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Comparison of post-emulsification freeze drying or spray drying processes for the microencapsulation of plasmid DNA

Majella E. Lane, Fiona S. Brennan and Owen I. Corrigan

Abstract

In this work, methods used to microencapsulate plasmid DNA in a biodegradable polymer were compared for their effects on the physicochemical characteristics of DNA-loaded microparticles and on the release and integrity of encapsulated DNA. Microparticles were formulated by either w/o/w emulsification and freeze-drying (EFD) or by w/o/w emulsification and spray-drying (ESD). The influence of both manufacturing processes on particle morphology, charge, release characteristics and biological activity of encapsulated DNA was evaluated. Particles produced by emulsification/spray-drying exhibited more diversity in shape and size than those produced by emulsification/freeze-drying. These particles also exhibited higher plasmid DNA encapsulation efficiency than particles produced by emulsification/freeze-drying. The fractional DNA release rates were similar over the first 25 days for both formulations, release rate declining more rapidly at later times for the ESD product. Mammalian cell transfection assays confirmed the biological activity of encapsulated DNA extracted from both types of particles, with significantly higher transfection levels being observed for ESD particles. Application of a double emulsion (w/o/w) before spray drying resulted in higher encapsulation levels (> 90%) relative to previous literature values, which used single (w/o) emulsions before spray drying. The emulsification/spray-drying technique described here appears to be a rapid and efficient method for the preparation of PLGA microparticles loaded with plasmid DNA.

Introduction

DNA encapsulation in polymer matrices has previously been reported as a formulation strategy to protect DNA from degradation and to control DNA release (Jones et al 1997). One of the most commonly used techniques for encapsulation of DNA is solvent evaporation (Wang et al 1999; Perez et al 2001); other techniques such as spray-drying (Walter et al 2001) and phase inversion nanoencapsulation (Mathiowitz et al 1997) have also been examined. However, aspects of these encapsulation methods, which involve high-speed homogenization or exposure to high temperatures, are themselves potentially damaging to the integrity of plasmid DNA. Changes in the physical structure of plasmid DNA (i.e. from a supercoiled to a linear or open circular form) are considered to be undesirable as the integrity and sequence of DNA is fundamental to its use as a viable vaccine. Common chemical processes, such as oxidation and deamination, may also lead to breakages in the phosphodiester backbone of the plasmid, converting supercoiled DNA into open circular DNA (Middaugh et al 1998).

In this study, microparticles were formulated by emulsification and freeze-drying (EFD) or emulsification and spray drying (ESD) to study the effect of microencapsulation technique on the morphology of DNA-loaded microparticles and on the release and biological activity of the encapsulated DNA. The influence of two different post-emulsification processes on the biological activity of samples of DNA extracted from microparticles was assessed following transfection into COS-7 cells.

Since the ultimate target of microparticles is the immune system, uptake by these cells in an in-vitro model of phagocytic cells was examined. Biodegradable poly(D, L-lactide-co-glycolide) (PLGA) microparticles have been shown to be effective in the

School of Pharmacy, Trinity College, Dublin 2, Ireland

Majella E. Lane, Fiona S. Brennan, Owen I. Corrigan

Correspondence: M. E. Lane, School of Pharmacy, Trinity College, Dublin 2, Ireland. E-mail: lanem@tcd.ie

delivery of proteins and nucleic acids and have been suggested to be ideal carrier systems for phagocytotic immune system cells, such as macrophage cells (Mϕ) (Walter et al 2001). DNA and protein-loaded microparticles have been shown to effectively transport DNA into the body inducing antibody responses (Challacombe et al 1997; McKeever et al 2002). The processes studied result in different particle size, morphology and zeta potential, properties that can impact on uptake of these microparticles to specific immune system cells (Ahsan et al 2002). A further aim of this work was, therefore, to investigate the ability of macrophage cells to take up microparticles produced by the different manufacturing processes.

Materials and Methods

Materials

Poly(D,L-lactide-co-glycolide) (75:25) (PLGA Resomer RG 752, MW 20 900; manufacturer's data) was obtained from Boehringer Ingelheim. pGL3 plasmid (5010 bp) and JM109 competent cells were purchased from Promega (USA). Plasmid DNA was purified using a Qiagen Mega Endofree plasmid purification kit according to manufacturer's instructions (Qiagen Ltd, UK). DNA release was quantified using a PicoGreen double-stranded DNA (dsDNA) quantitation kit (Molecular Probes, BV). Materials for cell culture experiments were obtained from Sigma (St Louis, MO) and from Gibco BRL (Paisley, UK).

