Review Article

Preparation of Porous Biodegradable Polymer and Its Nanocomposites by Supercritical CO_2 Foaming for Tissue Engineering

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Using supercritical carbon dioxide ($scCO_2$) as an alternative to conventional methods in the preparation of porous biodegradable polymer and polymer/nanocomposites for tissue engineering has attracted increasing interest in recent years due to the absence of using organic solvents and the ability to incorporate thermosensitive biologicals without loss of bioactivity. Additionally, $scCO_2$ can exert a high level of control over porosity and morphology of scaffolds by tuning the processing parameters. This paper describes the newly achievements on the preparation of porous polymer materials using $scCO_2$ foaming technology with focus on the porous biodegradable materials and its nanocomposites relevant to tissue engineering.

1. Introduction

Tissue engineering involves the use of cells to repair the damaged biological tissue, leaving only natural substances to reestablish organ function [1, 2]. One of the most important challenges in tissue engineering is the appropriate design and fabrication of porous, biodegradable, and biocompatible scaffolds made from polymers or its composites that can be utilized as temporary three-dimensional (3D) extracellular matrix analog for cells to grow into a new tissue [1–4]. Material, microstructure, and mechanical properties have all been shown to significantly affect scaffold bioactivity. Scaffolds must meet certain fundamental characteristics although the substitution of different biological materials requires different properties: [1, 2, 4–8] (1) high regular and reproducible 3D structure, (2) appropriate pore size for the specific tissue to be replaced, (3) sufficient porosity and high interconnectivity for uniform cell seeding, distribution, function, and tissue regeneration, (4) suitable surface chemistry for cell adhesion, proliferation, and differentiation, (5) adequate mechanical stability to maintain the predesigned tissue structure, and (6) good biodegradability, biocompatibility, and a tuned degradation rate to match the rate of the neo-tissue formation.

Numerous methods have been developed to generate highly porous biodegradable polymer or its composites for tissue engineering, which include phase separation, emulsion freeze-drying, solvent casting/salt leaching, fiber forming, and 3D printing [5, 9–14]. These conventional methods, however, generally involve the use of potentially harmful organic solvents in the fabrication process. Therefore, further purification and drying steps are often needed. On the other hand, the organic solvents may not be able to completely remove even after long time postprocessing. The residues of organic solvent remained in the matrix may promote bioincompatibility and inactivity of the growth factors. Moreover, some of these methods are often employed at high temperatures, which may degrade the incorporated thermosensitive components, such as pharmaceutical drugs and bioactive compounds. It is clearly desirable to use clean and environmentally friendly techniques operated under mild conditions for the processing of biodegradable materials for tissue engineering [15, 16].

Carbon dioxide (CO_2) used as a plasticizer and foaming agent to create 3D scaffolds is an attractive choice to overcome these limitations [16, 19, 22, 23]. The use of supercritical CO_2 ($scCO_2$) in the processing of biodegradable polymers

for tissue engineering has led to an increasing interest, and remarkable progress has been achieved over the last decade. In this paper, the applications of $scCO_2$ foaming technology in the preparation of biodegradable porous polymer, especially its use in generation of porous nanocomposite materials for tissue engineering will be introduced.

2. Supercritical CO$_2$ Foaming

Supercritical fluid (SCF) has received much attention and is currently being used as an environmentally friendly processing solvent for a range of polymer applications such as polymerization, purification, and fractionation, extraction, coating, blending, modification, composites formation, particle production, and foam generation [22, 23]. A supercritical fluid (SCF) can be defined as a dense phase, which is in a state above its critical temperature (T_c) and critical pressure (P_c) (Figure 1). At the critical point, liquid and gas coexist, and SCF shows unique properties of both gaseous and liquid states that are different from those of either liquid or gas under standard conditions. In general, SCF has similar solvating power to liquid solvents but offers mass transfer advantages over conventional organic solvents because of its gas-like diffusivity, liquid-like density, high compressibility, low viscosity, and low surface tension. Moreover, the physicochemical properties such as diffusivity, viscosity, polarity, and solvent strength of SCF can be easily tuned over a wide range by varying the system pressure and/or temperature.

CO_2 is the most commonly used supercritical fluid due to its many desirable attributes such as nontoxic, nonflammable, noncorrosive, abundant, inexpensive, commercially available in high purity, and readily accessible supercritical conditions (T_c = 31.1°C and P_c = 7.37 MPa) [22]. $scCO_2$ combines gas-like diffusivity with the liquid-like density and solvent power, which makes it a unique medium for polymer synthesis and processing. Especially its low critical temperature allows it very desirable for processing thermolabile compounds, such as pharmaceutical drugs and other bioactive factors. It was demonstrated that mammalian cells can survive for 5 min within $scCO_2$ (35°C, 7.4 MPa) [24].

Although $scCO_2$ is a weak solvent for polymers except for some fluoropolymers and silicones [25], it has substantial solubility in amorphous and semicrystalline polymers. The absorption of CO_2 in polymers can result in the decrease of glass transition temperature (T_g), viscosity, interfacial tension, and increase of polymer chain flexibility because of an increase in the free volume fraction.

