Micro-porous Paclitaxel-loaded PLGA foams - a new implant material for controlled release of chemotherapeutic agents

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Abstract

Supercritical gas foaming using CO_2 was used to fabricate blank poly DL lactide-co-glycolide (PLGA) micro-porous foams. Paclitaxel-loaded PLGA foams were also produced for the first time using a modification of the supercritical gas foaming technique whereby pacltitaxel-loaded PLGA microparticle powders obtained from spray drying was foamed. In this study, it was found that using polymer powders, more compact foams and smaller pores foams may be achieved with lower saturation pressures and time which is due to the much higher surface area to volume ratio of the microparticle powders. Experiments were carried out with varying lactide to glycolide ratio of the copolymer PLGA and it was shown that the pore size, *in vitro* swelling behavior and drug release profiles may be altered by changing the copolymer composition used. The foams fabricated also have good mechanical strength which makes it suitable to be applied as an implantation material for the post-surgical controlled delivery of chemotherapeutic drugs. The residual organic solvent content of the paclitaxel-foams were well below the allowable limit set by the US Pharmacopeia as shown in the present study. The *in vitro* release profiles over a period of 5 weeks showed close to linear release.

Index Terms **-** Keywords: Supercritical gas foaming technique, Poly DL lactide-co-glycolide (PLGA), Micro-porous foams, Controlled release, Paclitaxel

I. INTRODUCTION

Micro-porous polymer foams have important applications in tissue engineering as scaffolds [1-3] drug delivery systems [4-5]. PLGA is one of the most popular biodegradable polymers used in porous foam fabrication as it is biocompatible and FDA approved [1,3-5]. The degradation rate of the foam may also be controlled by careful selection of the copolymer composition and molecular weight [3]. Conventional methods of micro-porous foam formation such as solvent-casting, particulate leaching technique [3] are usually associated with the use of large amounts of organic solvents which may require extensive purification steps to remove the residual solvent. Using supercritical CO_2 as the foaming agent, the use of organic solvent may be minimized or even eliminated in the production of PLGA foams as illustrated in previous studies [5].

The mechanism of supercritical gas foaming technique is as follows [1]: Under high pressure CO_2, the glass transitin temperature (Tg) of PLGA is depressed and a polymer melt may be formed at lower temperatures with large amounts of dissolved CO_2. Upon rapid depressurization, thermodynamic instability takes place and the CO_2 molecules cluster and diffuse to form nuclei in the polymer matrix. As CO_2 leaves the polymer and pressure is lowered, the Tg of polymer increases to above the operating temperature and vitrifies to form a solid polymeric foam

The micro-porous foams can potentially be used in the post-surgical treatment of cancer cells by controlled release of chemotherapeutic agent. After a surgical removal of a tumor, not all the tumor cells will be removed. A controlled release device may be implanted immediately after the surgery to target the leftover cells to prevent proliferation of the leftover cells [6].

Paclitaxel is a promising chemotherapeutic agent against a wide spectrum of carcinomas [6-12]. It is a highly hydrophobic molecule with very low aqueous solubility. However, it was found to be associated with problems of extremely slow release when encapsulated in compressed discs [12].

In the present study, fabrication and characterization of blank and paclitaxel-loaded micro-porous foams as

treatment of gliomas as well as other forms of carcinomas

were studied and the operating conditions were optimized to achieve foams with good mechanical strength and controllable pore sizes.

II. Experimental

A. Materials

Polymer Poly DL lactide-co-glycolide (PLGA) for a range of copolymer composition was used in this study. PLGA 5050 Low IV was purchased from Lakeshore Biomaterials (Birmingham, AL). PLGA 5050 (Cat No. P2191 MW = 40,000 – 75,000 Da), PLGA 6535 (Cat No. 2066 MW = 40,000 – 75,000 Da), PLGA 7525 (Cat No. P1941 MW = 66,000 – 107,000 Da) and PLGA 8515