Preparation of microparticles

Plasmid-loaded DNA microparticles were manufactured using either emulsification/freeze drying (EFD) or emulsification/spray drying (ESD).

EFD particles

A modified w/o/w double emulsion technique, as described elsewhere (Barman et al 2000; Hao et al 2000), was used. Briefly, DNA ($1\,mg\,mL^{-1}$) was dissolved in Tris-edetate buffer (TE buffer) and dispersed in a 1% w/v solution of PLGA in dichloromethane. The resulting mixture was slowly added to 1 L of 1% polyvinyl alcohol (PVA) and further emulsified using a Silverson L4R Mixer (Silverson, Bucks, UK). After solvent evaporation by stirring at a controlled temperature for 12 h, microparticles were recovered by centrifugation and were subsequently washed three times in de-ionized water. Microparticles were lyophilized by immersion in a Hetofrig cooling bath (Heto) for 15 min, then transferred to a Hetosicc CD52 freeze-drier (Heto) and left overnight (O'Connor & Corrigan 2001).

ESD microencapsulation

The w/o/w emulsions were prepared as described above. Dispersions were spray dried in a Buchi mini Spray Dryer Model 191 (Buchi Laboritorium-Technik, AG, Flawil, Switzerland) using an inlet temperature of 78/79°C, an aspirator rate of 75%, a pump rate of 10% and an airflow rate of $600\,NL\,h^{-1}$ (Corrigan et al 2003).

Microparticle characterization

For morphological examination, the microparticles were viewed using a Hitachi S-7000 scanning electron microscope (Hitachi, Japan). The particle size of the microparticles was determined by laser diffraction using a Malvern 2600 particle size analyser (Malvern, UK). Zeta potential measurements of microparticles were carried out using a Malvern Zetasizer (Malvern, UK). Three samples of each batch were measured at 25°C in double-distilled water containing 0.01 M NaCl.

DNA extraction

Determination of loading and encapsulation efficiency was carried out after DNA extraction from microparticles. DNA was extracted from microparticles using a modified chloroform extraction method reported previously (Barman et al 2000; Hao et al 2000). DNA-loaded microparticles were weighed out and suspended in 2 mL of TE buffer (pH 8) as previously described (Barman et al 2000). An equal volume of chloroform was added and the tubes were rotated end-over-end for 120 min at ambient temperature. The two phases were separated following centrifugation for 10 min at $13\,000\,rev\,min^{-1}$ and 100 μL of the supernatant was removed. From the remaining supernatant, released DNA was precipitated following the addition of 0.1 volumes of 3 M NaOAc (sodium acetate) and 2 volumes of ice-cold ethanol.

The samples were incubated at −80°C for 20 min. The samples were then centrifuged at $13\,000\,rev\,min^{-1}$ for 20 min. The pellet was rinsed with 70% ethanol and resuspended in an appropriate amount of TE buffer. Extracted DNA was quantified in a UV-VIS spectrophotometer at 260 nm against a blank of TE buffer and these samples were subsequently diluted for analysis of approximately 200 ng of DNA agarose gel electrophoresis (Barman et al 2000; Hao et al 2000).

To ensure that 100% of the DNA was recoverable from the method outlined above, spiking experiments were carried out. DNA (1 mg in a total of 200 μL of TE buffer) was added to 10 mg of empty microparticles. Chloroform (500 μL) was added and the tubes were end-over-end rotated as a function of time. The samples were centrifuged for 5 min at $13\,000\,rev\,min^{-1}$. A 100-μL sample of the supernatant was removed and assayed using UV. Agarose gel electrophoresis was carried out on the supernatant to characterize the DNA retrieved (Hao et al 2000).

Cell culture

The biological activity of encapsulated DNA was evaluated firstly by transfection of extracted DNA in COS-7 cells and secondly by phagocytic studies in murine J774A.1 macrophage cells (ATCC Manessas, VA). COS-7 cells were obtained from ECACC (Porton Down, UK) and grown in supplemented Dulbecco's modified Eagles medium (DMEM). The

