentially heterogeneous and contains so-called unfrozen liquid microphase (UFLMP) along with the crystals of the frozen solvent.

(ii) Gel-forming reagents are concentrated in UFLMP, that is, cryoconcentration takes place. As UFLMP presents only a small portion of total initial volume, the concentration of gel precursors increases dramatically promoting the gel-formation. In fact, owing to cryo-concentration, the gel formation in such frozen systems proceeds sometimes faster than in liquid medium, when using the same initial concentration of precursors.

(iii) The crystals of frozen solvent perform as a pore-forming agent. When melted, they leave voids, macropores ûlled with the solvent. The surface tension between solvent and gel phase rounds the shape of the pores, making pore surface smoother.

(iv) When freezing, the solvent crystals grow till they meet the facets of other crystals, so after thawing a system of interconnected pores arises inside the gel. The dimensions and shape of the pores depend on many factors, the most important are the concentration of precursors and the regimes of cryogenic treatment.

(v) The polymer phase of the cryogel has, in turn,

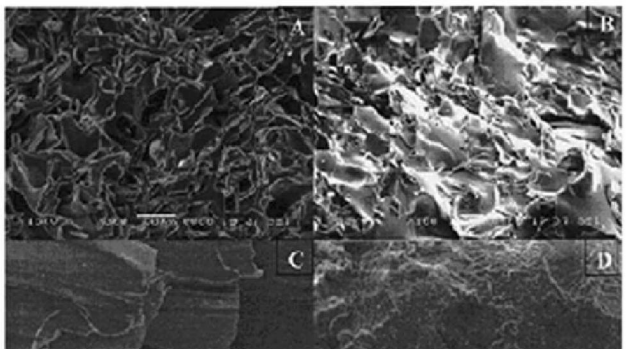

Figure 4 : SEM of the cryogels (A,B) and the hydrogels (C,D).

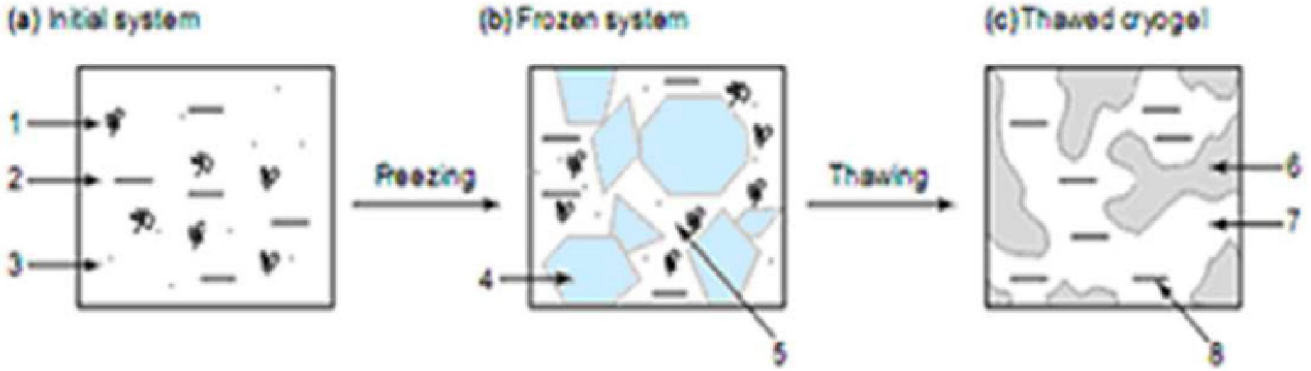

Figure 5 : 1 Macromolecules in a solution, 2 Solvent, 3 Low molecular solutes, 4 Polycrystals of frozen solvent, 5 Unfrozen liquid microphase, 6 Polymeric framework of a cryogel, 7 Macropores, 8 Solvent (A) Initial system (B) Frozen system (C) Thawed cryogel.

micropores formed in between the polymer chains. Thus, cryogels have both heterophase and heteroporous structure (Figure 5).

Advantages of freeze-thaw method

The hydrogels which were prepared from repeated freezing–thawing process were porous, spongy, rubbery and elastic and displayed good mechanical strength[25].

- Hydrogels show increased mechanical strength over most hydrogels because the crystalline regions are capable of better distributing a given mechanical load or stress.
- The gel show high elasticity and are capable of being extended to five or six times their initial length.
- For the entrapment and encapsulation of labile bioactive substances and living cells, physically crosslinked gels are of great interest especially when the gel formation occurs under mild conditions in the absence of organic solvents.
- Freezing thaw method free from toxicity issue because no initiator and crosslinking agents are used.
- Due to macro porous nature they can be used in delivery of macromolecule such as insulin etc without changing chemical nature of entrap compound.

Poly (vinyl alcohol) (PVA) gels that are prepared by freezing and thawing techniques have shown many improved properties over hydrogels prepared by traditional chemical crosslinking techniques. It has been shown that repeated cycles of freezing at –20°C and thawing at 25°C result in the formation of crystalline regions that remain intact upon being placed in contact with water or biological fluids at 37°C. These PVA hydrogels show increased mechanical strength over most hydrogels because the crystalline regions are capable of better distributing a given mechanical load or stress. Additionally, the gel show high elasticity and are capable of being extended to five or six times their initial length. Because of these characteristics, the potential for such materials for a variety of biomedical and pharmaceutical application is quite obvious[26].

