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PLGA microcapsules with novel dimpled surfaces for pulmonary delivery of DNA

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Abstract

We describe the fabrication of DNA-loaded poly(lactic-co-glycolic acid) (PLGA) microcapsules with novel surface morphologies that will be of use in pulmonary delivery. Our approach was to examine surface morphology and DNA encapsulation efficiency as a function of primary emulsion stability; using two surfactant series based on hydrophile–lipophile balance and hydrophobe molecular weight. Hydrophilic non-ionic surfactants yielded the most stable water-in-dichloromethane emulsions (HLB values >8). These surfactants normally favor convex (o/w) interfacial curvatures and therefore this atypical behavior suggested a relatively high surfactant solvation in the dichloromethane 'oil' phase. This was consistent with the large fall in the glass transition temperature for microspheres prepared with Tween 20, which therefore efficiently penetrated the PLGA matrix and acted as a plasiticizer. Blends of Pluronic triblock copolymers performed poorly as water-in-dichloromethane emulsifiers, and were therefore used to generate hollow microspheres ('microcapsules') with low densities (0.24 g/cm^3). Although the Pluronic-stabilized emulsions resulted in lower DNA loading (15–28%), microspheres (~8 μm) with novel dimpled surfaces were fabricated. The depth and definition of the dimples was greatest for triblock copolymers with high MW hydrophobe blocks. By cascade impaction, the geometric mean weight diameter of the microcapsules was 3.43 μm, suggesting that they will be of interest as biodegradable pulmonary delivery vehicles.
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Keywords: PLGA microcapsules; Non-ionic surfactants; Triblock copolymers; DNA encapsulation; Pulmonary delivery vehicles

1. Introduction

There is strong interest in developing PLGA microspheres for delivery of plasmid DNA for DNA-vaccines (Singh et al., 2000), or CpG DNA for stimulation of immune responses (Singh et al., 2001). Plasmid DNA (pDNA) encapsulated, either naked or condensed with polycationic polymers, in microspheres remains transcriptionally active following uptake in cultured macrophages (Benoit et al., 2001). In mice, DNA-vaccines released from microspheres (ca. 5 μm diameter) following their phagocytosis by antigen presenting cells stimulated natural humoral and cellular immune responses (Wang et al., 2004). Moreover, PLGA microspheres are themselves considered vaccine adjuvants by virtue of their controlled release properties (Thomasin et al., 1996; Audran et al., 2003; Zhou et al., 2003). It is apparent that encapsulation efficiencies for naked pDNA are quite low (Barman et al., 2000). This is sometimes overcome by prior DNA complexation with polycations such as protamine sulphate (Dunne et al., 2003). DNA 'nicking' (linearization) is clearly to be avoided and loss of supercoiled (to relaxed) isoforms is considered advantageous (Benoit et al., 2001). Optimization of DNA encapsulation has shown that the type and molecular weight of polyester, and surfactant concentration, are important considerations (Capan et al., 1999; Prabha and Labhasetwar, 2004). However, as for protein encapsulation, poly(vinylalcohol) is generally used as the emulsifier (Capan et al., 1999; Bouissou et al., 2004; Prabha and Labhasetwar, 2004). Surprisingly little work has described the stability of the primary emulsion with respect to the surfactant used and the efficiency of DNA encapsulation into microspheres.

Needleless vaccine delivery technologies generally focus on the nasal mucosae (van der Lubben et al., 2003; Yuki and Kiyono, 2003) or bronchus-associated lymphoid tissues (BALT), the latter demonstrated to trigger immunity against influenza virus (Smith et al., 2003). If the technological challenges can be overcome, good bioavailability of macromolecules can be achieved via the lung periphery employing particulate carriers. This has recently been demonstrated by spray-drying

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insulin-loaded chitosan nanoparticles suspended in mannitol solution to create hollow microspheres for pulmonary delivery (Grenha et al., 2005). Therefore, particles targeting the lung periphery and BALT could provide a needleless system able to test a range of vaccine concepts such as DNA-vaccines. If DNA-vaccines are to be delivered to the BALT and lung periphery, the PLGA vehicles would require an aerodynamic diameter above 3–5 μm, preferably with a narrow size distribution. 'Large porous PLGA particles', fabricated using emulsion–evaporation techniques, fit this criterion well, even though their size distribution is not strictly controlled (Edwards et al., 1997). The low densities of these particles results in an optimal aerodynamic diameter for a measured diameter of >8 μm. However, the dispersion and flow of PLGA aerosols could be further improved by minimizing particle–particle contact area. This is suitably demonstrated by albumin aerosols having external corrugations and common puffball spores which have surface protrusions (Geiser et al., 2000; Chew and Chan, 2001). Therefore, an alternative route to achieving PGLA aerosols would be to focus on engineering specific surfaces.

