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Production of drug-releasing biodegradable microporous scaffold using a two-step micro-encapsulation/supercritical foaming process

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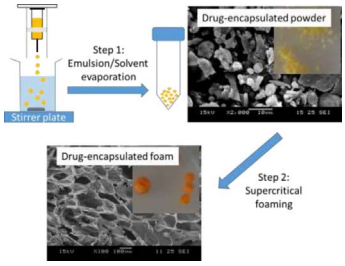
GRAPHICAL ABSTRACT



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ABSTRACT

A two-step fabrication process combining emulsification-solvent evaporation method for encapsulation of drug in PLGA microparticles followed by supercritical gas foaming was developed to produce drug-releasing biodegradable micro-porous foams. The encapsulation and release of model hydrophobic drug (Curcumin) and of model hydrophilic drug (Gentamicin) were investigated in this work. By utilizing a two-step fabrication process, a uniform dispersion of the drug in PLGA polymer matrix can be achieved and the method can be further adapted for the encapsulation of a wide range of active ingredient (both hydrophobic and hydrophilic) in biodegradable micro-porous scaffold.

The in vitro release profile of the drug-encapsulated PLGA foam was studied over a period of 2 weeks and it was observed that the drug release profile can be engineered by the selection of different PLGA polymer blend, varying lactic to glycolic ratio and molecular chain length of the polymer, and by addition of compatible biodegradable polymer such as Polyethylene Glycol (PEG) to the polymer matrix.

1. Introduction

Biodegradable polymer structures have important applications in biomedical and pharmaceutical applications as drug-releasing devices or implantable material in tissue engineering. Polylactic acid (PLA), polyglycolic acid PGA, and their copolymer poly (D,L-lactic-co-glycolic acid) (PLGA) were commonly used in various biomedical applications due to biocompatibility and ability to degrade into constituents that can be easily removed from the body [1,2].

Supercritical carbon dioxide processing has been applied in the development of various drug-delivery and biomedical materials using PLA and PLGA such as microparticles [3–6] and micro-porous biopolymer scaffold [7–14]. Microporous PLGA foams have potential applications as biodegradable and implantable drug delivery device [11], scaffold for DNA delivery [13] and tissue engineering [14]. Drug-releasing PLGA foams can be applied in tissue-engineering to support cell-growth as well as for implantable material for sustained release of medication. Microporous biopolymeric structures produced by

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supercritical CO_2 have high porosity (75–85%) [14], low residual organic solvent content [11], with good mechanical strength which allows easy handling without deformation [11].

Curcumin is a polyphenol compound derived from turmeric *(Curcumin longa)*. It has a distinct yellow-orange colour and has been found to display anti-inflammation, anti-oxidation, anticancer, antimicrobial and even anti-HIV [15–17] qualities. Current challenges in its biomedical application are mainly owing to its low aqueous solubility at physiological pH and low bioavailability [17]. Previous studies on formulation for curcumin using supercritical processing include using solution-enhanced dispersion via supercritical CO_2 (SEDS) to produce nano-curcumin [17,18], using atomized rapid injection solvent extraction (ARISE) system to produce inhalable curcumin with excipients such as polyvinylpyrrolidone (PVP) and hydroxypropyl-beta-cyclodextrin (HP-β-CD) [19], and co-precipitation of curcumin with PLGA to produce nanoparticles using a modified supercritical antisolvent (SAS) method [3].

On the other hand, Gentamicin is a commonly used highly hydrophilic antibiotic drug with antibacterial activity over a wide spectrum and excellent thermal stability [20–22]. Its application is limited by its hydrophilic properties which poses a challenge in developing prolonged antibiotic release implantable devices. Low encapsulation efficiency and short release times were some of the problems associated with its formulation. Gentamicin-loaded PLGA microspheres have been develop using water/oil/water emulsion [23,24] and also by spray drying methods [23]. Double-walled microspheres were also developed in an attempt to modify and prolong the release profile of gentamicin loaded samples for applications as implantable antibiotic treatment material [25].

Supercritical CO_2 foaming of biopolymers is an attractive method for production of microporous constructs for biomedical applications [7,9–11,13,14]. This is partly due to the non-toxic nature of CO_2 and its ability to effectively and cleanly remove residual organic solvents from the final product [11]. Encapsulation of active ingredient in microporous PLGA scaffold using supercritical foaming technique have been developed for compounds including chitosan [13], indomethacin [10], 5-fluorouracil [9] and paclitaxel [11]. Methods employed include a single-step impregnation process as presented by Cabezas et al. [9,10] and also a similar 2-step spray-drying/supercritical foaming process by Lee et al. [11] and Nie et al. [13].

In this work, drug encapsulation using established emulsification-solvent evaporation technique was performed to obtain a dispersion of drug compound in the PLGA polymer matrix. The 2-step process is adapted on both a model hydrophobic drug and a hydrophilic drug to demonstrate its potential application in a wide range of active ingredients that can be encapsulated in biodegradable foams using this technique. Due to the high affinity of supercritical CO_2 with organic solvents used in the microencapsulation step, the residual organic solvent content in the final product can be expected to be very low as shown in previous studies [11].