Solid freeform fabrication technique

Although the methods discussed previously have their own advantages and disadvantages, there are certain limitations, which cannot be overlooked and deserve attention. For example, almost all synthetic procedures are labor intensive; result in highly inconsistent macro- and micro- structural and material properties, involve toxic organic solvents, tedious and time consuming, and above all they do show shape limitations such as limited small pore sizes and interconnected pores etc. Thus, there must be engineered an approach which could promise not only to resolve the limitation problems effectively but also fabricate macroporous polymers of desired architecture and properties.

The introduction of solid freeform fabrication (SSF) technologies signals the start of a new revolutionary era for product design and manufacturing industries[27]. Also

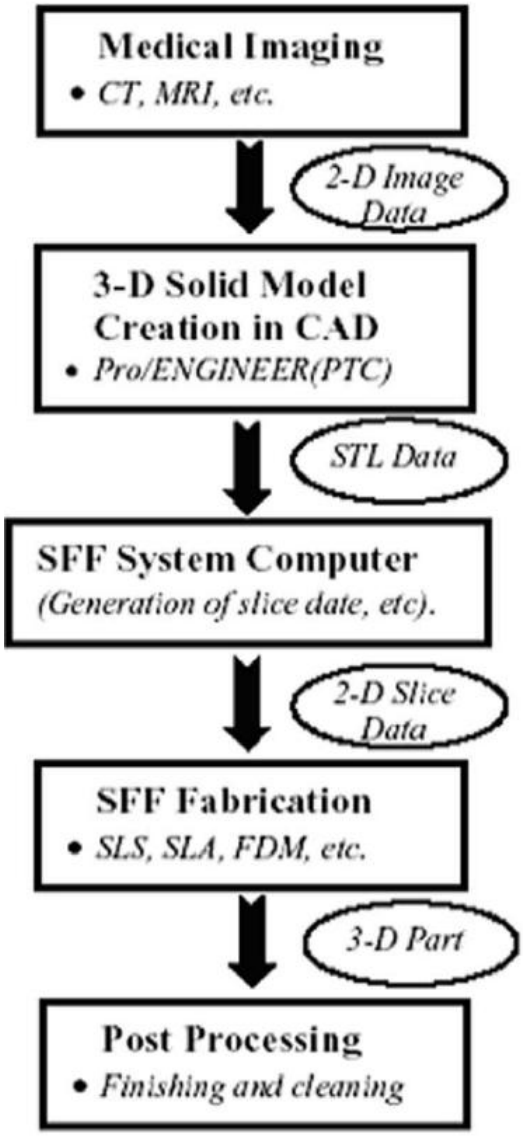

Figure 6 : A schematic chains showing solid freeform fabrication process[27].

called as Rapid prototyping (RP), SSF fabrication technologies represent a group of techniques that model a macroporous scaffold directly from a computer-aided design (CAD) data set. They are also regarded as "watershed" events[28] as they deliver tremendous time and cost savings with their application. SSF techniques are computerized fabrication techniques that can rapidly produce highly complex three-dimensional physical objects using data generated by CAD systems, computer-based medical imaging modalities, digitizers and other data makers[29]. hey build up a specific body shape by the selective addition of material, layer-by-layer, guided by a computer program[30]. The step-by-step construction of macroporous polymers facilitates improved reproducibility. In contract to conventional fabrication methods, parameters such as porosity, pore size and geometric stability, can be controlled more precisely[27]. Unlike conventional computerized machining processes which involve the removal of materials from a stock, SFF techniques use the underlying concept of layered manufacturing[31] whereby three dimensional objects are fabricated with layer-by-layer building via the processing of solid sheet, liquid or powder material stocks. The typical process chain for all SFF techniques is presented in Figure 6.

The techniques of SFF are quite outstanding and offer enormous capabilities of manufacturing which enable them to be employed for biomedical applications ranging from the production of scale replicas of human bones[32] and body organs[33] to advanced customized drug delivery systems[34] and other areas of medical sciences including anthropology[35], paleontology[36] and medical forensics[37]. It is worth mentioning here that although the applications of SFF for biomaterials formation is not yet widespread, its immense potential for producing porous biomedical polymers with highly complex macro- and microstructures is widely recognized and is receiving vast interests and attention from many researchers.

Advantages some significant advantages from SFF techniques may be outlined as below

A. Specific designs

Making use of CAD models it is possible to fabricate biomaterials with complex design and specific requirements of the patients.

B. Anisotropic microstructure

The SFF and CAD techniques allow to fabricate a porous material which could have different microstructure at different regions so that its multifunctional performance could be achieved which can not be realized by other conventional techniques. Thus having an anisotropic microstructural design is advantageous from viewpoint of many specific applications of the porous material.