all the experiments at 1/5 opening of the needle valve of the automatic back pressure regulator.
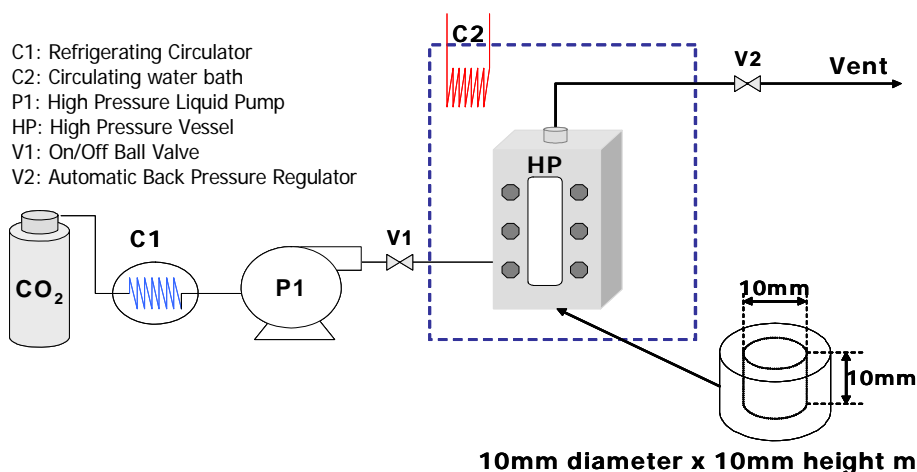
C. Spray Drying of paclitaxel-loaded microparticles

Paclitaxel-loaded PLGA powders were fabricated using a spray drying technique (Buchi Mini Spray Dryer) using as a solvent for paclitaxel and PLGA. For all the spray drying experiments, 2w/v% polymer solution, airflow rate of 800NL/min at 70°C, 100% aspirator ratio and 20% liquid feed rate was used.

D. Characterization and in vitro studies

Scanning Electron Microscopy (SEM, JEOL JSM-5600 LV, Japan) was used to study the porous structure and surface morphology of the polymeric foams fabricated in this study.

FIGURE 1. Experimental Setup for supercritical gas foaming technique

C1: Refrigerating Circulator
C2: Circulating water bath
P1: High Pressure Liquid Pump
HP: High Pressure Vessel
V1: On/Off Ball Valve
V2: Automatic Back Pressure Regulator

10mm diameter x 10mm height mold

(Cat No. 430,471 MW = 50,000 – 75,000 Da) were purchased from Sigma Aldrich (St Louis, MO, USA). Phosphate Buffered Saline (PBS, pH = 7.4) was purchased from Sigma Aldrich (St Louis, MO, USA). Paclitaxel was a generous gift from Bristol Myers Squibb. Compressed CO_2 (Airliquide Paris, France) was purchased from Soxal (Singapore Oxygen Air Liquide Pte Ltd). Dichloromethane (DCM) (DS1432, HPLC/Spectro Grade) and acetonitrile (ACN) (AS1122, HPLC/Spectro Grade) were purchased from Tedia (Farfield, OH, USA). Millipore water (Millipore Corporation, Billerica, MA) was used throughout the study.

B. Experimental Setup and foam fabrication

A custom-made mold with height and diameters of 10mm was machined from a ½" MNPT plug as shown in Figure 1. For each experiment, approximately 100 mg of polymer was loaded into the mold and sealed with a porous stainless steel wire mesh. The mold was then fitted onto the high pressure vessel and placed in a circulating water bath maintained at 35 ^{o}C. Saturation pressures from 8 – 10MPa and saturation times of 1 – 4 hrs was used in this study. The depressurization step was maintained consistently for

Differential Scanning Calorimetry (DSC, 2920 modulated, Universal V2.6D TA instruments) was carried out to determine the glass transition temperatures of the PLGA foams Approximately 2-10 mg of particles was loaded onto standard aluminum pans (40mg) with lids. The samples were purged with pure dry nitrogen at a flow rate of 20 ml/min. A blank aluminum pan was used as a reference in all of the analyses. The analysis was carried out using a temperature ramp of 10 ^{o}C/min from 20 – 200 ^{o}C for blank PLGA foams and from 20 – 250 ^{o}C for paclitaxel-loaded PLGA foams.

Gas Chromatography with Mass Spectrometer detector (GC-MSD) was used to determine the residual DCM content of the foams.

