

Nanoparticles for Drug and Gene Delivery via Electrohydrodynamic Spraying

Introduction

Nanoparticles—including solid lipid nanoparticles, liposomes, and polymeric particles or complexes—have been proposed as vehicles for drug and gene delivery. The goal is to control the size, composition and structure of the nanoparticles to improve the loading and release of drugs that are poorly soluble in water, as well as enhance the transfection efficiency of genes. Currently, many of these particles are made by bulk mixing (BM), solvent depletion, or high pressure homogenization (HPH). Although these methods are good for large-scale production, the energy input can be very high, and, thus, they are often unsuitable for use with temperature- and shear-sensitive biomolecules.

Electrohydrodynamic spraying (EHDS) is an alternate way to produce nanoparticles. Because the particles can be produced at or near room temperature, EHDS can be used to disperse sensitive biomolecules, such as DNA (Chen et al., 2000). The EHDS technique also complements the microfluidic approach used by other OSU NSEC researchers.

The goal of our work is to explore EHDS as a production method for a range of nanoparticles for use in drug and gene delivery. To date, we have demonstrated the feasibility of producing pure lipid (cholesterol/oleic acid) nanoparticles, liposomes, and DNA/PEI complexes, and have captured them in liquid media. We have used these particles to provide lipids to growing cells, encapsulate oligonucleotides (ODN) in liposomes, and transfect DNA that expresses Green Fluorescence Protein to NIT 3T3 cells. The projects described here are collaborative efforts with researchers in the NSEC Microfluidics core tech and the Biosensors testbed.

Experimental Methods

Figure 1 illustrates our basic experimental apparatus, in this case a concentric needle setup. Solutions are sprayed, and the nanoparticles are captured in a dish of liquid, such as distilled water or a dilute surfactant solution. Because the flow rates and compositions of the sprayed liquids are controlled independently, and both of these are independent of the composition of the collection liquid, the process is very flexible.

In the liquid phase, particle size distributions are measured (Fig. 2) using Dynamic Light Scattering (DLS). Alternatively, we can create the spray in an enclosed space and determine the size distribution of the aerosol using a scanning mobility particle sizer (SMPS, TSI Inc.).

Results

1. Single component nanoparticles - lipid nanoparticles

Single component oleic acid and cholesterol nanoparticles were produced as representative liquid and solid lipids, respectively, because both are Generally Regarded as Safe (GRAS). Cytotoxicity studies showed that there was no significant change in the cell viability

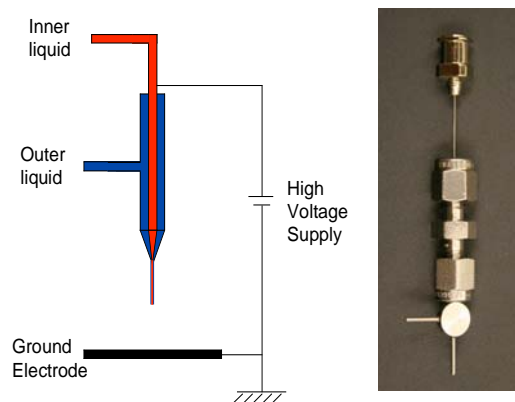


Figure 1. (left) A schematic diagram of the experimental setup using a concentric needle; (right) A photograph of the concentric needle assembly.

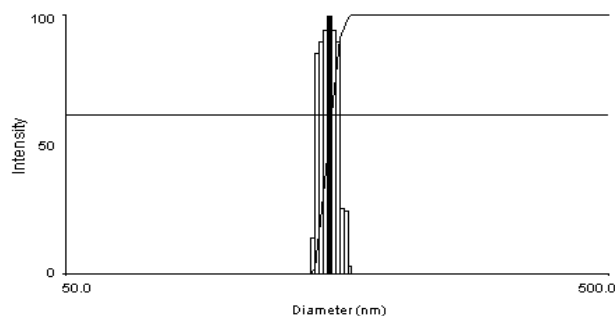


Figure 2. A typical size distribution of electrospayed cholesterol nanoparticles captured in 1% Pluronic F-68. The effective diameter, D_p is =152 nm.

when oleic acid nanoparticles were added to CHO cell culture medium at levels of 0.005% v/v and 0.015% v/v, nor when cholesterol nanoparticles were added to CHO cell culture medium or Hybridoma cell culture medium at levels of 0, 2, 4, and 8ug/mL, respectively.

The cholesterol particles were then tested to see if they could provide enough of this critical lipid to maintain the growth of cholesterol auxotrophic NS0 cells, and, thus, make nanoparticles a useful way to formulate a chemically defined medium. We compared the growth of NS0 cells in unsupplemented medium, as well as medium containing our cholesterol nanoparticles and a commercial cholesterol supplement, Synthechol (Sigma), respectively.