C. Controlled fabrication

Since the technique makes use of automated computerized fabrication, it is always easy to produce high throughput production with minimal manpower requirements. The specialty of SFF technique is to have high build resolution with the ability to control individual process parameters make it an attractive tool to fabricate complex biomedical architectures with consistent pore morphologies.

D. Amicable processing conditions

The novelty of the SFF technique rests upon the fact that a wide variety of process conditions are employed and none of the process involves either organic solvents or other toxic chemicals. Moreover, fabrication is carried out at room temperature so that the bioactive agents may be safely impregnated into the biomaterial devices[38].

Fiber bonding (unwoven meshes)

Scaffolds based on the use of PGA fibers were some of the earliest constructs proposed in tissue engineering[39-40]. These fibers, if bonded together in three-dimensions, provide large surface area for cell interaction and growth. The fibers can be attached to each other via two different techniques. In the first, developed by Mikos et al.[40], PGA fibers are immersed in a PLLA solution. When the solvent evaporates, the network of PGA fibers is embedded in PLLA. The composite is then heated to above the melting temperature of both polymers. The PLLA melts first and fills all voids left by the fibers. This helps retain the spatial arrangement of fibers so that when the PGA begins to melt, the fiber structure does not collapse. Instead, in order to minimize interfacial energy, fibers at the cross-points become "welded" (melted) together, forming highly porous foam. The PLLA is then removed by dissolution

with methylene chloride. This fabrication technique results in foams with porosities as high as 81% and pore diameters of up to 500 mm. Hepatocytes cultured for one week in these foams remained alive and began to interact with each other to form clusters[41]. A second method for bonding PGA fibers uses atomization of PLLA or PLGA to coat the fibers. PLLA or PLGA is dissolved in chloroform and sprayed onto the PGA fibers[42]. Since PGA is only weakly soluble in chloroform, the fibers remain unchanged during this process. The solvent is then evaporated, leaving the fibers glued with PLLA or PLGA. Although porosities were not reported, pore sizes similar to those of the previous technique were attained. When tubes made in this manner were implanted in rats for 17 days, fibrous tissue in growth was observed, indicating that constructs with these physical properties could encourage neotissue formation[42]. Although fiber bonding techniques produce highly porous scaffolds with interconnected pores that are suitable for tissue regeneration[39-40,42], both methods involve the use of solvents that could be toxic to cells if not completely removed. In order to extract these chemicals, the constructs must be vacuum dried for several hours, making it difficult to be used immediately in a clinical setting. In addition, the first method involves heating to high temperatures. The combination of toxic chemicals and extreme temperature presents difficulties if cells or bioactive molecules, such as growth factors, are to be included in the scaffold during processing.

Solvent casting/particulate leaching

Another method to create pores involves the use of a water- soluble porogen, such as salt (NaCl)[41,43]. The first step in this process is to dissolve the polymer (PLLA or PLGA) in chloroform or methylene chloride and then cast it onto a petri dish filled with the porogen. After evaporation of the solvent, the polymer/salt composite is leached in water for two days to remove the porogen. The resulting scaffold's porosity can be controlled by the amount of salt added, while the pore size is dependent on the size of the salt crystals. With 70 weight percent salt and above, the pores exhibited high interconnectivity[41]. Foams fabricated in this manner have been used extensively with various cell types and have shown no adverse effects on new tissue formation[39,43-46]. However, due to concerns that the side of the foam exposed to air had a different morphology (rougher) than that exposed to the petri dish, a modification of this technique has been developed[46-47]. In this case, pieces of the polymer/salt composite are compression molded into cylindrical form at temperatures just above the melting (PLLA) or glass transition temperature (PLGA). The cylinder is then cut into discs of desired thickness before leaching in water. This allows more precise control of scaffold thickness and increases uniformity of the foam surface. However, thermal degradation of the polymer during the compression molding step could be a concern. In an alternate form of the particulate leaching method, Shastri et al.[48] recently reported the fabrication of PLLA and PLGA scaffolds with up to 87% porosity and pores well over 100 mm in diameter using waxy hydrocarbons as porogens. After mixing the porogen and polymer (dissolved in methylene chloride or chloroform) into a paste, the composite is packed in a Teflon mold. The mold is immersed in a hydrocarbon solvent (pentane or hexane) to remove the wax without dissolving the PLLA/PLGA. The remaining foam is vacuum-dried for several days to extract any solvents. Thick samples (up to 2.5 cm) with interconnected pores can be created using this technique. This method also offers the possibility of adding a particulate phase to the paste to increase the strength or electrical conductivity of the final structure. When blended with polyethylene glycol (PEG) and seeded with bovine chondrocytes for four weeks, formation of cartilage-like tissue is seen in these foams, demonstrating their biocompatibility. With any solvent casting/particulate leaching procedure, organic solvents are used, which in many cases precludes the possibility of adding pharmacological agents to the scaffold during fabrication. Also, the leaching step for water-soluble porogens significantly increases the scaffold preparation time. However, in applications where prefabrication of cell-polymer constructs is suitable, promising results using a large range of cell types make these scaffolds very appealing.