High pressure liquid chromatography (HPLC with UV-visible detectors, Shimadzu) was used to determine the encapsulation efficiencies and in vitro release profile of the paclitaxel-loaded particles. Discs of geometry 3mm diameter by 1mm height were punched from the paclitaxel-loaded foams and placed in PBS solution in a shaking water bath at 37 ^{o}C and 120 rpm. At predetermined time intervals, PBS solution was removed for sampling and fresh buffer was replaced. An ACN: water (50:50 v/v) solution was used as the mobile phase for HPLC analysis.

A: PLGA 5050
B: PLGA 6535
C: PLGA 7525
D: PLGA 8515

	Mean (μm)	S.D (μm)
A	72.54	18.28
B	54.05	15.19
C	49.45	11.86
D	47.49	13.28
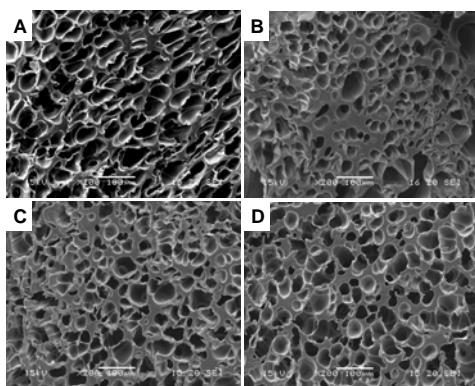
FIGURE 2. SEM observation of the interior pore structure of various blank PLGA foams obtained from polymer pellet (as obtained from Sigma Aldrich) using a saturation pressure of 12MPa and saturation time of 4hrs

III. RESULTS AND DISCUSSION

A. Micro-porous PLGA foams

Figure 2 shows the interior pore structure of blank PLGA foams with different lactide to glycolide content. A saturation pressure of 12MPa and saturation time of 4hrs was used to generate the foams. From Figure 2, it was observed that as the lactide content in the foams increase, the mean pore size decrease and which could be explained by the increased crystallinity of the polymer. It was also observed that PLGA 5050 yield open cell structures with interconnecting pores whereas PLGA 6535, PLGA 7525 and PLGA 8515 generally produced mostly closed cell structures.

The T_g of the foams were studied and Figure 3a and 3b shows the exotherm plot of the 4 different copolymers of PLGA before and after foaming respectively. The T_g of PLGA generally increases as lactide content increases. Comparing the T_g of PLGA before and after foaming, for all the 4 different copolymers, the T_g increases after foaming. This is due to the rapid solidification of the polymer during the depressurization step of the foaming process.

The *in vitro* swelling behavior of the 4 PLGA copolymers in PBS maintained in physiological conditions was studied. As shown in Figure 4, PLGA 5050 swelled by approximately 15% in the first 3 weeks. As the glycolide content in PLGA increase, its hydrophillicity increase. Therefore, PLGA 5050 is the most hydrophobic polymer which attributed to its most pronounced swelling. PLGA 6535 and PLGA 8515 showed little changes in volume. PLGA 7525 is observed to shrink and this may be due to the higher molecular weight (MW 66,000 – 107,000 Da) of the polymer as compared to the PLGA 5050, PLGA 6535 and PLGA 8515 used.
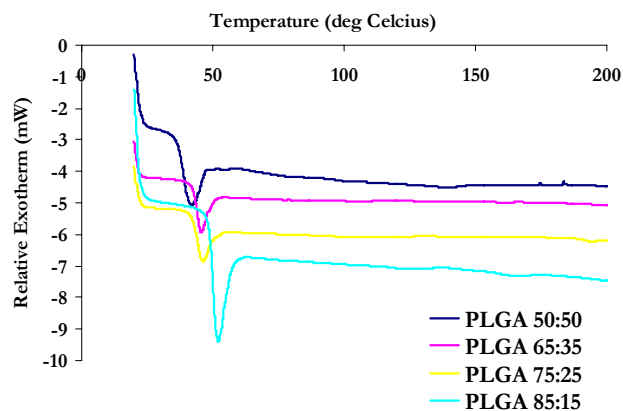

T_g (ºC)	PLGA 50:50	PLGA 65:35	PLGA 75:25	PLGA 85:15
Onset	36.50	42.37	42.18	48.46
MidPoint	37.34	42.23	42.55	48.72