2. Materials and methods

2.1. Materials

Polymers poly(D,L-lactic-co-glycolic acid) PLGA 75:25 (lactide: glycolide = 75:25; Product number: P1941; MW = 66–107 kDa; Tg = 45–50 °C), PLGA 5050 with Low MW (lactide: glycolide = 50:50 acid terminated; Product number: Resomer RG502H; MW = 7–17 kDa; Tg = 42–46 °C), PLGA 50:50 (lactide: glycolide = 50:50 ester terminated; Product number: Resomer RG505; MW = 54–69 kDa; Tg = 48–52 °C), polyethylene glycol (PEG 8000; Mw 7–9 kDa) were purchased from Sigma Aldrich (Singapore). Phosphate buffered saline (PBS) tablet (0.01 M phosphate buffer, 0.027 M potassium chloride and 0.137 M sodium chloride, pH7.4 at 25 °C, Curcumin (CM) from Turmeric Powder, Gentamicin sulfate (GS), Dichloromethane (DCM) anhydrous and O-Phthaldialdehyde (OPA) Reagent were purchased from Sigma Aldrich (Singapore). Acetone was purchased from Tedia (Fairfield, OH, USA). Ethanol (HPLC grade) was purchased from Fisher Chemical. Compressed carbon dioxide (CO_2) was purchased from Soxal (Singapore Oxygen Air Liquide Pte Ltd, Singapore).

2.2. Micro-encapsulation of curcumin or gentamicin in PLGA

Drug-encapsulated PLGA powder were prepared using an oil/water emulsion method as described in Section 2.2.1 and 2.2.2 for curcumin encapsulation and gentamicin encapsulation respectively.

2.2.1. Curcumin (model hydrophobic drug)

5 mg of curcumin and 500 mg of polymer (~1% w/w) were dissolved in 10 ml acetone. The solution was added dropwise to 100 ml of DI water at a 1.0 ml/min using a syringe pump fitted with BD luer-lokTM syringe with Terumo® needle size 25G (500 μm ID) and size 21G (800 μm ID) for formulations with PLGA 50:50 and PLGA 75:25 respectively The distance from the needle tip to the beaker was 5 cm. The emulsion mixture was constantly stirred at 400 rpm for 4 h at 37 °C in the fume hood. The precipitated drug-encapsulated polymeric particles suspended in DI water were centrifuged for 10 min at 10000 rpm at 10 °C (KUBOTA High Speed Refrigerated Centrifuge). The supernatant was removed while the moist particles were lyophilized using freeze drying (Christ Alpha 1–2 LO plus) for 24 h under −43 °C and vacuum pressure.

2.2.2. Gentamicin (model hydrophilic drug)

20 mg of Gentamicin and 500 mg of PLGA (~4% w/w) were dissolved in 10 ml of acetone. As Gentamicin is only slightly soluble in acetone, sonication was carried out to disperse it in the solution. This is to ensure that PLGA is fully dissolved while gentamicin sulfate is well dispersed in the solution. Solution was added dropwise to 100 ml of DI water at 60 ml/hr using a syringe pump. The emulsion mixture was constantly stirred at 400 rpm for 2 h at 37 °C in the fume hood. The drug-encapsulating particles were collected via centrifugation and freeze drying as described in Section 2.2.1.

2.3. Supercritical foaming to obtain drug-encapsulated foam

Approximate 100 mg of drug-encapsulated particles were weighted and packed in a 1 cm diameter, custom-made cylindrical shaped mold (aluminum) and loaded in the supercritical foaming chamber. The chamber was connected in the supercritical CO_2 foaming set-up as shown in Fig. 1. Compressed CO_2 was first liquefied (Polyscience refrigerated circulator) before delivered to the high pressure chamber (Constructed from a Swagelok 1½" stainless steel bulkhead connector) using high pressure liquid pump (Eldex BBB-4-2). The supercritical foaming chamber is maintained at 35 °C (Polyscience 712 immersion circulator) and 120 bar (using Automatic Back Pressure Regulator, Thar Technologies Inc.) during the foaming process for 4 h. At the end of the experiments, the vessel was depressurized by setting the backpressure valve to $^1/_{10}$ opening till 90 bar pressure and subsequently increasing the backpressure valve opening to $^2/_{15}$ opening till atmospheric pressure.

2.4. Size and surface morphology analysis

The surface morphology of the drug-encapsulated microparticles and microporous foams generated in this study were evaluated using scanning electron microscopy (SEM, JEOL JSM-5600 LV, Japan). Platinum coating (Autofine Coater, JEOL JFC-1300, Japan) at a current of 40 mA for 60 s was applied to all samples prior to analysis. The characteristic pore size and size distribution of the foams were estimated by measuring the equivalent projected area diameter of micropores observed on SEM images using image processing software ImageJ [26].