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Technical Note

Generation of porous microcellular 85/15 poly (DL-lactide-co-glycolide) foams for biomedical applications

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Abstract

Porous 85/15 poly (DL-lactide-co-glycolide) or PLGA foams were produced by the pressure quench method using supercritical CO_2 as the blowing agent. The rate of CO_2 uptake and CO_2 equilibrium concentration in PLGA at different processing conditions were studied by performing sorption experiments. The effects of saturation pressure and temperature on average cell size and relative density of the resulting foams were also studied. The time required to approach equilibrium exhibited a minimum with increasing saturation pressure. The diffusion coefficient and equilibrium concentration of CO_2 in PLGA increased with an increasing pressure in an approximately linear relationship. Porous PLGA foams were generated with relative densities ranging from 0.107 to 0.232. Foams showed evidence of interconnected cells with porosities as high as 89%. The pore size ranged from 30 to 100 μm.
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1. Introduction

Porous biodegradable polymer matrices are widely used in biomedical applications such as tissue engineering and guided tissue regeneration. Porous polymer matrices are used for both in vitro cell seeding and in vivo cell transplantation in tissue engineering studies [1–4]. The matrix provides a temporary support for cell growth and is also used to deliver growth factors to the growing cells. As the cells grow within the polymeric scaffold, they secrete their own support matrix and the polymer, which is no longer needed, degrades over time. In guided tissue regeneration a polymer scaffold is placed directly into the body to encourage cellular growth in vivo [5–8]. In this type of biomedical application, the porous biodegradable polymer functions as a size selective membrane and promotes cell growth at specific sites (e.g. bone regeneration) in the body by allowing nutrients and wastes to permeate while preventing the migration of undesirable cells and tissues to the healing site. Because the polymer's chemical composition and foam morphology (pore size, shape, and interconnectivity) can affect cellular growth, an ideal polymeric foam for tissue engineering and guided tissue regeneration should be highly porous to allow cell seeding and cell growth into the matrix. Overtime, the polymer matrix should also degrade into chemically benign components, which are not harmful to the growing cells.

Poly (lactic-co-glycolic) acid or PLGA is one of the most commonly used biodegradable polymers for fabricating porous foams for biomedical applications. PLGA is a desirable polymer because it biodegrades into lactic and glycolic acid, relatively harmless to the growing cells, and its use in other in vivo applications such as resorbable sutures has been approved by the Food and Drug Administration [9]. Also, the degradation rate of PLGA can be controlled by varying the ratio of its co-monomers, lactic acid and glycolic acid [10]. The techniques reported for generating porous PLGA foams include solvent casting–particulate leaching, fiber weaving, and phase separation [11–14]. Although,

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PLGA foams with porosities as high as 95% and cell sizes ranging from 20 to 500 μm have been reported, a big drawback to these techniques is that they utilize organic solvents in the fabrication process. Residues of organic solvents left in the polymer after processing may be harmful to the transplanted cells in biomedical studies and can inactivate many biologically active factors (e.g. growth factors). Therefore, a pressure quench method using supercritical CO_2 as the blowing agent was employed to fabricate PLGA foams in our investigation.

The pressure quench method was used to produce polymer foams because it does not involve the use of organic solvents, required by other techniques of fabricating polymers into foams e.g. solvent casting/particulate leaching and phase separation techniques. Fig. 1 shows a simplified schematic of the pressure quench method. This method has two steps. A thermoplastic sample is placed in a pressure vessel and saturated with an inert gas, typically CO_2 in the supercritical region. CO_2 is used because it is chemically inert and highly soluble in most polymers. Upon a prolonged exposure to supercritical CO_2 at high pressure, the polymer absorbs enough gas to lower its glass transition temperature below the processing temperature of the pressure vessel, resulting in a polymer/gas solution. The second step is to rapidly drop the pressure to ambient pressure. This rapid quench in pressure decreases the CO_2 solubility in polymer and causes bubble nucleation due to supersaturation. As the bubbles grow the gas concentration in the polymer drops until the effective T_g of the polymer is above the temperature in the pressure vessel. The rapid depressurization of the vessel also causes the temperature in the vessel to drop, possibly limiting cell growth.