DMEM was supplemented with 2 mM L-glutamine, 10% v/v foetal calf serum (FCS) and 100 mg mL^{-1} gentamicin. The transfection was carried out at approximately 70% confluency. COS-7 cells were transfected with DNA extracted from 1-mg samples of EFD and ESD microparticles as reported previously (Hao et al 2000; Prabha et al 2000). Each transfection experiment was carried out six times. Extracted DNA was used to transfect the COS-7 cells using DOTAP (Boehringer Mannheim, Germany) as the transfection agent. To measure the luciferase protein levels, the cells were washed twice using 1 × phosphate buffer saline (PBS) and lysed using 1 × Cell Culture Lysis Reagent (Promega, Madison, WI, USA). To each 20 μL of the cell lysate sample, 100 μL of the reconstituted luciferase assay substrate (Promega, Madison, WI) was added and the chemiluminiscence intensity was measured immediately using a luminometer (TD 20/20, Promega). The transfection efficiency was expressed as relative light units per mg of cell protein, the concentration of which was measured using a Micro-BCA assay (Pierce, Rockford, IL, USA).

In-vitro release studies

Microparticles (20 mg) were suspended in 2 mL of PBS buffer (phosphate buffer solution, pH 7.4) (Walter et al 1999, 2001). The microcentrifuge tubes were incubated at 37°C in a shaking water bath. At predetermined intervals the samples were centrifuged, the supernatant removed and replaced with fresh PBS. The DNA released from the microparticles was quantified using a PicoGreen double-stranded DNA quantitation kit. The resulting fluorescence of the reagent when bound to double-stranded DNA was measured by a spectrofluorophotometer according to the manufacturer's instructions (Molecular Probes, BV) (Barman et al 2000; Hao et al 2000).

Phagocytosis assay

Phagocytosis of PLGA particles was evaluated according to the method previously reported by Wang et al (1999). Macrophage cells were seeded at a density of 2×10^5 cells/well in 1 mL of DMEM media (Sigma, St Louis, MO) and incubated at 37°C in 5% CO_2. After 24 h incubation, 0.5 mg of microparticles were placed in 200 μL of DMEM media and added to each well. Wells were incubated at different time periods up to 24 h. At each time point the cells were photographed using phase contrast microscopy and then washed three times in PBS. They were then photographed again. The cells were scraped out and were either spun down onto a slide or were processed for transmission microscopy. The number of PLGA particles internalized per cell was evaluated by phase contrast microscopy. The magnification used was 63× oil immersion objective.

Statistical analysis

Differences in particle size, loading, encapsulation efficiency and macrophage uptake were analysed for both processes by the Mann Whitney U-test. The effects of incubation time on % recovery were analysed using the Kruskal–Wallis test followed by Dunn's test to determine individual differences. The in-vitro release data were fitted to the relevant mathematical model using the nonlinear curve fitting program Micromath Scientist for Windows Version 1.0 (Micromath Scientific Software). The goodness of fit and suitability of the model was evaluated using the coefficient of determination (CD) and model selection criterion (MSC). Differences between parameters were evaluated by the Mann Whitney U-test. Differences were considered significant at $P < 0.05$.

Results

Microparticle morphology and particle size

The morphology of typical microparticles formed via EFD is illustrated in Figure 1A and 1B. While plasmid-free microparticles had smooth and pore free surfaces (Figure 1A), plasmid-loaded microparticles produced by EFD were spherical with uneven surfaces and pores (Figure 1B). Figure 1C illustrates typical plasmid-loaded ESD particles.

Particle size will influence drug release from microparticles (Dunne et al 2000) as well as the potential for internalization of the microparticles by phagocytosis (Ahsan et al 2002). Particle size data are in agreement with the SEM images, with mean values in the range of 3–4 μm. The D[v,0.9] values for EFD particles (5.77 ± 0.65) and for the ESD particles (5.96 ± 1.30) were not significantly different ($\alpha = 0.05$, Table 1). In all of the microparticle batches in the study at least 90% of the microparticles were < 10 μm in diameter, consistent with the findings of Walter et al (1999) who reported particle sizes for microparticles produced by spray drying of a w/o emulsion, the majority of which were < 10 μm.

Loading and encapsulation efficiency

Analysis of loading and encapsulation efficiency (Table 1) indicated that the encapsulation efficiency for spray-dried systems was significantly higher ($\alpha = 0.05$) with a mean value of $91.13 \pm 2.85\%$ as compared with solvent evaporated systems with a mean value of $28.67 \pm 1.75\%$.

Comparison of processing methods on DNA integrity

Plasmid DNA exists in three major isoforms: supercoiled, representing the most compact stable plasmid isoform; nicked or open circle, occurring when there are breaks in the phosphate backbone of a single strand of the double-stranded molecule; and linear, indicating breaks at a single site in both strands of the circular plasmid. Depending on the stress or shear to which the plasmid has been subjected, degradation or conversion may progress from a supercoiled to nicked then linear isoform, and finally into smaller DNA fragments, which are represented by a smear in the agarose gel. Following standard plasmid preparation procedures, the majority of plasmid is in supercoiled form.

