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ScienceDirect
Acta Biomaterialia 4 (2008) 264–272
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

Novel fabrication techniques to produce microspheres by thermally induced phase separation for tissue engineering and drug delivery

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Received 6 August 2007; received in revised form 17 September 2007; accepted 25 September 2007
Available online 13 October 2007

Abstract

A novel application of thermally induced phase separation (TIPS) is described enabling the rapid formation of monodisperse porous microspheres. By taking advantage of TIPS processing parameters, the porosity, the pore morphology (bimodal/channel-like/radial towards the centre) and the presence of an open-pore or dense skin region can be tailored. Achievable sizes range from 10 to 2000 μm in diameter. The technique facilitates the homogeneous inclusion of particulate fillers and drugs. Moreover, the combined TIPS/oil-in-water emulsion technique allows for the production of microspheres with isotropic pore morphology with interconnected spherical pores of 30–70 μm and well-formed porous microspheres of 10–200 μm in diameter with an open porous surface. This method is advantageous over existing techniques by avoiding the use of long-term exposure to an aqueous continuous phase as used in oil-in-water or water-in-oil-in-water processing and therefore drug encapsulation efficiencies will be higher.

Keywords: Drug encapsulation; Scaffold; Thermally induced phase separation; Bioactive glass; Antibacterial

1. Introduction

Injectable scaffolds for tissue engineering to fill voids in damaged tissue are appealing due to their ability to conform to the implant site, whereas preformed scaffolds require prior knowledge of the defect or cavity to be filled and those with irregular size and shape can prove problematical. A microsphere network with sufficiently large interstices allows tissue to infiltrate the network to take over the mechanical loads as the scaffold degrades. Several microsphere-based injectable scaffold systems have been developed, including an injectable gas foaming polypropylene fumarate-based matrix containing microspheres [1], in situ formation of porosity in a matrix based on differential polymer degradation using microspheres [2], particulate leaching, gas foaming [3] and air entraining using surfactant molecules [4]. Solid poly(lactide-co-glycolide) (PLGA) microspheres have been investigated as injectable systems for cartilage [5] and adipose tissue engineering [6].

Biodegradable microspheres have also been widely investigated as local delivery and controlled release systems for bioactive compounds, such as low molecular weight and macromolecular therapeutics, proteins or DNA. This is probably because, unlike traditional small-molecule

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1742-7061/$ - see front matter © 2007 Published by Elsevier Ltd. on behalf of Acta Materialia Inc.
doi:10.1016/j.actbio.2007.09.011

drugs, proteins and peptides generally cannot be administered orally and may therefore require injection or infusion. Furthermore, because of their fragility and short in vivo half-lives, encapsulation of proteins in biodegradable polymeric devices enables a means of providing sustained and controlled release of the encapsulated bioactive compound, while the non-released bioactive material may be protected from degradation and physiological clearance. Microencapsulation processing is most often based on solvent extraction/evaporation methods. Various technologies have been used for microsphere preparation [7]. Among these are static mixing, extrusion through needles, membranes and microfabricated microchannel devices, dripping using electrostatic forces and ultrasonic jet excitation, as reviewed elsewhere [7]. A number of studies have investigated the preparation of biodegradable PLGA microspheres using water-in-oil-in-water (W/O/W) double emulsion methods aimed at delivering hydrophilic and macromolecular protein and peptide drugs in a sustained manner [8–10]. Sphere size and distributions are often poorly controllable with emulsion microsphere fabrication routes, with typical standard deviations of mean diameter being 25–50% of the target size, in addition to defect formations (such as hollow shells) [11]. Therefore a system capable of precise microsphere fabrication with high drug encapsulation efficacy is desirable to provide an efficient route to commercial manufacture and clinical implementation of drug-loaded microspheres. Fabrication of PLGA microspheres with precisely controlled and monodisperse size distributions has been achieved by spraying polymer-containing solutions through a nozzles with acoustic excitation to produce uniform droplets, or an annular non-solvent carrier system allowing further control of the droplet size [11], which has been applied to produce uniform spheres with average diameters from ~5 to >500 μm. Microspheres have also been formed by dropping polymer solutions containing dispersed protein particles via electrostatic forces into cold methanol (at −75 °C) for particle collection and solvent extraction [12]. To eliminate the initial burst and better control the release of the highly water-soluble cardiotoxic drug doxorubicin, double-walled microspheres with the drug encapsulated in the inner core have been fabricated with poly(L-lactide) shells and PLGA cores using the solvent evaporation technique – a modified oil–oil–water emulsion solvent evaporation technique, which involves the phase separation phenomenon of a binary composite of these two polymers [13].