Several different research groups have produced open cell foams for biomedical applications using the pressure quench method [15–17]. The materials used were PLA, PGA, and PLGA. These polymers typically have glass transition temperatures in the range of 40–50°C, and therefore supercritical pressures are not required to depress the T_g enough to use the pressure quench foaming method. These polymers are quickly degraded by heat and water, so it is desirable to use a foaming method that requires neither.

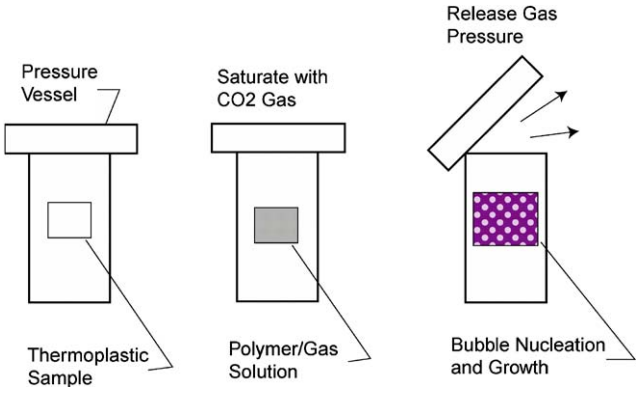

Fig. 1. The pressure quench foaming method.

2. Experimental

2.1. Material

The material used was 85/15 poly (DL-lactide-co-glycolide) acid or PLGA supplied by Birmingham Polymers Inc., Birmingham, AL 35211 (Lot # D96053). The material was received in the form of small white crystalline pellets with diameters ranging 3–5 mm, packaged under high purity nitrogen in polyethylene bags. Polyethylene bags were also heat-sealed and contained desiccant to absorb any moisture inside the bags. To prevent the hydrolysis of PLGA by moisture in the air, the pellets were stored in a vacuum chamber containing anhydrous calcium sulfate. According to the Certificate of Analysis accompanying the material, 85/15 PLGA has a glass transition temperature of 45°–50°C, a density of 1.2825 g/cm^3 and a molecular weight ranging from 50 to 75 kg/mol. The molar percentage of the material was 85% lactic acid and 15% glycolic acid.

PLGA pellets were compression molded into sheets using a Tetrahedron press. A force of 44,444 N (10,000 lb) was applied to the pellets for five minutes at 65°C. The sheets obtained were clear, 5–7 cm in diameter, ranged from 0.50 to 0.75 mm in thickness, and weighed 2.5–3.5 g each. The storing procedure described above was followed to prevent the hydrolysis of PLGA sheets by moisture in the air.

2.2. Sorption experiments

Sorption experiments were carried out to study the rate of uptake and the equilibrium concentration of CO_2 gas in PLGA sheets over a range of pressures and temperatures. Sorption experiments were performed at 25°C at pressures of 0.5, 3.0, and 5.0 MPa, and at 35°C at a pressure of 5 MPa. Samples measuring approximately 1×1 cm^2 were cut from the PLGA sheet and their weight and thickness was measured. The samples were then placed in the pressure vessel and saturated at the desired pressure and temperature. Temperature was controlled to ± 1°C and pressure was controlled to ± 0.01 MPa when saturating at 0.5, 3, and 5 MPa. Periodically, the samples were removed for weighing. The difference in weight of the sample is equal to the amount of CO_2 absorbed by the polymer. CO_2 uptake was calculated by dividing the difference in weight by the initial weight of the polymer. Sorption experiments were carried out until the CO_2 uptake stopped changing,

indicating that CO_2 concentration had reached equilibrium. If the sample foamed before the equilibrium CO_2 concentration was reached, a new sample was started at the same saturation conditions and left for a longer period of time in the vessel before weighing. After completing the sorption experiments, equilibrium concentrations and diffusion coefficients of CO_2 in PLGA were calculated for the different processing conditions. Sorption experiments at 0.5 and 3.0 MPa at 35°C were also carried out to determine the equilibrium CO_2 concentration in PLGA.

The rate of gas diffusion into a polymer can be described by a diffusion coefficient. The diffusion coefficient can be calculated from gas sorption data into an infinite sheet. Because our samples were very thin compared to their area, they were approximated as an infinite sheet for diffusion coefficient calculations. For early states of diffusion, when the CO_2 concentration is less than one half of the equilibrium concentration, the diffusion coefficient can be found by finding R, the slope of (M_t/M_∞) over $\sqrt{(t/l^2)}$, where: M_t is the concentration of gas in the polymer at time t, in mg of CO_2/g of polymer, M_∞ is the concentration of gas at equilibrium, l is the sample thickness in cm and, t is the elapsed time in s.