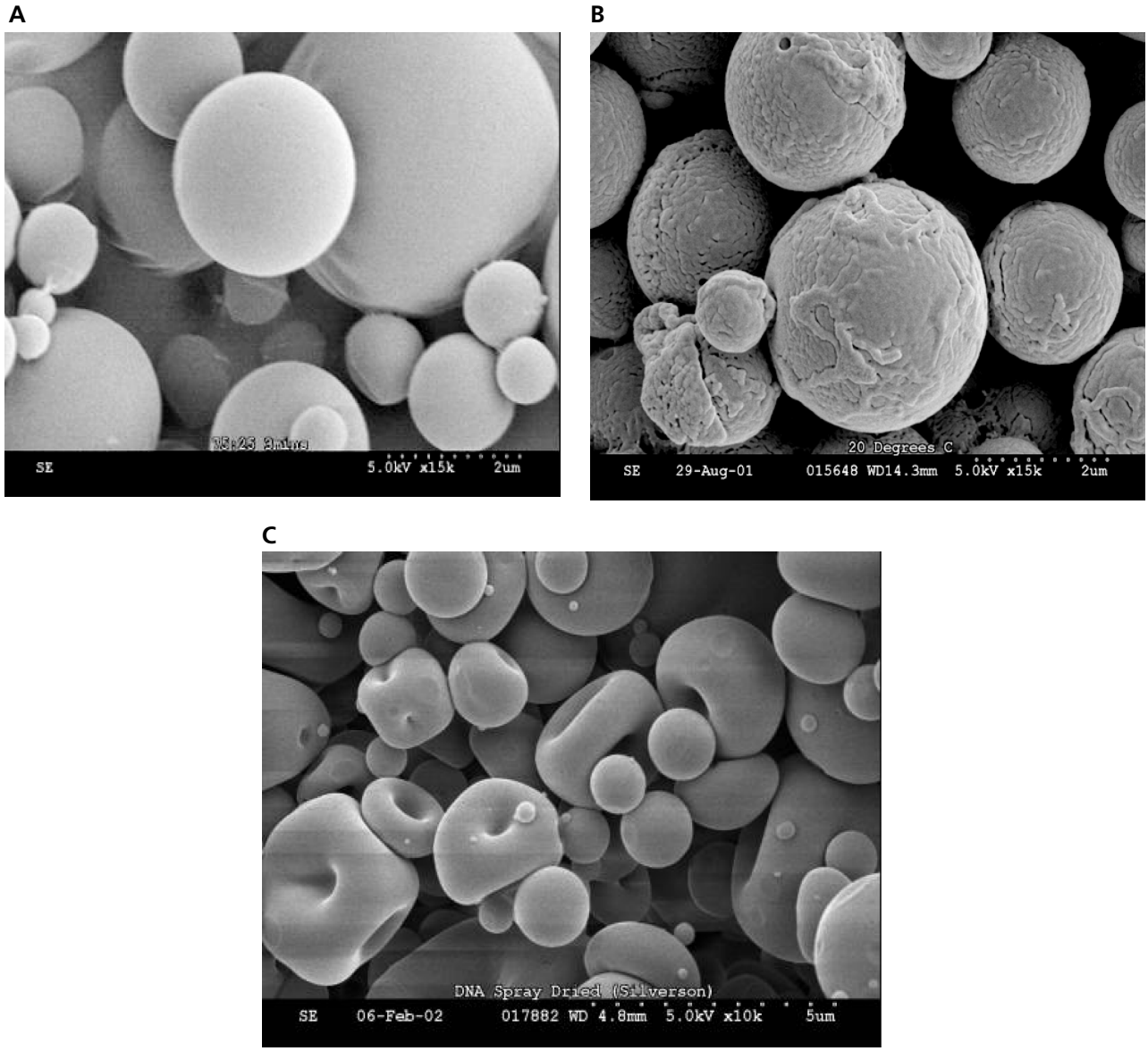

Figure 1 Scanning electron micrographs of plasmid-free EFD microparticles (A), plasmid-loaded EFD microparticles (B) and plasmid-loaded ESD microparticles (C).