Gas foaming

In order to eliminate the need for organic solvents in the pore-making process, a new technique involving gas as a porogen has been introduced[49]. The process

begins with the formation of solid discs of PGA, PLLA or PLGA using compression molding with a heated mold. The discs are placed in a chamber and exposed to high pressure CO2 (5.5 MPa) for three days, at which time the pressure is rapidly decreased to atmospheric pressure. Porosities of up to 93% and pore sizes of up to 100 mm can be obtained using this technique, but the pores are largely unconnected, especially on the surface of the foam. While this fabrication method requires no leaching step and uses no harsh chemical solvents, the high temperatures involved in the disc formation prohibit the incorporation of cells or bioactive molecules and the unconnected pore structure make cell seeding and migration within the foam difficult. Another approach to using gas a porogen was recently developed by Nam et al.[50-51]. This technique includes both gas foaming and particulate leaching aspects. Ammonium bicarbonate is added to a solution of polymer in methylene chloride or chloroform. The resultant mixture is highly viscous and can be shaped by hand or with a mold. The solvent is then evaporated and the composite is either vacuum dried or immersed in warm water. Vacuum drying causes the ammonium bicarbonate to sublime while immersion in water results in concurrent gas evolution and particle leaching. The latter method is preferred because it does not result in the creation of a nonporous outer skin, as seen in the vacuum-dried scaffolds. Porosities as high as 90% with pore sizes from 200-500 mm are attained using this technique. Rat liver cells were seeded in these foams and up to 40% remained viable over one week in culture, suggesting the scaffolds are biocompatible and facilitate adequate nutrient exchange[51]. In addition, the putty-like consistency of the polymer-salt mixture could be useful in molding constructs during surgery. However, concerns about the use of organic solvents and the long-term effects of residues of ammonium bicarbonate on cells may prevent these scaffolds from soon being employed in "on the spot" tissue engineering.

Thermal induced phase separation method

Thermally induced phase separation was first applied to PLA scaffolds[52]. Although several authors have applied this technique to composite scaffolds also[53]. It consists of inducing a solid-liquid or liquid-liquid phase separation which is done by dissolving the polymer in a solvent and quenching the solution at a certain temperature. Quenching induces a phase separation into a polymer-rich and a polymer-poor phase. The solvent must then be removed from the phase separated solutions either by freeze-drying, or by solvent extraction. 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. Its disadvantages include the use of

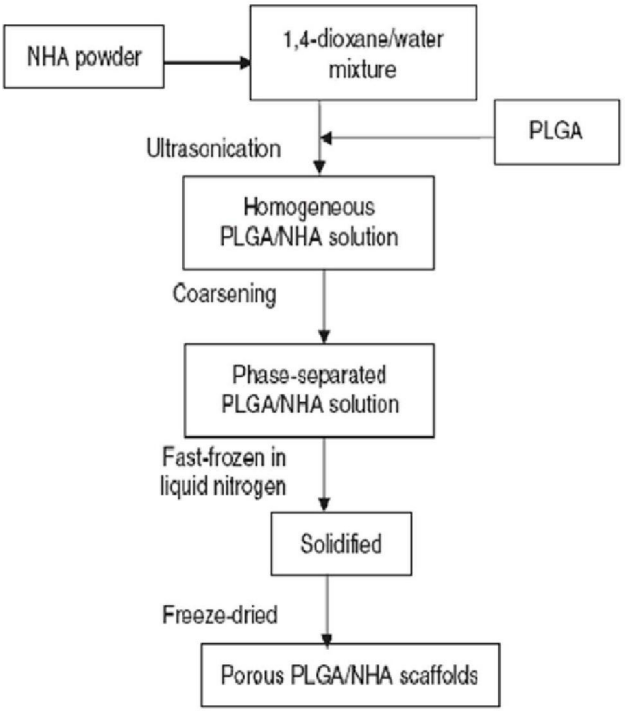

Figure 7 : The preparation of PLGA/NHA scaffolds by TIPS[56].

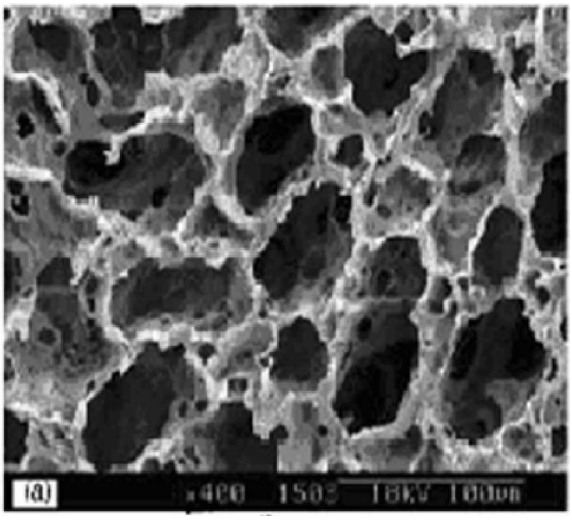

Figure 8 : SEM micrographs of PLGA/NHA scaffolds prepared from PLGA/dioxane/water[56].

potentially toxic solvents and a high degree of anisotropy of the porosity. The latter may actually be beneficial for certain biomedical and industrial applications such as nerve regeneration, filtration membranes, mechanically damping materials, packaging etc[54].