The average diffusion coefficient, D, can then be found from

$$D = (\pi R^2)/16.$$

2.3. Foaming experiments

Foaming experiments were carried out on PLGA at 35°C and 40°C. Foams were produced via the pressure quench method by saturating PLGA samples with CO_2 at 10, 14, 15, and 20 MPa at both temperatures. Temperature was controlled to ± 1°C and pressure was controlled to ± 0.5 MPa.

The densities of the foamed samples were measured using the weight displacement method, ASTM D792. To study the microstructure, the foamed samples were fractured using liquid nitrogen and sputter-coated with Au/Pd at 15 mA for 60 s to make them conductive. The prepared foam samples were studied using a JEOL scanning electron microscope (SEM), and micrographs were taken on Polaroid film. Porosity of two open-cell samples was calculated using a Beckman Air Pycnometer, ASTM D2856-94.

3. Results and discussion

3.1. Sorption results

Figs. 2 and 3 show the sorption results for CO_2 into PLGA sheet at 25°C at 0.5, 3.0, and 5.0 MPa and at 35°C at 5 MPa, respectively. Because sorption times in a sheet are proportional to the thickness squared, and because the samples used for sorption experiments had different thicknesses, the sorption plots have been normalized for sample thickness. To normalize for

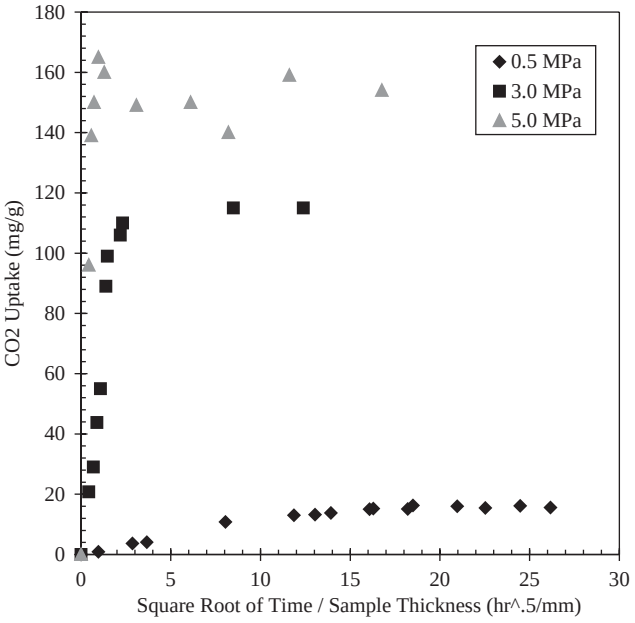

Fig. 2. Sorption data for 85/15 poly (DL-lactide-co-glycolide) sheets saturated at 25°C with CO_2 at pressures of 0.5, 3.0, and 5.0 MPa. The samples used had different thicknesses, so the X-axis has been normalized with respect to thickness.

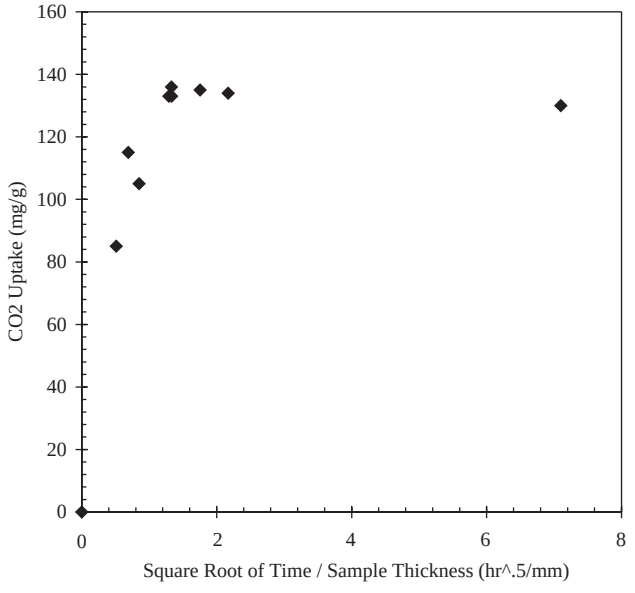

Fig. 3. Sorption data for 85/15 poly (DL-lactide-co-glycolide) sheet saturated at 35°C with CO_2 at a pressure of 5.0 MPa. The samples used had different thicknesses, so the X-axis has been normalized with respect to thickness.