Using $scCO_2$ to fabricate porous polymer materials is a well-documented process. In order to produce polymer foams, gas bubbles must nucleate and grow within the molten or plasticized polymer. In this process, firstly amorphous or semicrystalline polymer is saturated with CO_2 at a certain temperature and pressure. With the diffusion of gas into polymer matrix, it forms single-phase polymer/CO_2 solution [22]. The level of CO_2 sorption depends on the conditions such as temperature, pressure, and the intermolecular interactions between CO_2 and polymer [26, 27]. As a result, the density and viscosity of polymer reduce and the
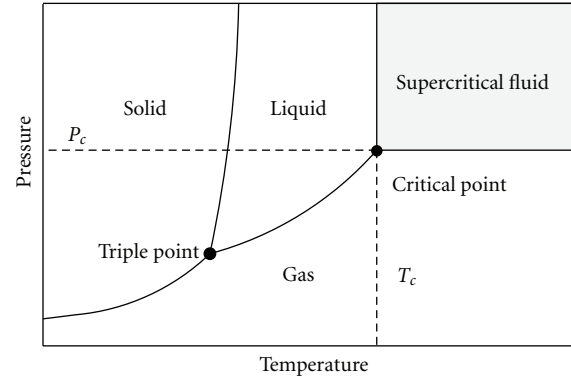

FIGURE 1: Pressure-temperature phase diagram of supercritical fluid.

mobility of polymer chains increases. Once the system reaches equilibrium, the phase separation can be induced by either reducing pressure (pressure quench method) or increasing temperature (temperature soak method). The sudden reduction in pressure or increase in temperature leads to the generation of nuclei due to the supersaturation. Finally, these nuclei grow to form the porous structure (Figure 2). During the foaming process, a second phase is generated from a meta-stable polymer/gas homogeneous mixture. With the depletion of CO_2, the T_g of polymer increases and the vitrification occurs with the foam structure fixed in the glassy state. The processing route to prepare porous materials can be achieved in a continuous or a discontinuous manner. According to classical nucleation theory [28–30], the properties of diffusivity of CO_2, gas concentration in polymer, interfacial tension in polymer/gas mixture, temperature, pressure drop rate, and the degree of supersaturation are the parameters that influence the nucleation rate. Therefore, manipulations of the processing parameters such as pressure, temperature, and pressure drop rate can control the foam morphology and structure.

After foaming process, the separation of the blowing agent from final product is simplified. No residue is left and a dry porous material is obtained because CO_2 can be easily removed from the polymer matrix by a simple depressurization step. In addition to its use as an environmentally friendly solvent, $scCO_2$ is also employed to purify sample by extraction. In this respect, fabrication of porous biodegradable and biocompatible polymer and its nanocomposites under organic solvent-free conditions, combined with possible extraction of impurities from the matrix, shows great potential for the applications in tissue engineering.

3. Preparation of Porous Biodegradable Materials for Tissue Engineering

Mooney et al. [31, 32] are the first to produce highly porous biodegradable polymer sponges for potential use in tissue engineering using high pressure CO_2 gas processing. Porous poly(D,L-lactic-co-glycolic acid) (PLGA) sponges with large pores (approximately 100 μm) and porosities of up to 93%
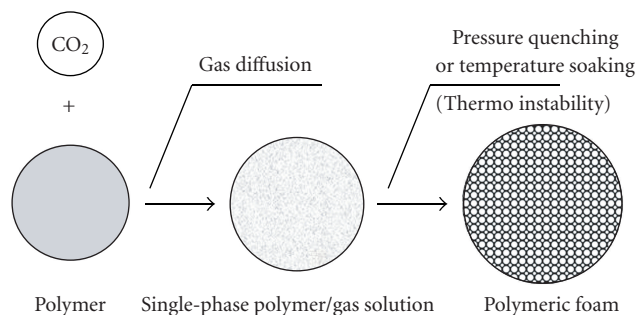
FIGURE 2: Foaming process via $scCO_2$.

were successfully fabricated. Much of the research in this field is focusing on poly(α-hydroxy acids), such as poly(ε-caprolactone) (PCL), poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and the copolymer PLGA as they are attractive candidates for fabrication of porous scaffolds for tissue engineering [33–37]. Singh et al. [38] have prepared interconnected porous microcellular 85/15 PLGA with pore sizes in the range from 30 to 100 μm and porosities as high as 89% by $scCO_2$ pressure quench method. Because of its ability to degrade to nonharmful lactic and glycolic acid and good foamability, 85/15 PLGA was considered to be an ideal biodegradable material in the generation of porous structures using $scCO_2$ technique for guided tissue regeneration and tissue engineering.

The CO_2 foaming process has been proven to be a very promising method for the preparation of scaffolds loaded with biological cues for further stimulation of cell behavior and enhancement of tissue regeneration. Howdle and coworkers [16] have encapsulated proteins in biocompatible and biodegradable polymers, such as PLA, PLGA, and PCL, at relatively low temperatures and moderate pressures. Hile et al. [39] have prepared microporous PLGA foams containing encapsulated proteins using $scCO_2$. The release and activity of fibroblast growth factor (bFGF) was determined in vitro from $scCO_2$-fabricated foams and compared with similar porous materials prepared by traditional solvent casting/salt leaching methods. The total protein release rate of the materials generated in $scCO_2$ was greater than that fabricated by the solvent casting/salt leaching technique. The release rate of active bFGF from the scaffolds prepared by salt leaching method was higher than that from the foams via CO_2 foaming. The capability to encapsulate and release protein at a controlled rate demonstrated that the porous scaffolds fabricated in $scCO_2$ are able to release active angiogenic factors for tissue engineering applications. Kanczler et al. [40, 41] investigated the bone regenerative potential of encapsulated vascular endothelial growth factor (VEGF165) poly(D,L-lactic acid) (PDLLA) scaffolds, which were fabricated using $scCO_2$ foaming technology to encapsulate and release solvent-sensitive and thermolabile growth factors in combination with human bone marrow stromal cells (HBMSC) implanted in a mouse femur segmental defect for 4 weeks. The ability to deliver, temporally, a combination of HBMSC and VEGF released from scaffolds prepared by

$scCO_2$ to sites of bone defects proved the enhancement of regeneration of a bone defect.