FIGURE 3a. Glass transition temperatures of PLGA 5050, PLGA 6535, PLGA 7525 and PLGA 8515 before foaming
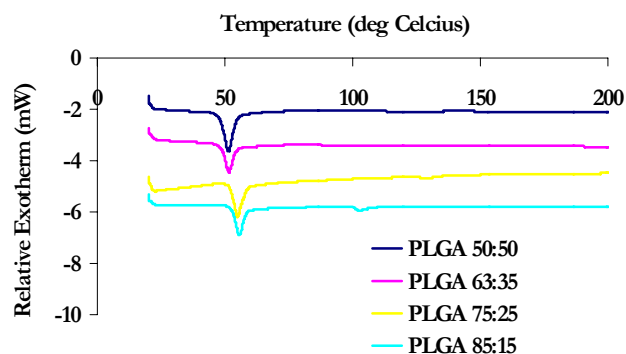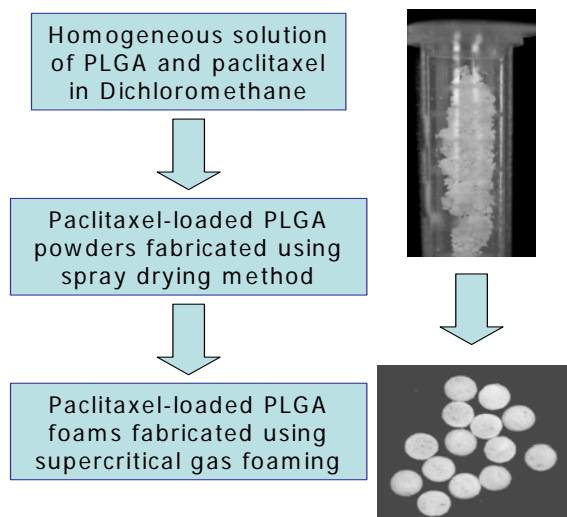

T_g (ºC)	PLGA 50:50	PLGA 65:35	PLGA 75:25	PLGA 85:15
Onset	48.08	48.51	52.23	52.57
MidPoint	46.13	47.66	51.65	52.09

FIGURE 3b. Glass transition temperatures of PLGA 5050, PLGA 6535, PLGA 7525 and PLGA 8515 after foaming
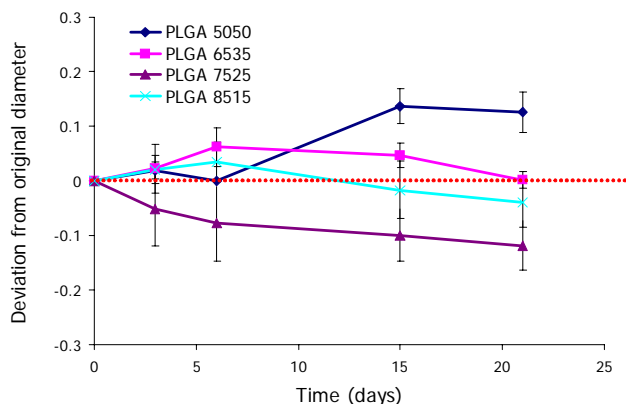
FIGURE 4. In vitro swelling behavior of PLGA foams

FIGURE 5. . 2-Step Fabrication process for paclitaxel-loaded PLGA foams using combined spray-drying and supercritical gas foaming technique

B. Paclitaxel-loaded PLGA foams

Paclitaxel-loaded PLGA foams were fabricated using a two-step process of spray drying followed by supercritical gas foaming of the microparticles as illustrated in figure 5. The purpose of the spray drying step was to achieve a molecular dispersion of paclitaxel in PLGA in the final foam product. The drug-loaded formulations studied were summarized in table 1 and the 5% drug loaded microparticle powders achieved from spray drying and the interior pore structures of 5% drug loaded foams are illustrated in figure 6a and 6b respectively. Figure 6a shows the spray dried powders with diameters ranging from 1 – 10 μm. From figure 6b, PLGA 5050 yield open cell structures and PLGA 8515 yield closed cell structures which are consistent with the results obtained for blank PLGA foams.