Porous biodegradable microspheres are desirable for tissue engineering and drug delivery applications because the constituent amount of polymer is reduced compared with solid microspheres, yet the scaffold volume is kept constant and the degradation mechanism is a more predictable erosion type occurring through water hydrolysis of ester bonds. Several techniques have been applied to fabricate porous microspheres, including rapid solvent removal by introducing a temperature gradient [14], gas foaming [15], double emulsification (W/O/W) [16] and solution induced phase separation [17]. However, many of these procedures are complicated for repeatable production, and with some the microspheres may still receive prolonged exposure to an aqueous continuous phase. Thermally induced phase separation (TIPS) has been applied to generate highly porous foams as monoliths [18], but to date this process has not been applied to the fabrication of porous microspheres.

The current study describes a novel technique to produce porous TIPS microspheres using poly(α-hydroxyesters) such as PLGA. The TIPS process offers control over pore morphology and porosity, the inclusion of particulates and drug inclusion. TIPS microspheres were produced from liquid–liquid phase separation of neat PLGA/dimethyl carbonate (DMC) and by an emulsion route, including water (to adjust the pore morphology), PLGA/silver-doped phosphate-based glasses by solid–liquid phase separation, and dispersion of protein particles by phase separation of PLGA/DMC in the presence of fluorescently labelled antibody.

2. Materials and methods

PLGA (75:25, inherent viscosity approximately 0.6 dl g^{-1}; Medisorb, Alkermes, USA) was used as the polymeric matrix. DMC (freezing point −1 °C, of >99.9% purity; Sigma Aldrich, UK) was used as a solvent for the PLGA. Hard particulate inclusions consisted of antimicrobial silver-doped phosphate-based glasses, prepared as previously reported [19] using NaH_2PO_4, $CaCO_3$, P_2O_5 (BDH, UK) and Ag_2SO_4 (Sigma Aldrich, UK). The glass compositions investigated had a fixed phosphate and CaO content of 50 mol% and 30 mol%, respectively, with Na_2O substituted with Ag at 1, 3 or 5 mol%. Glasses were ground using a Pascal rotating ball mill (Christison scientific, UK), sieved to <20 μm and stored in a cool, dark environment prior to use. Tetramethyl rhodamine isothocyanate (TRITC)-labelled goat antibody (Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD) was used as a model protein to indicate dispersion of additives in the microspheres.

2.1. Processing methodology for PLGA TIPS microspheres and particulate loading

0.83 g PLGA was dissolved in 5 ml DMC (1:6 w/v) for 2 h in a 50 ml Falcon™ tube, under magnetic stirring.

Phosphate-based glass was included at three concentrations, as summarized in Table 1. To ensure adequate mixing of the bioactive glass particulates and homogeneous distribution within the polymer solution, all mixtures were magnetically stirred for 2 h, sonicated for 15 min and subsequently vortexed prior to use. The polymer and composite solutions were manually dropped from a 1 ml syringe fitted with a 23G needle (nominal inner diameter (ID) 320 μm) into liquid nitrogen (~40 ml in a Falcon™ tube) to rapidly induce the phase separation. The distance between the needle tip and the liquid nitrogen surface

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