The scaffold with suitable pore size, high porosity, and interconnectivity determines the ability of bone to regenerate within this environment. In conditions required for mechanical function, balance must be attained between optimizing porosity and maximizing mechanical properties in the porous scaffold. $scCO_2$ foaming process has been found to be a viable technology to develop porous, resorbable polymer scaffolds with structural and mechanical properties similar to human bone. White et al. [42] fabricated PDLLA foams with varying structural and mechanical properties from different molecular weights (57, 25, and 15 kDa). Scaffolds with homogeneous pore distributions and some closed pores were produced by rapid depressurization. With the decrease in depressurization rate, scaffolds with wider distributions of pore size, and larger, higher interconnected pore structures were obtained. In compressive testing, 57 kDa PDLLA foams exhibited typical stress-strain curves for elastomeric open-cell foams. The structures and mechanical properties of 57 kDa PDLLA foams demonstrated that such scaffolds prepared by $scCO_2$ foaming are suitable for potential bone tissue engineering applications. Baker and coworkers [43] have synthesized porous resorbable poly(D-lactic-co-glycolic acid) (PDLGA) constructs with different glycolic acid compositions. The scaffolds exhibited a dense skin layer about 15–20 μm and an interconnected porous core with the pore size in the range 236–239 μm, which is similar to iliac crest bone grafts in spinal fusion process. Mechanical properties and the hydrophilicity of the constructs were found to depend on the glycolic acid composition. Gualandi et al. [44] fabricated porous scaffolds of a random copolymer of ω-pentadecalactone (PDL) and ε-caprolactone (CL) (poly(PDL-CL)) by $scCO_2$ foaming and demonstrated that the copolymer with highly crystallinity could be successfully foamed. Scaffolds with porosity in the range 42–76% and average pore size of 100–375 μm could be generated by tuning the foaming processing parameters such as soaking time, depressurization rate, and cooling rate. The obtained poly(PDL-CL) scaffolds showed mechanical properties suitable for potential applications in cartilage tissue regeneration.

$scCO_2$ foaming technology has many advantages in generation of porous biodegradable polymer, which can avoid the use of organic solvent and high temperature and decrease the saturation time compared with the conventional methods. However, for biomedical applications, the disadvantage of the CO_2 foaming process is that the foams are often lack of interconnectivity between pores, which lowers viability of seeded cells and hence results in a nonuniform distribution of seeded cells throughout the matrix [31]. To improve pore interconnectivity, a combination technique of CO_2 gas foaming with particulate leaching [32, 45–50] or particle seeding [51] was recently utilized. These techniques allow creating porous matrix with well-controlled porosity and pore morphology. Well-interconnected macroporous PLLA scaffolds with average pore diameters of around 300–400 μm were fabricated, which could be ideal for high-density cell seeding. 95% seeding efficiency and up to 40% viability at 1

day after the seeding were obtained for rat hepatocytes seeded in scaffolds [46]. It also reported that by varying the concentration of micrometric sodium chloride particles, it was possible to control the porosity and pore size spatial distribution of the open pore PCL foams [45]. However, the limitation of this process is long fabricating time and difficulty in completely porogen elimination. The use of ultrasound technique as a postprocessing step to control and improve the interconnectivity of the pores has been proposed by Wang et al. [52]. The foams fabricated by high-pressure CO_2 were exposed ultrasound at a frequency of 20 kHz and an average power input of 100 W. Porous structure with slightly increased pore size and substantially improved interconnectivity was obtained as a result of pore wall broken by applying ultrasound treatment. Ethanol used as a cosolvent [53] with supercritical CO_2 was also applied in the preparation of the skinless poly(methyl methacrylate) (PMMA) foams [54]. The addition of $scCO_2$ could enhance the affinity between semipolar PMMA and $scCO_2$ which resulted in the delayed release of absorbed CO_2 from PMMA film. Therefore, the obtained PMMA foams exhibited a low density and pores were connected to each other to make channels through the skin layer.

It has been reported that highly porous and well-interconnected scaffolds with controlled pore size, distribution and number of pores could be obtained by only modifying CO_2 processing parameters. Barry et al. [55, 56] prepared methacrylate scaffolds by $scCO_2$ foaming technology and reported that the degree of porosity and interconnectivity of materials could be controlled by simply changing the depressurization rate. Scaffolds with high porosity (about 89%) and high interconnectivity (74% open pores) were obtained. Jenkins et al. [57] also stated that the depressurization time had strong influence over the mechanical properties and the pore size and pore morphologies of the semicrystalline PCL foams. Fujiwara et al. [58] reported that not only the processing temperature and pressure but also the crystallinity of polymer sample could control the pore size and porosity. Liao et al. [17, 27] claimed that by controlling CO_2 processing parameters and hence the physical properties of PLLA by exposure to CO_2, various novel foam morphologies such as core-skin, layered, and interconnected could be generated. Different pore morphologies and sizes in the core as well as open and interconnected pores in the skin were obtained with small changes in the processing parameters (Figure 3). The core with larger stretched pores was considered to be suitable for cell growth, and the skin with open and interconnected nano pores was thought to be an excellent passageway for the flow of nutrients.

The generation of intriguing porous morphologies is not only dependent on the processing conditions but also the types of modifications applied to polymer chains. It was found that the presence of the metal chromophores in the PCL seemed to have generated sites within the polymer whereby nucleation of gas bubbles was promoted as compared with the pure PCL macroligand. Biodegradable telechelic poly(ε-caprolactone) foams with open pore diameters of about 10 μm via subcritical CO_2 processing were obtained. Due to the low metal content in the polymer

microstructure, the porous polymer with open pore structures tagged with luminescent chromophores were anticipated to act as an ideal niche for cell culture and cell attachment in tissue skin regeneration as well as good candidates in diagnostic molecular imaging as optical sensors for potential applications for biomedical use [59]. Changing the blend composition is another way to control the pore morphology. Barry et al. [60] investigated the foaming behavior of blends of (tetrahydrofurfuryl methacrylate) THFMA with styrene-isoprene-styrene (SIS) copolymer elastomer for soft prosthetic applications. By modifying the blend ratio and the processing temperature, the porosity and interconnectivity of the blends foams could be controlled. Since higher percentages of THFMA in the blend resulted in greater swelling of polymer in CO_2, the blend with 70% THFMA exhibited a higher degree of open pores and different pore size and distribution in comparison to the blends containing 30% and 50% THFMA. Initial biocompatibility assessments indicated that SIS/THFMA foams consisting of 70% THFMA could maintain the adhesion and growth of chondrocytes and neuron-like cells throughout the porous network.