The TIPS technique provides a scaffold with a uniform pore size and high degree of interconnection, good mechanical properties and various morphologies. The morphology can be controlled by several experimental parameters such as the quenching temperature, quenching rate, quenching period or aging time, polymer concentration, solvent to non-solvent ratio, molecular structure, and added surfactant or porogen[55]. The regular and highly interconnected porous polyLactide-co-glycolic acid (PLGA) / nano hydroxyapatite (NHA) scaffolds have been fabricated[56]. he effect of solvent composition, polymer concentration and coarsening time as well as NHA content on micro morphology, mechanical properties of PLGA / NHA have also been investigated. he results show that pore size scaffolds surface is developed for efficient bone tissue engineering. The preparation of scaffolds can be divided into two parts, the first is about determination of cloud point temperature solution and the second is about the preparation of scaffolds. Figure 7 is the flow chart process of TIPS techniques. The SEM micrograph (Figure 8) of PLGA / NHA scaffolds shows that most of the pores are connected with their neighbors and have many small pores on the big pore's wall which entitles the foam's novel connectivity and makes it more suitable to serve as scaffolds for cell seeding and nutrients transporting in tissue engineering.

A regular and highly interconnected macroporous poly (l-lactic acid) (PLLA) scaffold was fabricated[57] from a PLLA – dioxane – water ternary system with added polyethylene glycol (PEG)–PLLA diblocks. The morphology of the scaffold was investigated in detail by controlling the following TIPS parameters: quenching temperature, aging time, polymer concentration, molecular structure, and diblocks concentration. The regular and highly interconnected macroporous scaffolds fabricated ranging in size 50 to 150 μm were fabricates from PLGA - dioxane - water ternary systems without any surfactant or other additive[58]. The effect of scaffolds morphology on processing parameters including quenching temperature, polymer concentration, solvent composition and molecular weight was investigated as a function time.

In principle the final porous morphology is thought to rely on the thermodynamic state of the solution to be quenched, as schematized in the temperature-composition phase diagram in Figure 9.

A nucleation and growth mechanism of phase separation results in a poorly connected stringy or beady structure. In contrast, spinodal phase separation is expected to give rise to a highly interconnected structure. The initial pore size is determined by the concentration fluctuations induced by the quenching, the polymer con-

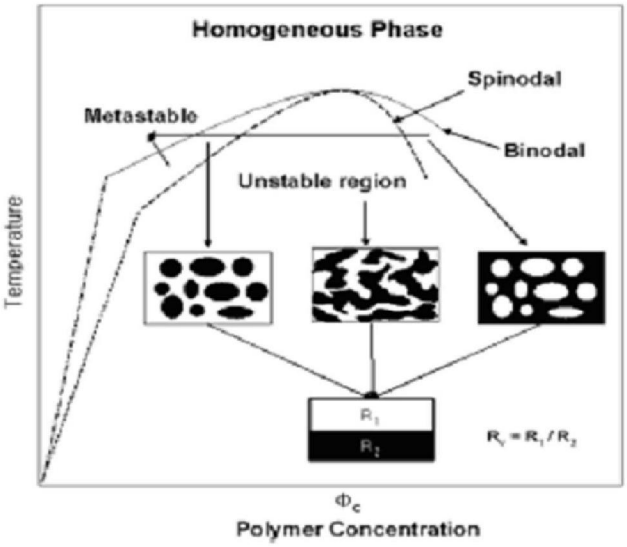

Figure 9 : Schematic presentation of a typical polymer-solvent-nonsolvent ternary phase diagram (R1: polymer lean phase, R2: polymer rich phase, Fv: volume ratio[58].

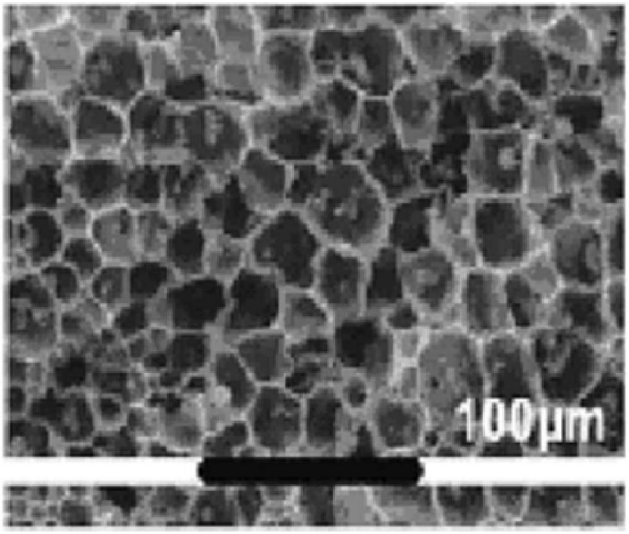

Figure 10 : SEM images of the scaffold prepared from PLGA/ PLLA solution in dioxane/water with additive[59].

centration in the solution, and fluctuations associated with thermal flow. Therefore, the final porous morphology can be controlled to some extent either by varying processing parameters such as the quenching rate, quench temperature exposed to the atmosphere, and quenching period, or by varying formulation parameters such as the polymer concentration, solvent composition, crystalline and molecular.