thickness, the *X*-axis is plotted as the square root of the sorption time divided by the sample thickness. As expected, after a prolonged exposure to CO_2 at a constant pressure and temperature, the polymer stops absorbing more gas as CO_2 concentration reaches equilibrium. Since pressure can affect the rate of diffusion of gas into the polymer, pressure also affects the time needed for the CO_2 concentration to reach equilibrium. Fig. 2 illustrates that the time needed for the polymer to reach equilibrium decreases with increasing pressure. Thus, increasing the pressure also increases the rate of diffusion of CO_2 into the polymer. Fig. 5 shows a plot of diffusion coefficients against saturation pressure. Diffusion coefficient of CO_2 in PLGA increases with increasing saturating pressure. Therefore, a sample saturated at 5 MPa will reach equilibrium faster than the sample saturated at 3 MPa.

The pressure and temperature used to saturate the sample also affects the equilibrium CO_2 concentration in the polymer. Equilibrium CO_2 concentrations for 25°C and 35°C are plotted against the saturation pressure in Fig. 4. Fig. 4 shows that an increasing pressure results in an increase in equilibrium concentration and an increasing saturation temperature results in a decrease in equilibrium concentration. This could be explained by the facts that at higher pressure more CO_2 molecules can be forced into the polymer than at lower pressure, and CO_2 solubility in a polymer decreases with an increasing saturation temperature (Figs. 4 and 5).

3.2. Foaming results

Data on PLGA foams produced is presented in Table 1. For each saturation condition, the foam density, foam relative density, and the approximate cell diameter is given. Foam relative density is defined as the foam density divided by the density of the unfoamed polymer $-1.2825\,g/cm^3$ for PLGA. Foams were produced with a range of relative densities from 0.107 to 0.232. As illustrated in Fig. 6, there is a trend of foam density increasing with increasing saturation pressure. Approximate average cell diameter was estimated from SEM micrographs.

Fig. 7 shows SEM micrographs of six foamed samples. Foams in images A, B, and C were produced by saturating the polymer at 35°C at 10, 15, and 20 MPa, respectively. Samples in images D, E, and F were saturated at 40°C at 10, 15, and 20 MPa, respectively. The pores were roughly spherical and the average diameter ranged from 30 to 100 μm. Although, every foamed sample presented in Fig. 7 shows some level of interconnectivity between pores, the foams produced at a saturation temperature of 35°C exhibit a greater amount of interconnectivity and a larger pore size than the foams produced at 40°C. This can be attributed to the fact that CO_2 solubility into a polymer drops with increasing saturation temperature. Therefore, the samples saturated at 35°C absorbed more gas than the ones saturated at 40°C, and this difference in the amount of gas and nucleation force available to the samples saturated at 35°C resulted in a greater interconnectivity and a larger pore size. The samples shown in images B and C were analyzed with a Beckman air pycnometer to measure the open cell content or porosity. Samples B and C had an open cell content of 73% and 89%, respectively.