4. Preparation of Porous Biodegradable Polymer/Nanocomposites for Tissue Engineering

The important properties of the porous biodegradable polymer used in tissue engineering are biocompatible surfaces and sufficient mechanical strength [61]. However, no single component polymer materials can meet all the functional requirements. Therefore, the design and fabrication of multiphase polymer systems represent a viable strategy to develop multifunctional biodegradable scaffolds [62, 63]. In particular, this paper deals with the introduction of nanostructures in biodegradable polymer matrix to obtain porous nanocomposites with specific properties able to be used in tissue engineering.

Nanotechnology involves materials that possess one or more of the dimensions in the nanometer length scale [64, 65]. Generally, polymer nanocomposite is defined as a material consisting of two immiscible phases of which one is a polymer and another is a filler (dispersed phase) with a nanoscale feature in at least one dimension. Polymer nanocomposites often show an excellent balance between strength and toughness in comparison with their individual components [66–69]. The addition of nanoparticles in polymer matrix provides the possibility to increase and modulate thermal stability, mechanical and gas barrier properties, melt viscosity, biocompatibility, biodegradation rate, and so forth [67, 69–72]. In fact, natural bone is an organic/inorganic composite material consisted of collagen and apatites. From this point, composites with both organic and inorganic components are excellent choices for tissue engineering [73].

The mechanical properties of available porous resorbable polymer scaffolds are often not suitable for hard tissue regeneration as the stiffness and compressive strength is much lower than that of native bone. Porous polymer nanocomposites for tissue regeneration are developed to
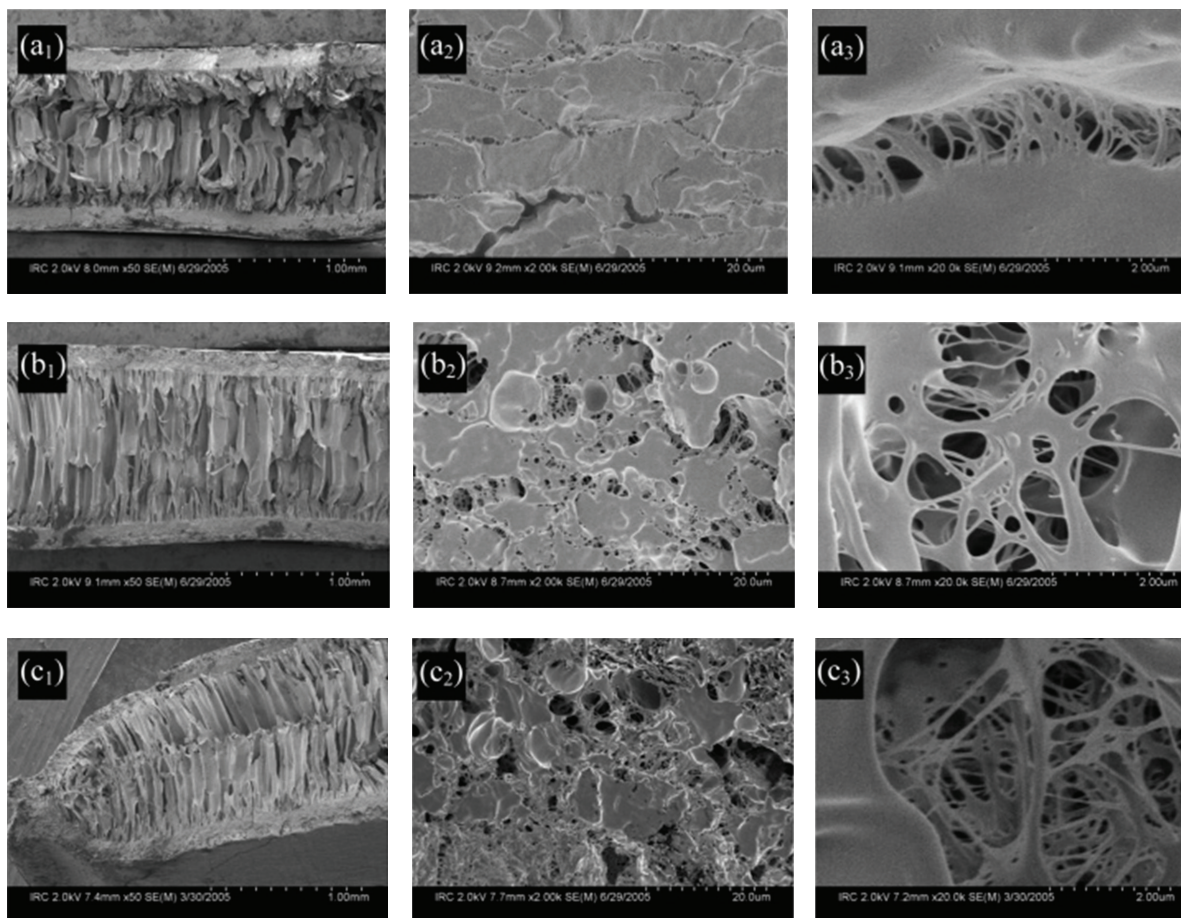
FIGURE 3: SEM microphotographs of CO_2-foamed PLLA samples saturated at 25°C and 5.5 MPa for 5 min and foamed at (a$_1$) 70°C, ×50; (a$_2$) 70°C, skin ×2000; (a$_3$) 70°C, skin ×20000; (b$_1$) 80°C, ×50; (b$_2$) 80°C, skin ×2000; (b$_3$) 80°C, skin ×20000; (c$_1$) 90°C, ×50; (c$_2$) 90°C, skin ×2000; (c$_3$) 90°C, skin ×20000. (From [17]. Copyright (2006) with permission from American Chemical Society.)

