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Preparation of porous microsphere-scaffolds by electrohydrodynamic forming and thermally induced phase separation

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ABSTRACT

The availability of forming technologies able to mass produce porous polymeric microspheres with diameters ranging from 150 to 300 μm is significant for some biomedical applications where tissue augmentation is required. Moreover, appropriate assembly of microspheres into scaffolds is an important challenge to enable direct usage of the as-formed structures in treatments. This work reports the production of poly (glycolic-co-lactic acid) and poly (ε-caprolactone) microspheres under ambient conditions using one-step electrohydrodynamic jetting (traditionally known as atomisation) and thermally induced phase separation (TIPS). To ensure robust production for practical uses, this work presents 12 comprehensive parametric mode mappings of the diameter distribution profiles of the microspheres obtained over a broad range of key processing parameters and correlating of this with the material parameters of 5 different polymer solutions of various concentrations. Poly (glycolic-co-lactic acid) (PLGA) in Dimethyl carbonate (DMC), a low toxicity solvent with moderate conductivity and low dielectric constant, generated microspheres within the targeted diameter range of 150–300 μm. The fabrication of the microspheres suitable for formation of the scaffold structure is achieved by changing the collection method from distilled water to liquid nitrogen and lyophilisation in a freeze dryer.

1. Introduction

Microspheres have been widely used in medical and pharmaceutical applications as effective carriers of encapsulated drugs [1,2]. Spheres made of a biodegradable polymer enable the encapsulated drug to be released in a time-controlled manner, maintaining a constant therapeutic concentration in body fluids over a desirable period (hours or days) from the moment of administration [3]. Polymeric spheres are achievable with special characteristics such as high surface-to-volume ratio, low density, and low coefficient of thermal expansion [4]. Control of the internal and external morphology of the microspheres can be used to influence their interactions with the encapsulated drug as well as the microenvironment after their delivery into the body. Microspheres with surface porosity show a better rate of drug release compared with their smooth surface counterparts. This is because the porous membrane will lead to a slow homeostatic level of the encapsulated drug and prevent the spheres from initial sudden burst [4].

Common methods available for generating drug-encapsulated polymeric porous spheres include emulsion polymerization, thermal phase separation and spray-drying [5–8]. However, there are disadvantages with these methods. For example, emulsion polymerization produces polydisperse spheres with a broad size distribution profile [8]. Non-degradable additives such as surfactants or polymers are also typically required as emulsifiers [9]. Residual solvent toxicity is another issue and the purifying process to separate the spheres from the solvent is slow and costly for pharmaceutical applications [9]. Most importantly, due to exposure of drug for instance to elevated temperatures and high shear stresses in the emulsion method, the biological activity of the drug can be significantly reduced during processing [10–12]. A thermal phase separation technique can generate spherical particles with rigid outer surfaces and a long shelf life. However, the method requires time-consuming multiple processing stages, and suffers from poor control over the diameter distribution of the fabricated particles. In addition, the spheres often stick to each other during formation and before the completion of the processing stages, resulting in large aggregates [13]. Although this technique suffers from a number of disadvantages, the use of solvents with low-boiling point such as dimethyl carbonate (DMC) combined with freeze-drying can reduce the drawbacks in generation of therapeutic products. Thermally induced phase separation (TIPS) followed by freeze-drying has been widely adopted for fabrication of the porous drug vehicles for applications in chronic wound therapy, drug delivery and also tissue engineering [14–18]. Spray-drying is a robust sphere generation method and the processing conditions to control the generated particles diameters are relatively straightforward. However, solvent removal is an issue in spray-drying, which often produces large aggregates. In addition, a large number of spheres

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are often lost during production due to the products sticking to the walls of the spray drier. [19–21].

Single-nozzle electrohydrodynamic atomisation (EHDA) can be used to generate near-monodisperse micro- and nano-spheres for applications in drug delivery systems [22,23]. It is a versatile method capable of processing a variety of solutions and emulsions of different polymers and/or therapeutic agents under ambient conditions via a single-stage production process, with flexibility to monitor the product quality at any time without delay during the production stage, without affecting the continuity of the process or having to wait for a multi-stage production process to complete before inspection [4,24,25].

An EHDA process subjects a liquid to a high electric field (in the range of kilovolts), which causes charges to build up within the liquid. When the applied electrostatic force overcomes the surface tension of the liquid, the meniscus of the liquid held at the tip of a nozzle elongates into a conical shape and a fine jet generates from the apex of the cone. The jet subsequently breaks up and deposits on an electrically grounded collector as fine polymeric droplets [26]. A stable cone-jet is the most desirable electrohydrodynamic jetting condition for near-monodisperse spherical particle generation [26]. The mean sphere diameter generated can be changed from the micrometre to the nanometre scale by varying EHDA processing parameters, especially flow rate and applied voltage as well as solution properties, e.g. concentration and physical properties of the polymer solution [27–30]. To maintain a stable cone-jet, the rate of mass transfer to the nozzle exit (controlled by the liquid flow rate) should be coupled and balanced by that out of the nozzle (controlled by the applied electric field, which is responsible for the force causing jet formation). Hence, the flow rate and the applied voltage used for each polymer liquid should be carefully coupled within a defined range [27].