The integrity of DNA encapsulated in PLGA microspheres manufactured by EFD and ESD processes was examined. Before the extraction of DNA encapsulated in PLGA microparticles, a spiking experiment was carried out to determine the effect of the extraction procedure on plasmid DNA, as reported by Barman et al (2000). As can be seen from Table 2, recovery of DNA following the spiking experiment indicated that > 80% of the DNA was recovered following 60, 90 and 120 min incubation followed by chloroform extraction. Extending the incubation time beyond 60 min did not influence the percentage of DNA recovered (Mann Whitney U-test, $P > 0.05$).

Extracted DNA samples from PLGA microspheres manufactured by EFD and ESD processes were examined. The results from agarose gel electrophoresis of the two samples analysed by this method are shown in Figure 2. From Figure 2 it can be seen that both lanes of extracted plasmid DNA contain both open circular (oc) and supercoiled (sc) DNA. DNA extracted from ESD microparticles also contained supercoiled (dsc) denatured products, possibly reflecting additional thermal and shear stress associated with the manufacture of the ESD particles.

The migration patterns of the unencapsulated control and DNA extracted from EFD microparticles appeared to be identical. Unencapsulated DNA was primarily in supercoiled form (as indicated by the higher intensity of the supercoiled band when compared with the open circular band), while DNA extracted from EFD microparticles show nearly equal intensities between the supercoiled and open circular band. However, extracted DNA from ESD

Table 1 Particle size data, zeta potential, loading and encapsulation efficiency for spray-dried and solvent-evaporated microparticles

Process	Particle size D[v, 0.9] (μm)	Zeta potential (mV)	Nominal load (μg DNA/mg particles)	Actual load (μg DNA/mg particles)	Encapsulation efficiency (%)
EFD	5.77 ± 0.65	0.9 ± 0.4	2.86	0.82 ± 0.05	$28.67 \pm 1.75\%$
ESD	5.96 ± 1.30	-5.2 ± 3.2	3.16	2.88 ± 0.09	$91.13 \pm 2.85\%$

Data are means $\pm$ s.e.m.

microparticles shows conversion to a number of DNA isoforms. This can be seen from the smearing that occurred in the gel during the run, which would indicate the presence of denatured DNA. Jilek et al (2004) prepared spray-dried microparticles of plasmid DNA from w/o formulations produced by sonication. Significant conversion of extracted plasmid DNA from supercoiled to the open circular form was observed after agarose gel electrophoresis of DNA. This observation was attributed to the rapid removal of solvents that occurs during the spray drying process. In addition, denatured DNA is also likely to reflect the temperature stress of the spray drying process (Gander et al 1995).

COS-7 cell transfection

COS-7 cells were transfected with DNA using the cationic lipid DOTAP. Cells were lysed and luciferase levels were determined using a luminometer and were measured as amount of luciferase per mg of total cell protein. Significantly more luciferase activity ($P < 0.05$) resulted from the transfection of DNA extracted from ESD microparticles (4642.47 ± 221.09 fg (mg total cellular protein)$^{-1}$) when compared with the DNA extracted from EFD microparticles (605 ± 55.38 fg (mg total cellular protein)$^{-1}$) (Figure 3). Hao et al (2000) microencapsulated plasmid DNA in PLGA 50:50 using a double emulsion solvent evaporation technique and reported conversion of a significant fraction of plasmid DNA to the nicked isoform. Transfection efficiency, however, did not appear to be affected by the changes in plasmid conformation in contrast to more sensitive bacterial transformation assays. Transfection efficiency was superior for DNA extracted from the ESD systems in this study in comparison with the EFD formulations. This is in line with the much higher encapsulation efficiency of the spray-drying process.

Table 2 Recovery of DNA following spiking experiment

Incubation time (min)	Extracted DNA (μg)	% Recovery
60	2.25 ± 0.09	86.21 ± 4.45
90	2.39 ± 0.16	91.57 ± 6.18
120	2.11 ± 0.14	90.84 ± 6.62

Data are means $\pm$ s.e.m.