improve the mechanical properties of the polymer matrix with nanoparticle reinforcement and to enhance the overall biological response of the implant. Incorporation of a small amount of high aspect ratio nanoparticles, such as clay or nanotube, to a polymer matrix results in a high improvement in stiffness and strength without significant changes in the density. Water contact angle measurements indicated that the wettability of the PLA-based nanocomposites was improved by the addition of nanoclay to the polymer matrix [74]. In an attempt to improve osteoconductivity and to some extent mechanical strength, bioactive calcium particles such as hydroxyapatite (HA) or β-tricalcium phosphate (β-TCP) have received a lot of attention because bone itself consists of around 60 wt% ceramic nanocrystals. It has been reported that incorporation of a certain amount of HA to polymer may convert a nonbioactive polymer into a bone-bonding composite [75]. Using nano-HA as filling material in PCL/chitosan porous composite scaffolds has been reported and improved mechanical properties and bioactivity were obtained [76]. Liu et al. [77] reported that

introduction of clay strengthened the PCL matrix, and thus the compressive property of PCL/clay nanocomposite foams prepared using a chemical foam agent was improved. With increasing clay content, the mean pore size decreased and pore wall thickness almost remained the same at low clay loadings. On contrast, at high loadings, both the average pore size and pore wall thickness increased. This phenomenon was attributed to the change of melt viscosity of nanocomposites and the heterogeneous nucleation behavior of clay at low loadings [28, 78].

In scCO$_2$ foaming process, either a pressure drop or a temperature soaking method is used to make the polymer/gas solution supersaturated. When clusters of gas molecules are greater than the critical size, the activation energy is overcome and gas bubble nucleation occurs. The greater the supersaturation is, the lower the activation barrier is. Nanoparticles are commonly used to adjust the nucleation rate and hence the pore morphology by significantly decreasing the barrier of activation energy [79]. Compared to conventional micron-sized fillers, the extremely fine dimensions, large
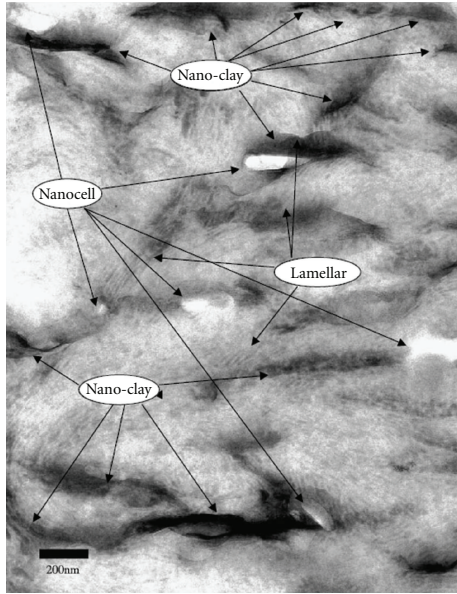
FIGURE 4: TEM micrograph for the structure of PLA/MMT-ODA cell wall foamed at 100°C under 28 MPa. (From [18]. Copyright (2006) with permission from Elsevier.)

surface area, and intimate contact between nanoparticles and polymer matrix may dramatically affect the bubble nucleation and growth of CO_2 gas. The addition of a small amount of intercalated nanoclay greatly reduces cell size and increases cell density. Compared with unmodified polymer foams, the nanocomposite foams exhibit higher tensile modulus, improved fire retardance, and better barrier properties.

Okamoto and coworkers [70, 80–89] have done a series of studies on the effect of nanofillers, type of surfactant and compatibilizer, and the content and distribution of organically modified layered silicates on the properties of PLA. The nanocomposites exhibited significant improvement in crystallization behavior [86], mechanical and flexural properties [70], heat distortion temperature, O_2 gas permeability [80], and improved biodegradability [70, 81, 82] as compared with pure PLA. They first reported the possibility of preparing biodegradable nanocellular polymer/nanocomposite foams via scCO$_2$ technology. The effect of distribution and type of silicate nanoparticles on the porous structure of PLA has been investigated [18, 33]. A controlled structure of the nanocomposite foams from microcellular to nanocellular morphology was produced by changing the surfactants for clay modification and the processing conditions [33]. The incorporation of nanoclay-induced heterogeneous nucleation because of a lower activation energy barrier compared to homogeneous nucleation. The grown pores with diameter of $\sim$ 200 nm were localized along the dispersed nanoclay particles on the wall of pores. The dispersed nano-clay particles served as nucleating sites for pore nucleation and growth happened on the surfaces of the clays as shown in Figure 4.

Liao et al. [90] investigated the presence of nano-clay on the foaming behaviors and pore structures of PLA using subcritical CO_2 as a foaming agent. It was found that

smaller crystallites and lower crystallinity were obtained in the nanocomposites compared to the pure PLA since the nano-clay acted as nucleation site to facilitate the crystallization process of the PLA matrix. By controlling the crystallinity, novel skin-core foam morphologies in PLA-clay nanocomposites were obtained. With the addition of a small amount of organically modified clay, the specific strength of PLA foams increased.