The encapsulation efficiencies of the paclitaxel-loaded microparticles and foams were studied using HPLC and The encapsulation efficiency of paclitaxel from the spray dried powders to the final foamed product was determined as:

$$Encapsulation\ efficiency\ (\%) = \frac{drug\ loading\ in\ foam\left(\frac{mg}{mg}\right)}{drug\ loading\ in\ powders\left(\frac{mg}{mg}\right)} \times 100\%$$

-- (1)

From table 2, the drug encapsulation using the supercritical gas foaming technique has very high efficiency between 90 – 100%. High encapsulation efficiency is a very important factor in most pharmaceutical processes as the active ingredient is normally very rare and expensive.

To determine whether paclitaxel encapsulated was molecularly dispersed in the polymer matrix within the foam [13-14], a thermogram analysis was carried out on the 10% paclitaxel-loaded powders and foams as shown in figure 6. Crystalline paclitaxel has an endothermic melting peak at 223 °C [15] and from figure 7, no paclitaxel melting peak was observed for both the powders and foams. This showed that no crystalline paclitaxel was present in both formulations and the drug was molecularly dispersed in the PLGA polymer matrix.

Figure 8 shows the release profile of paclitaxel from the 5% formulations. The release from 5% drug-loaded PLGA 5050 (Low IV) has the fastest release rate which can be explained by its high affinity for water. Using a more hydrophobic polymer PLGA 8515, a much slower release rate was achieved. This showed that by selection of the copolymer used, the *in vitro* release of drug from the foams may be altered.

As DCM was used as a solvent during the spray drying step, it is important to ensure that the residual DCM content in the foams was low enough within the limits of 500ppm as stated by the US Pharmacopeia. From Figure 9 it was shown that the spray dried microparticles has a high residual DCM content of approximately 300ppm. After freeze drying, the DCM content reduced by approximately 100ppm. Foaming with supercritical CO_2 removed approximately 150 ppm of residual DCM from the powders which shows the supercritical CO_2 removes DCM more effectively in much shorter times as compared to freeze drying.

**TABLE 1.** Composition of paclitaxel-loaded foams fabricated

Sample	Polymer	Drug loading (%)
A*-5%	PLGA 5050 (Low IV)	5
D-5%	PLGA 8515 (Sigma)	5
A-10%	PLGA 5050 (Sigma)	10

**TABLE 2**. Encapsulation efficiencies of paclitaxel loaded powders and foams

Sample	A*-5%	A-10%	D-5%
Drug loading in powders (mg/mg)	0.0478	0.1072	0.0490
Drug loading in foam (mg/mg)	0.0442	0.1072	0.0487
Encapsulation efficiency (%)	92.6	~100	99.4

IV. CONCLUSION

Paclitaxel-loaded PLGA foams were fabricated for controlled delivery applications. These foams provide an alternative to conventional methods of making implants
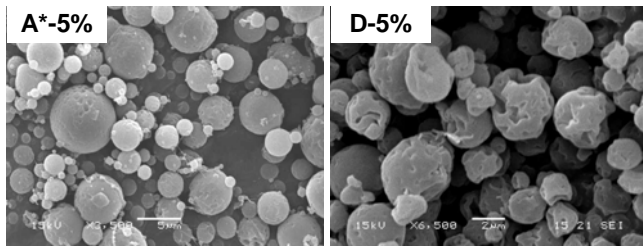
FIGURE 6a. SEM observation of the 5% paclitaxel-loaded PLGA spray dried microparticle powders
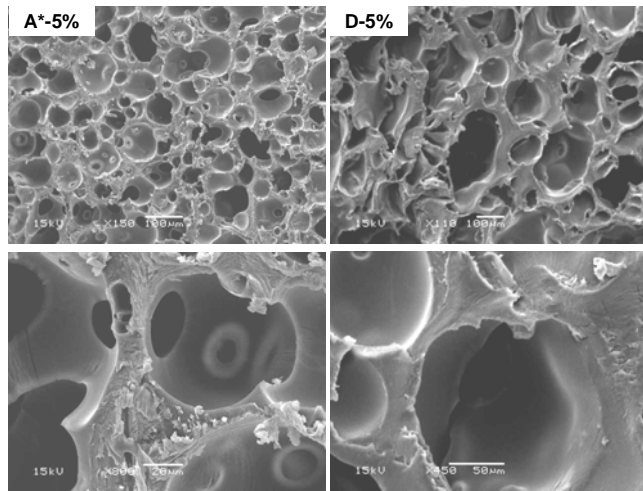
FIGURE 6b. SEM observation of the interior pore structure of 5% paclitaxel-loaded PLGA foams obtained powders using a saturation pressure of 8MPa and saturation time of 1hr