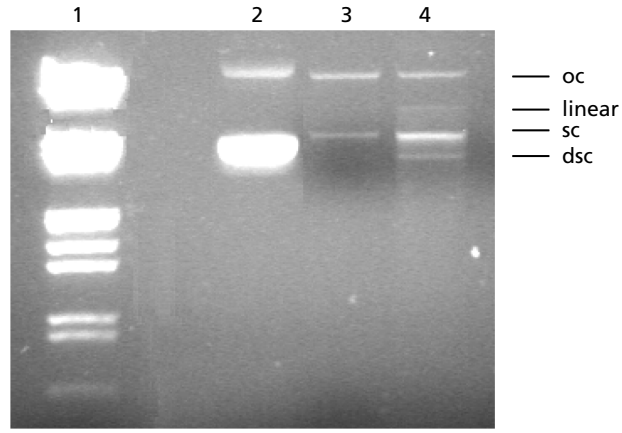

Figure 2 Structure of DNA post-encapsulation. Approximately 200 ng of DNA extracted from EFD and ESD microparticles was analysed by agarose gel electrophoresis. oc, open circular; sc, supercoiled; dsc, denatured supercoiled. Lane 1, molecular weight markers (MW range: 23 130 base pairs to 125 base pairs); lane 2, control DNA; lane 3, DNA extracted from microspheres produced following freeze-drying; lane 4, DNA extracted from microspheres produced following spray-drying.

DNA release

In-vitro plasmid DNA release studies were carried out over a 100-day period as previously described (Panyam et al 2003). There are different analytical methods available to determine DNA concentration. The most commonly used technique is the determination of the absorbance at 260 nm (OD_{260}), with the major disadvantages being the inability to distinguish between nucleotides, single-stranded DNA (ssDNA) and double-stranded (dsDNA), the interference by contaminants and the relative insensitivity of the assay. The PicoGreen reagent is an ultrasensitive fluorescent nucleic acid stain that has been used for quantitating

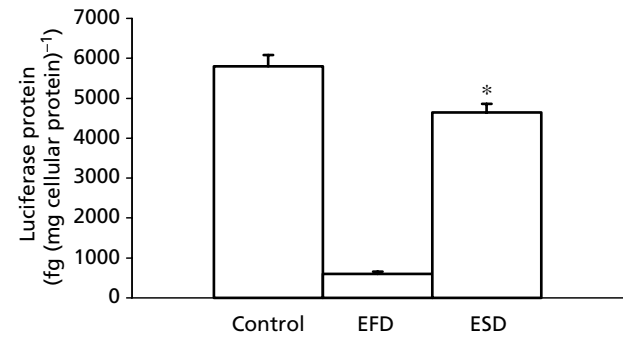

Figure 3 Transfection of COS-7 cells with DNA extracted from EFD and ESD microparticles and unencapsulated DNA as control. All wells received 1 μg of DNA or equivalent based on microparticle loading. Results are expressed as mean $\pm$ s.e.m. for six experiments (*$P < 0.05$ compared with EFD).

dsDNA in solution (Ferrari et al 1998). Free dye is essentially non-fluorescent and exhibits fluorescence upon binding to dsDNA. It is highly selective for dsDNA over ssDNA and oligonucleotides. In Figure 4 the fractional release of DNA from EFD microparticles is compared with that from ESD microparticles. The release profiles are non-linear and the fraction released is similar for both products over the first $\sim$ 25 days. Slower release is evident at later times from the ESD product. The data were fitted to the Sinclair-Peppas general power law expression (Equation 1) (Peppas & Sinclair 1984).

$$M_t/M_\infty = Kt^n \quad (1)$$

where K is the diffusional constant, and n is the diffusional exponent. The parameters obtained are given in Table 3. A higher MSC value for the EFD product, which gave a diffusional exponent (n) of 0.465, suggests that DNA release is diffusion controlled from these microparticles. The poorer fit and lower n value ($P < 0.05$) obtained for the ESD product is consistent with an initial burst release from this product, which may be linked to the greater loading efficiency obtained by the ESD process. The three washes in de-ionized water might also be expected to contribute to a lower burst effect for EFD particles relative to ESD particles, which are not subject to any washing procedure.

PicoGreen analysis reveals significantly lower amount of dsDNA released compared with OD_{260} measurements as reported previously (Walter et al 1999). Walter et al (2001) have reported the extent of the burst release to be of the order of 10–12% of total plasmid material from spray-dried particles prepared from single emulsion systems. Both EFD and ESD systems exhibited a sustained release of DNA over the one-hundred days of the trial, with 40% of plasmid release occurring for EFD systems by day 100, whereas 25% of plasmid release is observed for ESD particles. The release profile from ESD particles may be a function of the less porous morphology of these particles when compared with particles produced by EFD.