Tsimpliaraki et al. [91] studied the effect of surface chemistrys and nano-clay content on the porous structure of PDLLA nanocomposites prepared by scCO$_2$. The results suggested that the foam properties were not only depended on the content of clay but were also strongly related to the chemical nature of the modifier and the extent of modification. The addition of organically modified clay led to heterogeneous nucleation and hence greatly reduced pore size and increased pore density. In most cases, the bulk foam density increased as well. By controlling the interaction between CO_2 and the clay surface as well as the loading content of clay, controlled foam morphology of nanocomposites could be obtained. The more the clay content, the longer the alkylammonium surfactant used, and the higher is the modification level of the clay, the more foams with smaller average pore diameters and larger pore density could be produced. Moreover, they found that scCO$_2$ not only acted as a processing/foaming agent but also played a role as a clay dispersion medium.

Baker et al. [92] investigated the use of scCO$_2$ technology to simultaneously disperse a nano-clay in PLA and prepare suitable porous matrix for load-bearing applications. Compared with pure polymer constructs, the compressive strength of porous resorbable nanocomposites increased 2.5-fold, which were considered to be related to the dispersion of nano-clay in the PLA matrix and a subsequent reduction in polymer chain mobility [77, 79]. Besides the significant increase in mechanical properties, the porous nanocomposite exhibited a greater biocompatibility than that of polystyrene culture plate controls. Additionally, calcium phosphate-rich deposits could clearly be observed not only on the surface but also at the center of the cultured scaffolds, which indicated that osteoblasts were able to penetrate the porous nanocomposite network. The pore size of $\sim$200 μm suggested that these porous nanocomposites prepared in scCO$_2$ would also support neovascularization.

A complex hybrid release system of 3D PDLLA/chitosan/nanochondroitin sulfate composites was developed by scCO$_2$ foaming. The hybrid porous biomaterial displayed adequate porosity and interconnectivity for cell culture, and sufficient mechanical strength for cell adhesion and support. By adjusting the loading parameters of both scaffolds and nanoparticles, it was possible to design different release profiles. The novel hybrid porous structure composed PDLLA/chondroitin sulfate nanoparticles, which offered adequate morphological parameters necessary for cell culture as well as the ability to deliver bioactive molecules at different rates for further stimulation of cell/tissue behavior, could be a promising approach as a multifunctional cell template for potential tissue engineering use [93]. Ginty et al. [94] developed protein loaded polymer/calcium alginate fibers
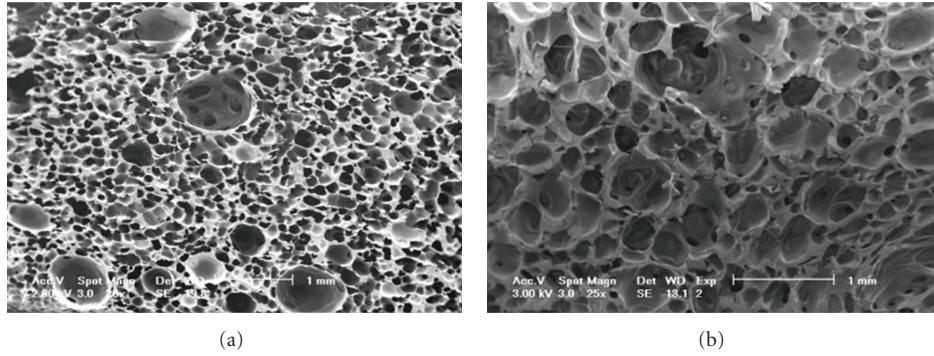
FIGURE 5: The effect of filler on composite foam morphology: (a) neat PLA; (b) 5 wt% HA PLA. (From [19]. Copyright (2005) with permission from John Wiley and Sons.)
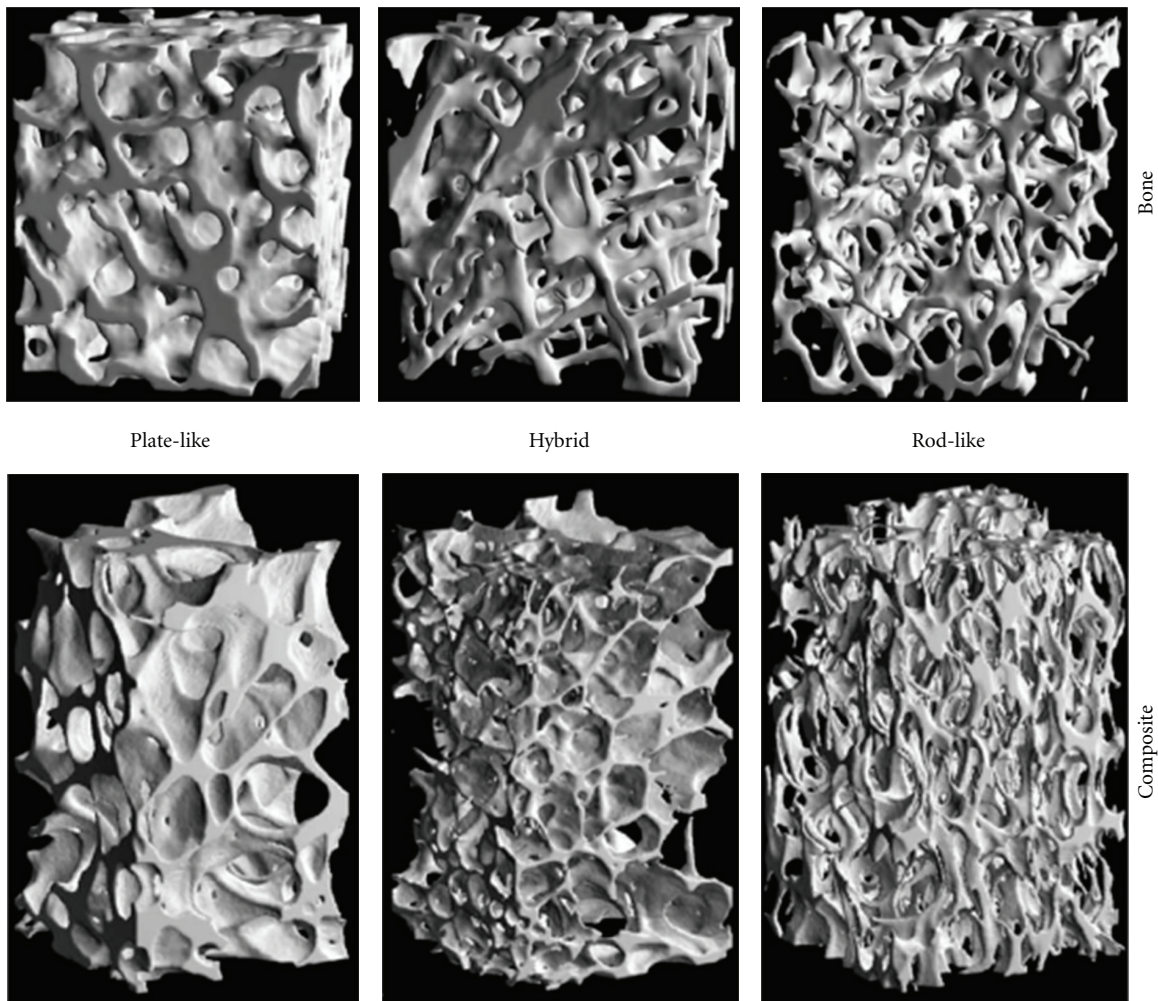