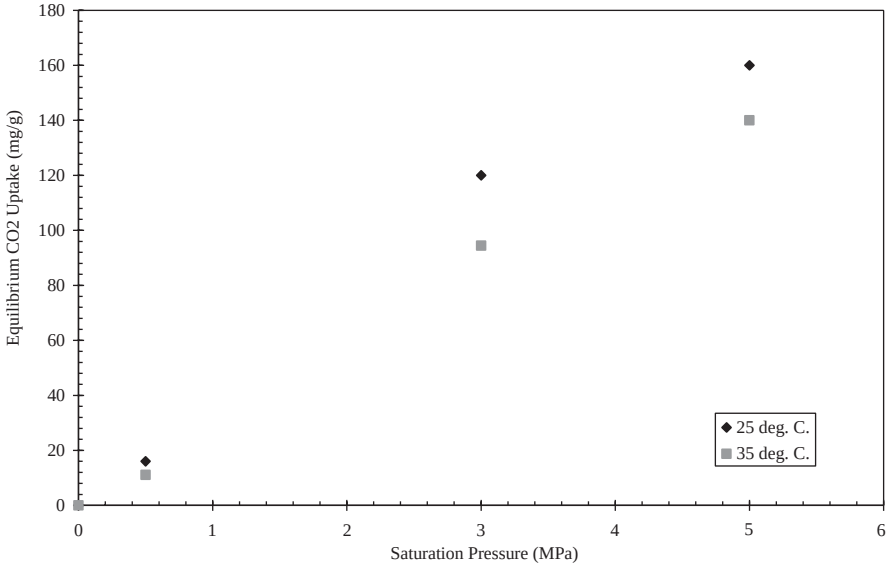

Fig. 4. Equilibrium gas concentrations for 85/15 poly (DL-lactide-co-glycolide) sheet saturated with CO_2 at 25°C and 35°C at 0.5, 3.0, and 5.0 MPa. Note that increasing pressure and decreasing temperature lead to greater equilibrium concentration.

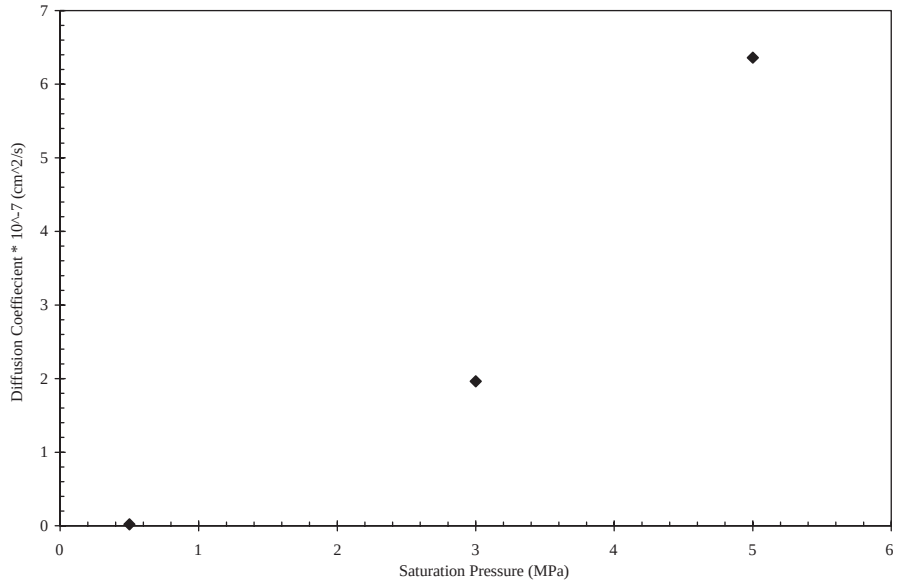

Fig. 5. Sorption diffusion coefficients for CO_2 into 85/15 poly (DL-lactide-co-glycolide)sheet at 0.5, 3.0, and 5.0 MPa and 25°C. The diffusion coefficient increases with increasing pressure.

Table 1
Foaming data for 85/15 poly (DL-lactide-co-glycolide) in CO_2. Foam density, foam relative density, and an estimated average cell size are given

Saturation temperature (°C)	Saturation pressure (MPa)	Foam density (g/cm^3)	Foam relative density	Approximate average cell diameter (μm)
35	10	0.143	0.111	70–100
35	14	0.253	0.197	—
35	15	0.254	0.198	40
35	15	0.254	0.198	30
35	20	0.277	0.216	70
40	10	0.138	0.107	60
40	14	0.267	0.208	70
40	15	0.298	0.232	40
40	20	0.275	0.214	30
40	20	0.284	0.221	—

Relative density is defined as the foam density divided by the density of the unfoamed material, 1.2825 g/cm^3.