using compression discs. Micro-porous foams have higher surface-to-volume ratios, allowing more efficient drug release. The release profiles for the micro-porous foams fabricated as 3mm discs showed continuous and relatively linear release profile up to 5 weeks *in vitro*. The foams are mechanically strong enough to be used as an implant in surgical experiments. In addition, by altering the co-polymer ratio of the PLGA used, the release rate of paclitaxel may be modified to address therapeutic requirements.
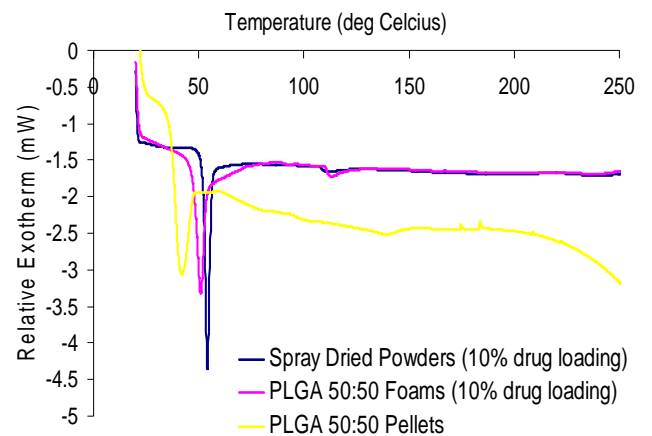
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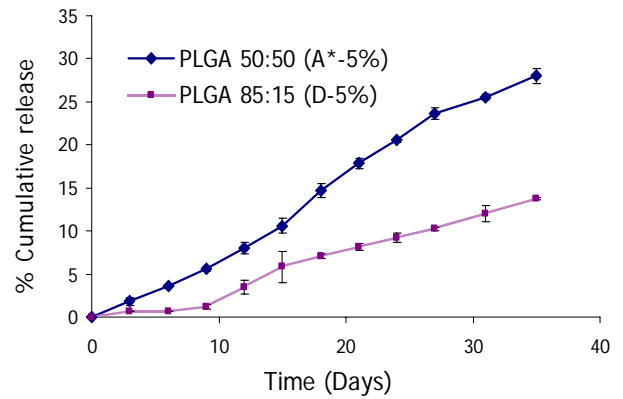
FIGURE 7. In vitro release of paclitaxel from PLGA 5050 (Low IV) and PLGA 8515 (Sigma Aldrich)

FIGURE 8. Thermogram properties of 10% paclitaxel-loaded PLGA 5050 powders and foams and blank PLGA before foaming
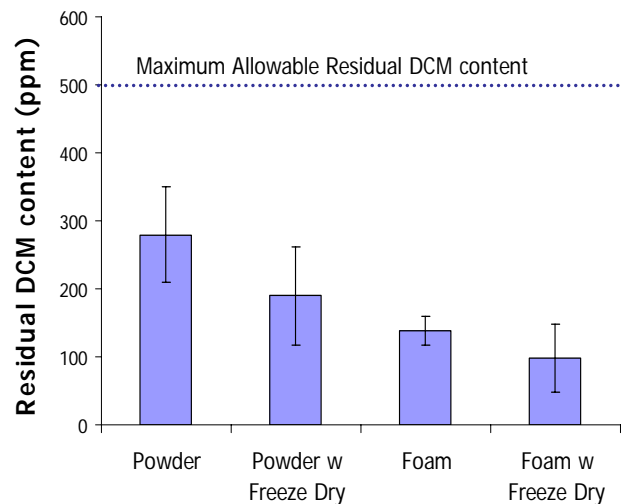
FIGURE 9. Residual DCM content in paclitaxel-loaded powders and foams measured using GC-MSD

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