Poly (L-lactic acid) (PLLA) was blended with PLGA to increase the viscosity of polymer solution; a block copolymer of poly (ethylene glycol) (PEG) with PLGA was added as a surfactant to decrease the interfacial tension between the polymer-rich and polymer-lean phases. The effect of TIPS parameters including the concentration of diblocks copolymer and PLGA/PLLA ratio was also studied[59]. By these fabrication methods a regular and well connected macroporous ranging from 50 to 200 μm was prepared and their morphology was controlled by the process parameters such as quenching temperature, aging time, polymer concentration, composition and length of diblocks. The combination of these two methods yields a new method to prepare open, regular and well inter connected macroporous scaffolds of PLGA (Figure 10)

Freeze-drying of Polylactide solutions in 1, 4 dioxane has been studied as a way to produce microcellular foams[60]. The thermally induced phase separation has been studied in relation to several processing and formulation parameters. The effects of several processing and formulation parameters such as polymer concentration, chain stereoregularity, polymer molecular weight and cooling rate have been investigated in connection with the porous morphology and the physico-mechanical characteristics of the final foams. As a rule, bundles of channels are formed with a diameter of 100 μm. They have a preferential orientation that fits the cooling direction. A porous substructure (10 μm) is observed in the internal walls of the tubular macropores.

TIPS is[61] also used for the fabrication of porous foams based on various biodegradable polymers of poly (L-lactic acid) and its copolymers with D-lactic acid and/or glycolic acid. Diverse foam morphologies were obtained by systematically changing several parameters involved in the TIPS process, such as polymer type and concentration, coarsening conditions, solvent/ non solvent composition, and the presence of an additive. The produced foams had microcellular structures with average pore diameters ranging from 1 to 30 μm depending on the process parameters, which were characterized by scanning electron microscopy (SEM) and mercury intrusion porosimetry. Additionally, Pluronic F127 was used as an additive porogen to control the pore geometry and size. In particular, the addition of polymeric surfactant in the TIPS formulation enhanced the size of pores and improved their inter-connectivity. The prepared foams could find applications in controlled drug delivery.

A method for preparing regular and highly interconnected macroporous poly(l-lactic acid) (PLLA) scaffolds[62] with pore sizes ranging from 50 to 300 μm were fabricated from a PLLA/1,4-dioxane/water ternary system via a thermally induced phase separation (TIPS) in the presence of small amounts of NaCl. The addition of salt has raised the cloud-point temperature because of a salt-out effect. Spinodal decomposition was induced at a higher temperature with a large quench depth. Gelation due to the crystallization of PLLA was prevented to a large extent, which led to the creation of highly interconnected macropores required for effective cell penetration. An optimal quenching route was found for the fabrication of well-designed macroporous structures.

APPLICATIONS OF MACROPOROUS MATERIALS

In many biotechnological and biomedical applications a biocompatible and hydrophilic structure with desired porosity is required which are best provided by a macroporous matrix of polymeric nature. Thus, some specific prominent applications of macroporous materials may be briefly discussed as below:

Bioseparation

Interconnected systems of macropores and sponge-like morphology are typical for cryogels, allowing unhindered diffusion of solutes of practically any size. Most of the water present in spongy cryogels is capillary bound and can be removed mechanically by squeezing. The properties of cryogels can be regulated by the temperature of cryogelation, the time the sample is kept in a frozen state and freezing/thawing rates, by the na-

ture of the solvent and by the use of soluble and insoluble additives. The unique macroporous morphology of cryogels, in combination with osmotic, chemical and mechanical stability, makes them attractive matrices for chromatography of large entities such as protein aggregates, membrane fragments, viruses, cell organells and even whole cells. Special attention is given to immunosorption of viruses on cryogel-based sorbents. As chromatographic materials, cryogels can be used both in bead form and as spongy cylindrical blocks (monoliths) synthesized inside the chromatographic column. The macroporous nature of cryogels is also advantageous for their application as matrices in the immobilization of biocatalysts operating in both aqueous and organic solvents[63]. Chromatography, both analytical and large-scale, is the predominant technology in downstream separations. Traditional packed-bed chromatography with immobile stationary phase, despite its elegance and high resolving power, has a major limitation: incapability of processing particulate-containing ûuids, for example, cell suspensions or non-clariûed crude cell homogenates. Particulate material is trapped between the beads of the chromatographic carrier resulting in increased ûow resistance of the column and complete blockage of the ûow. To address this drawback, expanded-bed chromatography has been proposed[64]. However, despite all its advantages, expanded bed chromatography requires a special type of columns and equipment and cannot be ûtted in traditional packed bed chromatographic systems. The porosity of cryogels makes them appropriate candidates as the basis for such supermacroporous chromatographic materials. For instance, a continuous chromatographic column based on cryoPAAG with grafted metal chelating iminodiacetic ligands has been prepared[65]. Owing to supermacroporosity and interconnected pore-structure, such a chromatographic matrix has a very low ûow resistance. Water passes freely through the 4 cm high column at linear ûow rate (volumetric ûow rate divided by cross-section area of the column) of, 750–2000 cm h^{-1} at a pressure of, 0.01 MPa. For comparison, HPLC operates at ûow rates of 300–1700 cm h^{-1} at excessive pressures of 2–10 MPa[66] and expanded bed chromatography at ûow rates of 200–400 cm h^{-1} at excessive pressure, 0.01 MPa[64]. Thus, it is reasonable to assume that continuous supermacroporous beds produced by the cryotropic gelation would allow chromatographic process at ûow rates comparable with those in HPLC and exceeding those used in expanded bed chromatography, while using only minimal pressures typical for low-pressure protein chromatography.