FIGURE 6: Similarity of cancellous bone structures and polymer foam macrostructures. (From [20]. Copyright (2006) with permission from Elsevier.)

composites to modify the release kinetics of protein from PDLLA scaffolds. The rapid release rate of protein from the hydrophilic alginate fibers in the initial stages could be a complement for the slower release of protein from the slower degrading PDLLA scaffolds. The release kinetics of either a single protein or two separate proteins could be controlled by adding calcium alginate fibers in PDLLA matrix, which could provide opportunity to modify the single or dual release of growth factors for specific application.

PLLA is a semicrystalline thermoplastic whose mechanical properties and biodegradable behavior are dependent on the crystal structure and morphology. Therefore, it is important to understand how the presence of the nanoparticles influences crystallization kinetics and the crystallinity of

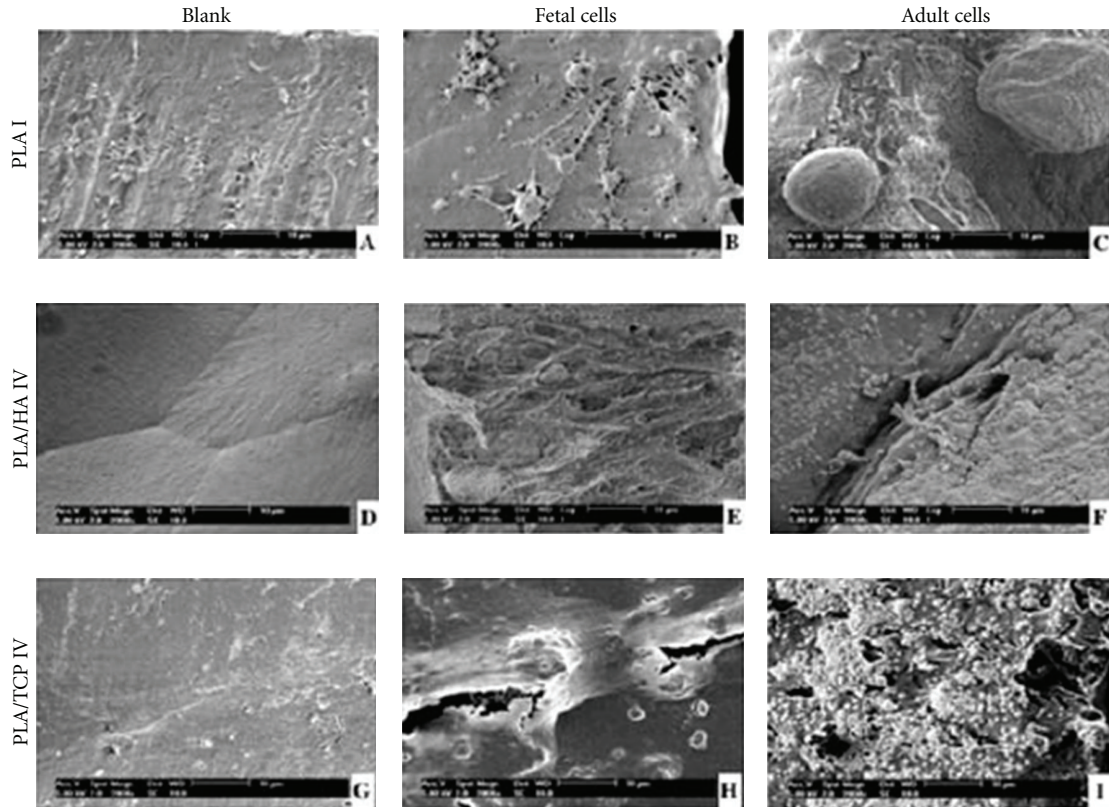| Blank	Fetal cells	Adult cells

FIGURE 7: SEM micrographs of uncolonized scaffolds (left) and of scaffolds colonized by fetal cells (middle) and adult cells (right). After 4 weeks of treatment with osteogenic factors, cells were visible on the scaffold surfaces, with extracellular matrix deposition and mineral crystals. The cells exhibited multiple cytoplasmic extensions that interconnected, wrapping the polymer surface. Macropores were gradually obturated by a multilayered cover of cells. The width scale was chosen in order to focus on cells lying on pore wall cross-sections and inside pores. Scale bars: $10\,\mu$m. (From [21]. Copyright (2005) with permission from Mary Ann Liebert, Inc.)