Experiments (Fig. 3) confirmed that NS0 cells do not grow without cholesterol and that our nanoparticles supported NS0 cell growth. Although much higher nominal concentrations were required than for the commercial product, in one case we were able to eliminate the use of methyl β -cyclodextran. This molecule is known to solubilize cholesterol but can also extract lipids from cell membranes and, thus, be toxic to cells. Finally, to ensure that the cells were not growing simply

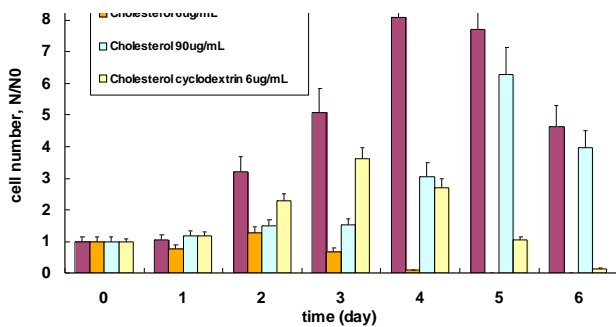


Figure 3. The growth of NS0 cells in medium supplemented by cholesterol nanoparticles or the commercial product, Synthecol. The structure of methyl β -cyclodextran is shown in the inset.

because of cholesterol carried over from the initial medium, we conducted a long-term culturing study that demonstrated both good growth and cell viability for 10 passages.

II. Two component nanoparticles

Particles containing two components are readily made using the concentric needle assembly. In addition to producing structured nanoparticles, such as liposomes, this setup can also be used to rapidly contact two liquids to make, for example, DNA/PEI polyplexes.

Liposome encapsulation of ODN

Liposomes were produced by flowing lipid mixtures (DC-chol, EggPC and PEG-DSPE in ethanol) in the outer needle and ODN-PBS solutions in the inner needle. Compared with the solvent depletion method, EHDS produces liposome in a single step, and eliminates the need for dialysis. Gel electrophoresis (Fig. 4) clearly shows that the ODN is tied up in the liposomes. Particle sizes, measured by DLS, ranged from 100nm – 290nm as the ODN/lipid ratio varied, suggesting that multi-layer liposomes may form because of interactions between the ODN and lipids. Cryo TEM studies are planned to confirm this.

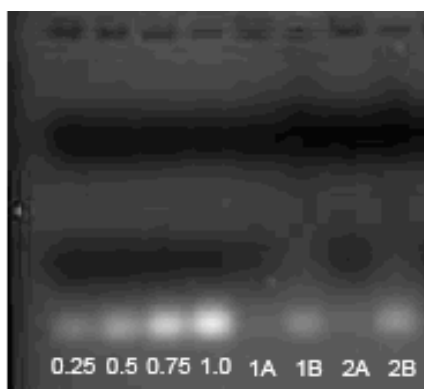


Figure 4. Gel electrophoresis of ODN liposomes. The lanes labeled 0.25-1.0 are the ODN standards in mg/mL. Lanes 1A and 2A: ODN encapsulated in lipids at ODN/lipid ratios of 1:40 and 1:30. Lanes 1B and 2B: the ODN is released by the addition of 1% Triton X100.

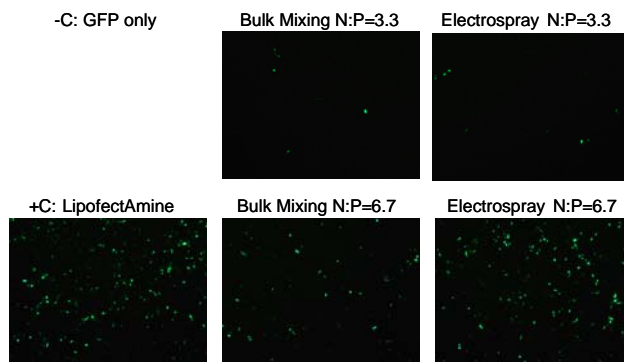


Figure 5. Transfection of GFP into NIH 3T3 cells using GFP/PEI particles produced by bulk mixing and EHDS at two N:P ratios. Particles produced by EHDS at an N/P ratio of 6.7 transfect cells at levels comparable to LipofectAmine (Invitrogen).

DNA/PEI nanoparticles for gene delivery

DNA/PEI nanoparticles were produced by flowing DNA through the inner needle and PEI through the outer needle. As illustrated in Figure 5, at an N:P ratio of 6.7, the transfection efficiency of particles produced by EHDS is comparable to that of the commercial product, LipofectAmine (Invitrogen). Systematic quantitative studies to examine the transfection efficiency of these particles, as a function of particle size and production methods, are currently underway.

Conclusions

EHDS is a simple but robust technique to produce nanoparticles for drug and gene delivery. EHDS does not only produce single component particles, such as oleic acid and cholesterol nanoparticles, but also creates multicomponent and structured particles, such as ODN encapsulated in liposome and DNA/PEI polyplexes.

EHDS is also a gentle enough method for use with temperature and shear sensitive biomolecules. The bioactivity of cholesterol nanoparticles was demonstrated by providing cholesterol to support NS0 cell growth. EHDS does not damage DNA at all. The transfection efficiency of electrosprayed DNA/PEI polyplexes is comparable to LipofectAmine.

References

Chen, D. R. Wendt, C. H. Pui, D. Y. H. (2000). A novel approach for introducing biomaterials into cells. *Journal of Nanoparticle Research*, 2, 133-139

Publications

1. “The Use of Electrohydrodynamic Spraying to Disperse Hydrophobic Compounds in Aqueous Medium” *Wu, Yun, Chalmers, Jeffrey, Wyslouzil, Barbara* (submitted Biotechnology and Bioengineering)