Pore size in traditional chromatographic adsorbents is usually so that 95% of convective ûow takes place in the liquid in between the beads of the chromatographic matrix. Even in the ideal case of most densely packed spheres, the interparticle volume is,27%of the total column volume in practice it is higher owing to the irregularities of size and shape of the beads. To improve the convectional transport, the columns with large pore size, as well as minimal dead volume, are required. The enforced convectional transport is realized in monolithic columns[67-69].

Macroporous inorganic chromatographic matrices, similar to porous glasses, seem to meet these requirements. They are available with a variety of pore sizes in the micrometer range, mechanically stable and sustain thermal sterilization. Although the use of these matrices for virus-speciûc immunosorbents has been reported[70], the brittleness, pronounced non-speciûc sorption and chemical instability in alkaline media limit their application. Thus, cryogels present a very interesting chromatographic material allowing the direct separation of proteins from unprocessed crude extracts or even from fermentation broth in the case of extracellulary expressed proteins. Cryogel-based chromatographic materials open a whole new area of bioseparation, in which bioparticles, similar to viruses, microbial cells and even mammalian cells, are isolated and separated in chromatographic mode. In recent years, a new and promising affinity-based cell separation chromatography technique has been developed using highly interconnected supermacroporous cryogels. Due to the importance and relevance of cell-based therapy and stem cell separation in the present scenario, cryogels present a new tool for preparative scale cell separation[71].

Adenosine triphosphate (ATP) is an important high-energy compound widely used in biological and therapeutic fields. It can be produced by phosphorylation of adenosine monophosphate (AMP) with microbial cells in industrial scale and the effective isolation of ATP from microbial fermentation broth is a challenging work. In this work, Yun et al[72] developed a

novel one-step method to directly separate ATP from fermentation broth of Saccharomyces cerevisiae by anion-exchange chromatography using super-macroporous cryogel.

Bioreactor

Separation into stable aqueous compartments is a promising concept to enable the synthetic application of biocatalysts in unfavorable non-aqueous media, and might provide the key for successful application of complex multistep synthesis. This study describes and evaluates the practical utility of polyvinyl alcohol (PVA) cryogels as a matrix for such compartmentation. The protocol for enzyme entrapment in the gels was highly efficient, giving an immobilization yield of >99% and a total loss of material less than 10%. The resulting gel compartments were between 2.2 and 4 mm in size and had high mechanical strength. Shrinking occurred in solvents with the ability for water uptake. The matrix enabled the synthetic use of benzaldehyde lyase and alcohol dehydrogenase in hexane, in which equilibrium concentrations were comparable to a conventional two-phase system. The results suggest a general suitability of PVA gels for the compartmentation of biocatalyzed reactions in non-aqueous media[73].

Enzyme immobilization

Immobilization of enzymes has attained significant interest for the separation of the enzymes from reaction mixtures. It imparts greater stability to the enzyme, allowing greater use in the development of continuous processing, enabling greater control of the catalytic process and, therefore, an economical utilization of an otherwise cost-prohibitive enzyme application. Many porous polymers have been studied extensively as supports for immobilization as the porosity of these polymer supports has a direct bearing on diffusion of substrate and product through them, which affects rate of enzymatic conversion. It is well recognized that presence of meso and /or macrospores is a key requirement to minimize diffusional limitations[74]. Reactive functional groups of macroporous matrix assist the formation of permanent covalent bond between the support and enzyme without affecting its tertiary structure. A number of such macroporous polymer supports are cited in literature with different types of enzymes[75].

Tissue engineering

The incidence of organ and tissue loss or failure is increasing steadily, whereas the traditional surgical treatment of implantation of a healthy organ from a donor is limited by immune rejection and the number of donors. As an application of tissue engineering, the use of cell transplantation is now being investigated as an alternative therapeutic strategy for tissue repair and organ replacement[76]. In culturing of the cells, shape of the scaffold, a temporary substrate to allow growth and specialization of the cell culture, plays an important role[77]. Polymeric scaffolds must be porous enough to allow a high density of cells to be seeded, yet also possess sufficient mechanical stability and a well defined network of interconnected pores to permit growth into the implanted structure. Thus, macroporous polymer materials meet all the desired qualities and, therefore, have been extensively utilized in tissue engineering[78].