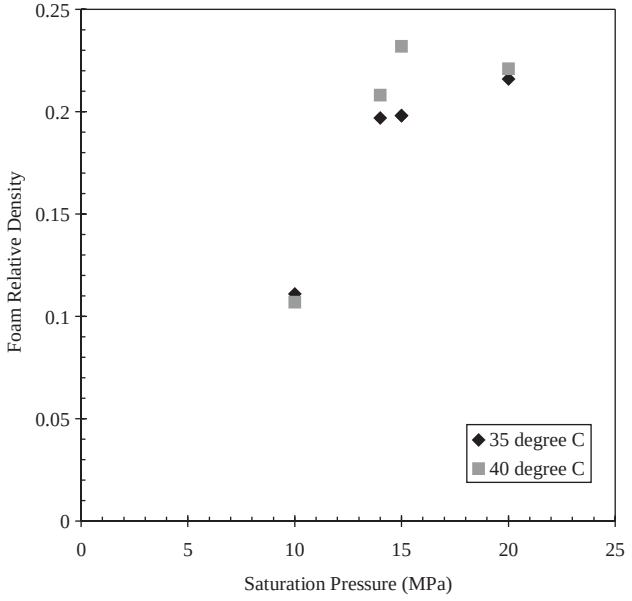

Fig. 6. 85/15 poly (DL-lactide-co-glycolide) foam relative density as a function of saturation pressure for saturation temperatures of 35°C and 40°C. Note that foam relative density increases with increasing saturation pressures.

4. Conclusion

Porous 85/15 poly (DL-lactide-co-glycolide) or PLGA foams were produced by the pressure quench method using supercritical CO_2 as the blowing agent. The time required to approach equilibrium exhibited a minimum with increasing saturation pressure. The diffusion coefficient and equilibrium concentration of CO_2 in PLGA increased with an increasing pressure in an approximately linear relationship. Foams generated had relative densities ranging from 0.107 to 0.232. Foams showed evidence of interconnected cells with porosities as high as 89%. The pore size ranged from 30 to 100 μm. Because of its good foaming properties, which include interconnected cells and its ability to degrade to lactic and glycolic acid, not harmful to the body, 85/15 poly (DL-lactide-co-glycolide) is an ideal biodegradable polymer for fabricating porous matrices for biomedical applications such as guided tissue regeneration and tissue engineering.

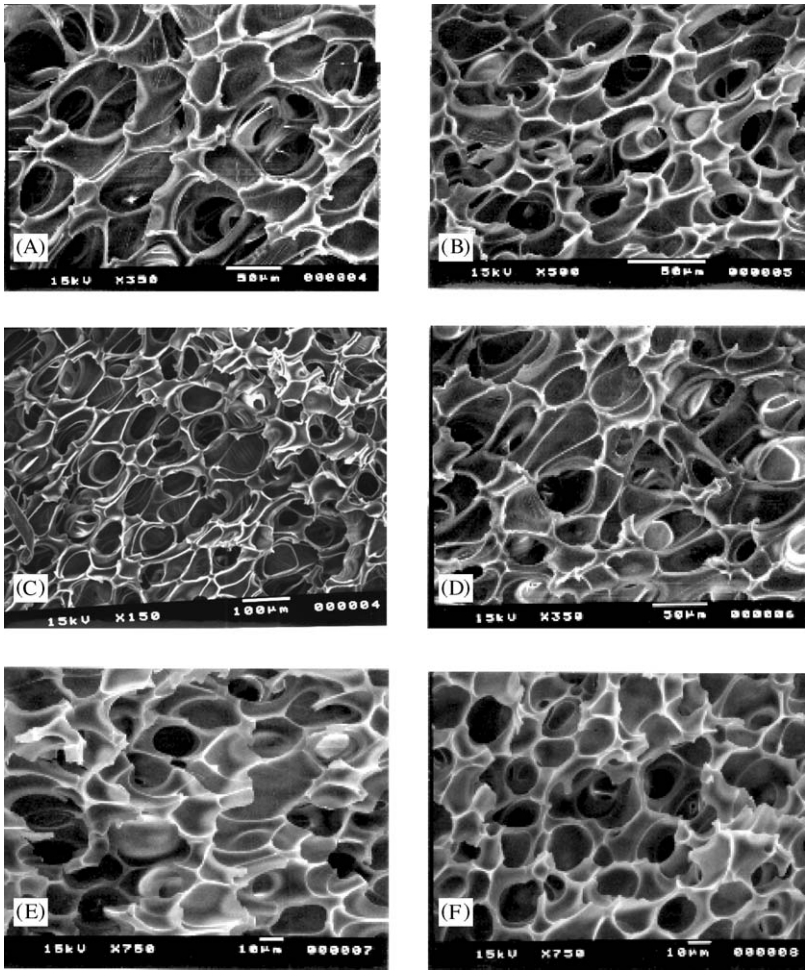

Fig. 7. Scanning Electron Micrographs of six 85/15 poly (DL-lactide-co-glycolide) foams produced using the pressure quench method. The samples in images A, B, and C were saturated at 35°C at 10, 15, and 20 MPa, respectively. The samples in images D, E, and F.

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