polymer matrix. The presence of nanocrystalline HA (nHA) could enhance osteoblast adhesion, and surface deposition of calcium-containing materials in comparison with microcrystalline HA [95]. It was found that the number of spherulites per unit volume increased during isothermal crystallization process in the presence of nHA. [96]. With the increase of nHA ratio, there was a considerable decrease in the growth rate of spherulites in the temperature range 100–130°C, which was probably because of the significant increase in the melt viscosity of the nanocomposties. Interconnected porous structure with a diameter of 200–400 μm and compressive moduli between 10 and 180 MPa for porosities from 78 to 92% for neat PLA could be generated. With ceramic fillers, a more heterogeneous structure was obtained with nHA than with micro-β-TCP due to the tendency of nano-HA particles to agglomerate. The presence of fillers resulted in an increased foam density and larger pore diameter (Figure 5). For a given porosity, with fillers an improved modulus up to 250 MPa were obtained. Biocompatibility studies demonstrated that both neat and composite scaffolds were biocompatible, allowing cell colonization

and differentiation. These composite scaffolds should be adequate for use in bone tissue engineering [19, 20, 97, 98]. Bone from different sites around the body is anisotropic, both morphologically and mechanically. The scCO$_2$ foaming technique has been proved to be a flexible method to fabricate various macrostructures for replacing different types of porous bones. Mathieu et al. [20] produced open pore PLLA composite foam using HA and β-TCP as fillers by scCO$_2$ foaming. It was demonstrated that the foam showed anisotropy in morphology and in mechanical behavior, which was thought to be suitable for bone tissue engineering applications. Figure 6 displays the structures of three polymer foams and three different types of trabecular bones. That anisotropy was more or less pronounced, which was depended on the bubble nucleation density and cooling rate. Rapid cooling rate resulted in large numbers of spherical pores, whereas a slower cooling rate facilitated pore elongation. Phosphate glass (PG) of the composition 0.46(CaO)–0.04(Na$_2$O)–0.5(P$_2$O$_5$) was also used as filler in PLLA foams developed in scCO$_2$ for bone tissue engineering. However, no significant improvement in

compressive moduli and biocompatibility was observed with glass incorporation [99].

Use of scCO$_2$ allows the incorporation of biological agents in polymer/nanocomposite and the manufacture of open and interconnected porous materials. Bioresorbable ceramic-reinforced PLLA scaffolds obtained by scCO$_2$ foaming were recently described as suitable environments for tissue engineering, which provide biocompatibility with primary osteoblasts in vitro and strong mechanical properties. The introducing of HA or β-TCP in PLLA matrix led to higher alkaline phosphatase (ALP) enzymatic activity for fetal bone cells and a stronger production of Gla-osteocalcin for adult bone cells (Figure 7) [21]. The in vivo host-tissue induced reactions and osteoconductive properties of different PLA/ceramic composite scaffolds obtained via scCO$_2$ foaming were evaluated in a critical size defect craniotomy model in Sprague-Dawley rats. As assessed by histology and radiography, complete bone bridging was observed 18 weeks after implantation with PLA/5 wt% β-TCP and PLA/5 wt% HA scaffolds. scCO$_2$ foaming, therefore proved to be a promising method to create porous composite structures for tissue engineering, especially for applications in maxillofacial bone repair and during revision of total joint replacement [100].

To improve the interconnectivity, salt leaching method was introduced in preparation of porous polymer/nanocomposites for tissue engineering in scCO$_2$ [101]. High-porosity PDLLA/HA composite foams (up to 90% $\pm$ 2% porosity) with pore sizes ranging from 100 to 300 μm were fabricated, which is suitable for cell seeding. By tuning the saturation pressure, saturation time, and temperature as well as amount of NaCl particles, the microstructure and morphology of the composite foams could be controlled. PDLLA/nHA composites foams showed better hydrophilic ability, enhanced mechanical properties, and excellent biocompatibility than that of PDLLA foams. Moreover, the inflammatory response of tissue after implanting was lightened by incorporation of nHA in PDLLA matrix [102, 103].

5. Summary

In recent years, scCO$_2$ foaming technology has been utilized to overcome the drawbacks of conventional scaffold fabrication methods. As scCO$_2$ foaming method can operate under mild conditions without the use of organic solvents and take advantage of the tunable properties of the fluid phase, an increasing interest in preparing porous scaffold using scCO$_2$ foaming technology for tissue engineering is continuously growing. The novelty of scCO$_2$ foaming technology opens a wide range of opportunities for the preparation of scaffolds of biodegradable polymer and its nanocomposites with enhanced and multifunctional properties. The polymer/nanocomposites scaffolds prepared using this process have demonstrated improved mechanical strength, biocompatibility, and biodegradation rate of polymer matrix. Despite the obvious advantages, however, the challenge remains to design a porous polymer nanocomposite with adequate interconnected pores via scCO$_2$ foaming. Several attempts such as salt leaching, ultrasound posttreatment, cosolvent, and processing parameters controlling have been employed combined with gas foaming technology to enhance the connectivity of the pores, but further deep investigation and process optimization are still required to control over the scaffold architecture and properties.
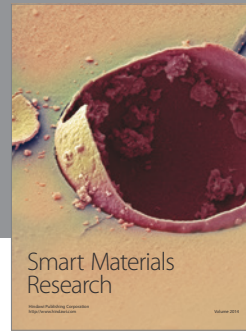
Acknowledgments

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