Surface engineering of PLGA microspheres is not currently possible due to a lack of understanding of parameters controlling the evolution of microsphere surface morphology. Work has focused on emulsion parameters (extraction of the oil phase, homogenization, PLGA molecular weight and concentration, etc.) and drying techniques controlling the internal morphology and drug loading-release (Nihant et al., 1994; Yang et al., 2000; Kim and Park, 2004b). For example: (i) lowering the emulsion stability alters the internal pore-morphology from matrix-like to multivesicular (Nihant et al., 1994); (ii) increasing the rate of solvent extraction increases microsphere diameter and size distribution (Yang et al., 2000). In contrast, no mechanism has been proposed for the appearance of differing surface morphologies, although the formation of the surface pores is thought to occur on water extrusion from osmotic imbalance (Yang et al., 2001). Our previous work has shown, empirically, that the type of surfactant used in the primary emulsion influences the microsphere external morphology (Bouissou et al., 2004). Also, a 'defective skin surface' has been described as a consequence of low oil-phase volumes (Yang et al., 2001); and tricaprin oil loaded microspheres unexpectedly yielded microspheres with dimpled surfaces (Schaefer and Singh, 2002).

Here we aim to address the issue of DNA encapsulation as a function of primary emulsion (water-in-dichloromethane) stability, and employ the various emulsion systems to investigate alternative routes to engineering microsphere surface morphology. The dichloromethane–water interfacial stability will be characterized for a series of non-ionic surfactant blends guided by their HLB values. Although an empirical value, HLB has been shown to be a predictive variable for the stability of polar/non-polar organic solvent emulsions (Cameron and Sherrington, 1996), and may therefore be of use in respect of the primary emulsions used in microsphere fabrication. The non-ionic surfactant series will be compared to HLB-matched Pluronic® PEO–PPO–PEO triblock copolymers (where PEO is poly(ethylene oxide) and PPO is poly(propylene oxide)); in principle imparting a steric contribution to the desorption kinetics from the interface (Cameron and Sherrington, 1996). A possible experimental route to PLGA particles with novel surfaces would be to use emulsion systems involving self-assembling 'biocolloids'; as applied to the fabrication of artificial spores from polystyrene (Hemsley and Griffiths, 2000; Hemsley et al., 2003). Although, the polystyrene chains aggregated orders of magnitude slower than does PLGA during solvent extraction, the work inspires the investigation of surfactant penetration into the PLGA matrix upon solvent extraction and surface evolution. We used differential scanning calorimetry to assess the glass transition of the microspheres as an indication of surfactant penetration of the PLGA matrix. The external and internal morphologies of microspheres, and DNA encapsulation efficiency were compared for non-ionic surfactants and triblock copolymers series. The work is of interest to the development of inhalable DNA-vaccines and in a wider context to the development of generic pulmonary drug delivery vehicles.

2. Materials and methods

2.1. Materials

PLGA (50:50 DL-lactide:glycolide, inherent viscosity 0.88 dl/g) was purchased from Purac Biochem, Netherlands. Poly(vinylalcohol) (PVA) (MW 25 000, 88% hydrolysed), Tween® and Span® sorbitan surfactants were purchased from Sigma Chemical Company. Water was purified to >16 MΩ cm. Dichloromethane (DCM) (analytical grade) was obtained Fischer Scientific, UK. Pluronic® series triblock copolymers were received as a kind gift from BASF, USA. Plasmid DNA (pGL3-control, Promega, UK) was prepared using a modified method of Sambrook and Russell (2001a); purified to an A_{260}:A_{280} ratio of 1.8 and analyzed by agarose gel electrophoresis (1% agarose gel, stained with ethidium bromide, following protocols described in Sambrook and Russell, 2001b) for RNA contamination. Hard gelatin capsules and the Monodose dry powder inhaler (DPI) were received as kind gifts from Capsugel, Belgium and MIAT SpA, Italy, respectively.

2.2. Preparation of water-in-DCM (w/o) emulsions

Water containing surfactants blends (0.2 to 1%, v/v) were emulsified in DCM using a homogenizer (IKA, T18 basic) at ratios of 1:5 and 1:10 (w:o). Visual inspection at set time points was made to record phase separation (creaming—the first formation of water globules). Stock surfactant blends were made to a specific HLB values as described in Table 1 and diluted appropriately in water before homogenization (stock solutions of Span were dissolved in DCM rather than in water).

2.3. Preparation of microspheres

A water-in-oil-in-water (w/o/w) double emulsion–solvent evaporation technique was employed. One hundred microliters of aqueous phase, constituting 300 μg of pGL3-basic plasmid, 1% surfactant or surfactant blend in TE buffer (10 mM Tris, 5 mM EDTA, pH 8.0), was injected into 1 ml DCM containing

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