The intrinsic material properties such as mechanical[79] or thermal[80] and structural features like pore size, amount of porosity, morphology etc. play a vital role in shaping suitability of the macroporous materials for a specific application. For instance, from tissue engineering viewpoint the optimal pore size should be 20 μm for the ingrowths of fibroblasts and hepatocytes, between 20 and 150 μm for the skin repensration, and in the range of 100-150 μm for bone regeneration[81]. Similarly when the pore size of polymer particles lays in the range 100-150 A^0 a longer time will be required to separate the components because of their slow diffusion rate[82]. It is, therefore, recommended that the size of the pores must be almost 10 or 20 times of solute molecular size.

Thus, for applications of macroporous polymers as scaffolds in tissue engineering the following macro and micro structural properties have to be taken into consideration while designing synthetic routes of the porous polymers[83]. Figure 11 shows SEM micrograph of a salt-leached scaffold used to create the macroporous architecture. (*d* and *e*) SEM micrographs of an isotropic hydrogel (*d*) and a macroporous hydrogel (*e*).[84].

Controlled drug delivery

Both the conventional macroporous polymers and recently designed High Internal Phase Emulsion Poly-

Review

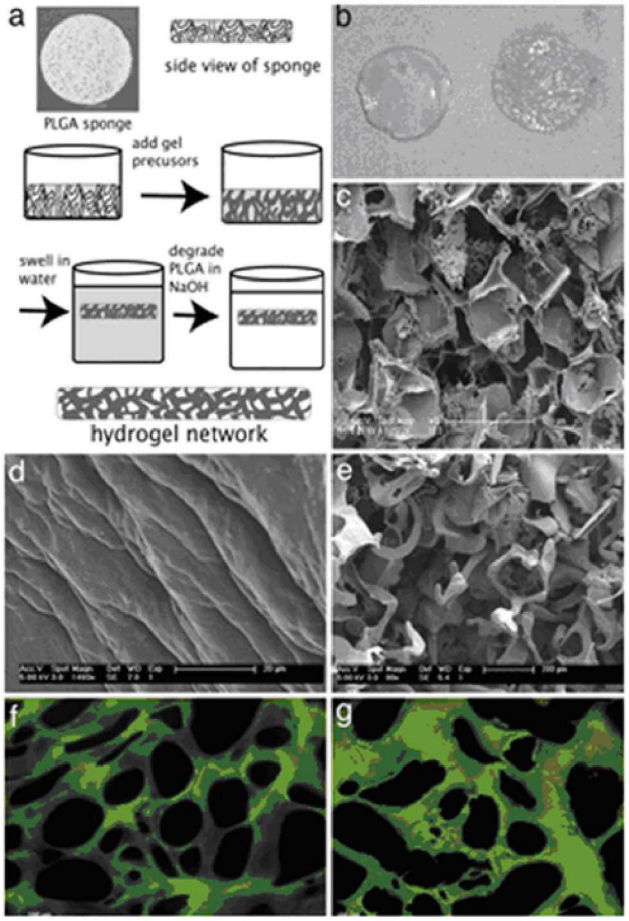

Figure 11 : Formation of the macroporous hydrogel. (*a*) Schematic of the macroporous process. (*b*) Photograph of isotropic hydrogel (*Left*) and macroporous hydrogel (*Right*). The gels are 5 mm in diameter. (*c*) SEM micrograph of a salt-leached scaffold used to create the macroporous architecture. (*d* and *e*) SEM micrographs of an isotropic hydrogel (*d*) and a macroporous hydrogel (*e*). The macropores, created by casting the hydrogel around the salt-leached scaffold, are evident. (*f* and *g*) Cross sections of the isotropic (*f*) and macroporous (*g*) hydrogel labeled with FITC to demonstrate the pore structure of the gels. The FITC labels the amines in the polylysine component of the hydrogel[84].

mers (HIPE) have been used in achieving zero order release dynamics for a variety of drugs[85]. Whereas the former architecture contains irregular pores of Angstrom dimensions that terminate within solid matrix, the later one contain large cavities of micrometer dimensions. Another significant difference is that in the conventional macroporous matrix total porosity is typically 50% while in the later matrix it is more than 70%. part from these applications the macroporous polymers find multitude application in biomedical and pharmaceutical fields.

OUTLOOK

The concept of supermacroporous gels offers many interesting possibilities to the biotechnologist. Separation of particulate matter, capturing of soluble material in particle containing media as well as preparation of efûcient immobilized biocatalysts are the areas of applications that will become more abundant when the gels become more easily available.

The macroporous polymers have shown their potential in biomedical and allied fields and emerged as one of the most promising materials with high performance applications. However, at present the regenerative biology has shown promise to be one of the biomedical revolutions of the current century. It includes implantation of bioartificial tissues, cell transplantation and stimulation of regeneration from residual tissues in vivo. To achieve these approaches macroporous polymeric materials with tailor made functional groups could be of great use and may enable researchers to explore the interfaces of living tissues and biomaterials. Thus, designing new and newer materials with multifunctional activities poses challenges to polymer chemists and engineers who have to be aware of the intimate relation between the synthetic approaches of macroporous matrices and their structural architectures which are the ultimate factors to control and decide the performance of the material.

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