A number of polymers with different properties have been investigated in EHDA studies for their potential application in drug delivery systems. However, only a few of them such as poly (ε-caprolactone) (PCL) and poly (lactic-co-glycolic acid) (PLGA) have been extensively used due to their biocompatibility, biodegradability and versatile degradation kinetics [31,32]. PLGA co-polymers have higher rate of biodegradation by hydrolysis under physiological conditions compared with PCL as they contain more ester groups per polymer molecular chain [33]. The biodegradation products of these two polymers have been shown to be non-toxic, non-immunogenic, non-teratogenic and non-carcinogenic [34]. Furthermore, the composition of these polymers can be varied in order to obtain a desirable release profile based on the rate of hydrolytic degradation. Because of their wide usage in the development of drug delivery systems [35], PLGA and PCL were selected for this investigation.

A suitable sphere diameter and morphology is one of the crucial requirements in a drug delivery system [25,36–38]. The diameter and morphology of the spheres (from the micrometre to the nanometre range) determines their surface area to volume ratio for biochemical reactions and physicochemical interactions with biological agents and cells [34,35]. The diameter, surface morphology and membrane porosity of the spheres influence the physical and chemical interactions as well as the anisotropy of the spheres in a physiological environment, and their ability to penetrate tissue structures in vivo. These characteristics also influence intercellular trafficking as well as drug release, endowing them additional promising advantages in different medical and pharmaceutical applications [39–41].

Microspheres are being considered for a number of biomedical applications where minimally invasive delivery combined with in-situ scaffold formation technology is required [14–18]. For example with chronic wounds, it is suggested that biodegradable microspheres with surface porosity and diameter range of 150–300 μm will provide a conformable structure capable of filling irregular shaped cavities caused by chronic wounds. The interstices formed between the packed microspheres need to be large enough to allow cell migration into the scaffold to facilitate wound healing. This study investigated the feasibility of producing microspheres using one-step fabrication by single-nozzle EHDA and the subsequent assembly into scaffold structures with TIPS.

To ensure robust EHDA production, this work mapped EHDA parameters using a variety of polymer solutions for controlled generation of near-monodisperse microspheres of a targeted diameter. Comprehensive sets of data on the diameter distribution profile of the particles obtained over a broad range of flow rate and applied voltages are presented.

2. Materials and method

2.1. Materials

PLGA (copolymer 50:50, Resomer RG503H, number average molecular weight (Mn) = 33000 g/mol), was purchased from Boehringer Ingelheim (Ingelheim, Germany). PCL of two molecular weights, PCL10000 (Mn = 10000 g/mol) and PCL45000 (Mn = 45000 g/mol) were obtained from Sigma Aldrich (Poole, UK). Dimethyl acetomide (DMAc), dimethyl foramide (DMF), dimethyl carbonate (DMC) and toluene were obtained from Sigma Aldrich (Poole, UK). Liquid nitrogen was purchased from British Oxygon Company (London, UK). All materials were used as received.

2.2. Solution preparation

2.2.1. PLGA

5, 10 and 20%w/w PLGA solutions were prepared for two solvents of PLGA: DMAc and DMF. PLGA solutions of 5, 10 and 15%w/w were also prepared with combination of the polymer and DMC. Each solution was mechanically stirred for 900 s to ensure complete dissolution of PLGA.

2.2.2. PCL

15, 20 and 25%w/w PCL10000 and 5, 7 and 10%w/w concentrations of PCL45000 solutions were prepared by dissolving the respective polymer in toluene and mechanically stirring the solutions for 1800 s. Lower concentrations were used for PCL45000 to ensure the solutions remain in the dilute concentration regime for the higher molecular weight PCL polymer, to be able to generate spheres instead of fibres [42] during EHDA.

2.3. Polymer solution characteristics

The density, viscosity, surface tension, electrical conductivity and pH of each polymer solution were characterized at atmospheric pressure and ambient temperature (~20–24 °C). Density was measured with a 25 ml specific gravity bottle (VWR International, Lutterworth, UK). The mass of the empty bottle and the mass of the bottle filled with the solution were measured using an electronic balance (A&D HF-1200G A&D Instruments Ltd., Japan). Viscosity was measured using a rheometer (DV III Ultra Rheometer, Brookfield viscometers, USA). The electrical conductivity was estimated using a standard conductivity probe (pHenomenal PC 5000H, VWR, UK). The pH of the solution was measured by dipping a standard pH probe in the solution (pHenomenal CO11, VWR, UK). Surface tension was characterized using a Kruss tensiometer (plate method). Distilled and deionised water was used for calibrations of the instruments.

2.4. Droplet formation

Single-nozzle EHDA was used to prepare polymer droplets with different size ranges (Fig. 1). A stainless steel nozzle with 1.18 mm orifice (Stainless Tube & Needle Co Ltd, Tamworth UK) was coupled to a high-power voltage supply (Glassman Europe Ltd, Tadley, UK) to provide the applied voltage at the tip of the nozzle. The nozzle was supplied with the polymer solution by a silicon tube (with inner diameter