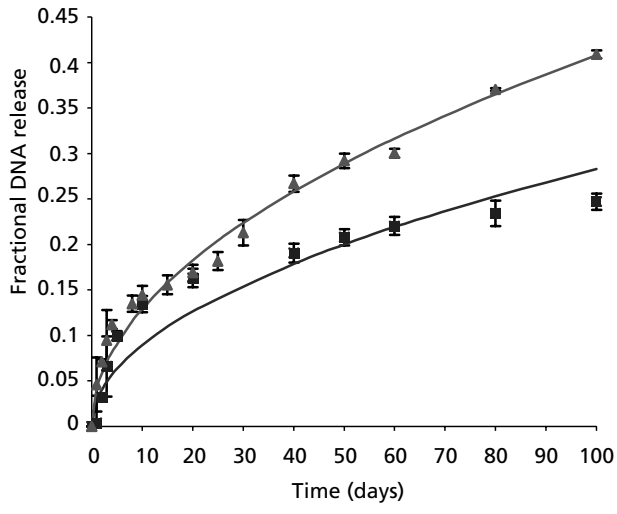

Figure 4 Fractional release of DNA from EFD (▲) and ESD (■) microparticles fitted to Equation 1. Results are expressed as mean s.e.m. for three batches.

Table 3 Parameters K and n obtained for the fitting of release of DNA from microspheres manufactured following emulsification/freeze-drying (EFD) and emulsification/spray drying (ESD) to Equation 1

Process	K	n	CD	MSC
EFD	0.047 ± 0.011	0.465 ± 0.007**	0.985	3.93
ESD	0.046 ± 0.008	0.378 ± 0.003**	0.954	2.75

Also shown are associated coefficients of determination (CD) and model selection criterion (MSC) values. Data are means $\pm$ s.e.m. **$P < 0.05$ as measured with Mann Whitney U-test.

Murine macrophage cell uptake of microparticles

Phagocytosis of microparticles by macrophage (Mϕ) cells has been reported to be greatly influenced by the physicochemical properties of the microparticles' surface and especially by the surface charge and the particle size (Tabata & Ikada 1990). In this study, particle sizes for microparticles produced using both processes were found to be comparable and to be below 10 μm (Table 1). The zeta potential of microparticles produced by EFD or ESD is reported in Table 1, where ESD microparticles are observed to have a stronger negative zeta potential (-5.2 ± 0.3 mV) than EFD particles (0.9 ± 0.4 mV). Microparticles produced from both processes were readily taken up by macrophages. Lacasse et al (1998) reported that microparticles with no drug loading, produced by spray-drying o/w emulsions of pure PLGA polymer, exhibited stronger negative zeta potential values when compared with microparticles produced from solvent evaporation of similar o/w emulsion formulations. This was attributed to residual polyvinyl alcohol on the surface of microparticles produced by solvent evaporation. The zeta potential values for plasmid-loaded microparticles produced by ESD in this study are less strongly negative than the values reported by Lacasse et al (1998) for microparticles with no drug loading. Higher numbers of ESD microparticles were observed to be phagocytosed over 24 h relative to EFD microparticles ($P < 0.05$) (Figure 5). The average number of particles per cell determined from three different experiments was 1.87 ± 0.45 particles for ESD particles and 1.11 ± 0.13 particles for EFD particles. This is in line with observations that masking of the negative zeta potential of particles will reduce their interaction with phagocytic macrophages (Lacasse et al 1998). The dependence of macrophage phagocytosis on the size of microparticles was examined by Tabata & Ikada (1990). Using monodisperse polystyrene particles, they reported that the number of microparticles phagocytosed per macrophage cell had a maximum at a diameter of 1–2 μm. The size of particles produced by both

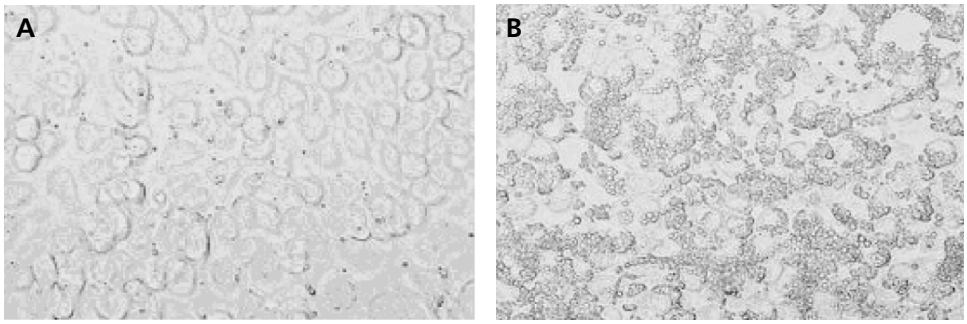

Figure 5 Macrophage uptake of EFD microparticles (A) and ESD microparticles (B).

processes did not appear to differ significantly as observed from particle size analysis, suggesting that particle charge may be the predominant influence on particle uptake. In the current study, DNA-loaded particles produced by ESD also appeared to aggregate more in cell culture medium when compared with (EFD) solvent evaporated microparticles (Figure 5). This aggregation may be attributed to the observation that spray-dried microparticles appeared to agglomerate at increased inlet temperature during spray drying (Stahl et al 2002).

Discussion

The manufacture of microparticles by spray-drying of oil-in-water (o/w) formulations for a wide range of drugs and bioactive molecules is well documented in the literature. However, to date, spray drying of water-in-oil-water (w/o/w) emulsions for microencapsulation of drug molecules has not been reported. This study was carried out to compare physicochemical characteristics of microparticles manufactured by freeze-drying or spray drying of a double emulsion formulation of plasmid DNA.

Particles formed following solvent evaporation were uniform in shape and size in comparison with the folding and indentation observed for microparticles produced from spray drying of w/o/w emulsions. In this study, particle size values for microparticles produced using both processes were comparable and less than 10 μm.

DNA encapsulation efficiency levels were found to be highest for the emulsification/spray-drying process with encapsulation efficiency levels > 90%. In contrast, microparticles produced via the double emulsion/evaporation process exhibited encapsulation efficiencies of less than 30%. Encapsulation efficiencies in this study for microparticles produced by emulsification/solvent evaporation are in line with other researchers; Wang et al (1999) encapsulated plasmid DNA using solvent evaporation and reported encapsulation efficiencies of approximately 30%.

Levels of plasmid DNA encapsulation in PLGA 752 following spray drying were found to be higher in our study, at 91.11%, when compared with previous studies by Walter et al (1999) who reported encapsulation efficiencies in the range of 21.2–79.5% for encapsulation of plasmid DNA in PLGA 50:50. These workers also observed that co-encapsulation of suitable buffering agents with plasmid DNA was required to achieve encapsulation efficiencies greater than 80%.

Luo et al (1999) have reported the formulation of both small and large DNA molecules (herring sperm DNA, 0.1–0.6 kb; GFP, 1.9 kb; lambda DNA, 48.5 kb) in polyethylene-co-vinyl acetate (EVAc) matrices, and in PLGA or PLA microspheres. The EVAc matrices displayed a biphasic pattern of DNA release; an initial burst release (phase I) and a period of slow but continuous release (phase II). The mechanism of plasmid release from these systems was reported to be diffusion controlled, consistent with our findings.

DNA integrity following encapsulation was examined following extraction of DNA from microparticles and gel electrophoresis. Results indicated a process-related denaturation of the plasmid DNA from supercoiled to open circular and linear forms of DNA. This change in DNA isoform might be attributed to the high temperatures used during spray drying, which may induce nicking of one or more DNA strands, thus converting the supercoiled form into open circular or linear DNA.

Despite a greater conversion of plasmid DNA to open circular and degraded DNA for the emulsification/spray-drying process, DNA extracted from both systems transfected COS-7 cells successfully, resulting in the production of luciferase enzyme as determined by luminometer results. Bacterial transformation assays, however, confirmed that post-encapsulation, the ability of plasmid DNA to transform competent cells is comparable for both processes. The transfection and transformation results demonstrate that the manufacturing procedures examined in this study allow for the encapsulation of biologically active and potent DNA. ESD particles are produced in a shorter time-period and, potentially, might represent a more efficient process for plasmid DNA encapsulation relative to EFD particles.

Immune system cells, such as macrophage cells, represent attractive targets in the development of drug delivery systems. In this work we examined the uptake by macrophage cells of solvent-evaporated and spray-dried microparticles. Both spray-dried and solvent-evaporated DNA-loaded RG 752 microparticles were readily taken up by macrophages

with zeta potential readings of -5.2 ± 3.2 and 0.9 ± 0.4 mV, respectively. Higher numbers of the spray-dried microparticles were phagocytosed over 24 h.

We conclude that, as for any therapeutic agent, effective DNA formulations must allow for administration of the DNA in a manner that optimizes its stability. We suggest that microencapsulation of DNA by spray-drying would seem to be the optimal method to prepare delivery systems for DNA vaccination. Direct targeting of phagocytic cells is a potential mechanism of action. Gene expression in target cells will be necessary to confirm that such dosage forms offer a realistic option as novel, efficacious vaccine systems.

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