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Research Paper

Probing the interaction of nanoparticles with mucin for drug delivery applications using dynamic light scattering

Peter Charles Griffiths *, Beatrice Cattoz, Mervat Shafik Ibrahim, Josephine Chibuzor Anuonye

Department of Pharmaceutical, Chemical and Environmental Sciences, Faculty of Engineering and Science, University of Greenwich, Medway Campus, Central Avenue, Chatham Maritime, Kent ME4 4TB, UK

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ABSTRACT

Drug delivery *via* the eye, nose, gastrointestinal tract and lung is of great interest as they represent patient-compliant and facile methods to administer drugs. However, for a drug to reach the systemic circulation it must penetrate the "mucus barrier". An understanding of the characteristics of the mucus barrier is therefore important in the design of mucus penetrating drug delivery vehicles *e.g.* nanoparticles. Here, a range of nanoparticles – silica, aluminium coated silica, poly (lactic-co-glycolic acid) (PLGA) and PEGylated PLGA – each with known but different physicochemical characteristics were examined in the presence of mucin to identify those characteristics that engender nanoparticle/mucin interactions and thus, to define "design rules" for mucus penetrating (nano)particles (MPP), at least in terms of the surface characteristics of charge and hydrophilicity. Dynamic light scattering (DLS) and rheology have been used to assess the interaction between such nanoparticles and mucin. It was found that negatively charged and hydrophilic nanoparticles do not exhibit an interaction with mucin whereas positively charged and hydrophobic nanoparticles show a strong interaction. Surface grafted poly (ethylene glycol) (PEG) chains significantly reduced this interaction. This study clearly demonstrates that the established colloid science techniques of DLS and rheology are very powerful screening tools to probe nanoparticle/mucin interactions.

1. Introduction

One of the targets that has become of great interest to scientists is drug delivery through the eye, nose, and gastrointestinal (GI) tract and lung mucosal surfaces, these being a compliant and facile method to administer drugs. This delivery route does show high delivery efficiencies with fewer side effects for a wide range of therapeutics [1], but in order for the therapeutic agent to gain access to the systemic circulation and be absorbed, it must traverse the mucus barrier [2].

Mucus is a viscoelastic gel that lines the lumen of the gastrointestinal, urogenital, respiratory and eye tissues [3]. The major component of mucus is mucin. The term "mucin" represents a family of glycosylated proteins secreted by goblet cells and the seromucous glands of lamina propria at the apical epithelium [1]. The dry weight of typical mucus contains mucin (5 wt%), lipids (37 wt%), proteins (39 wt%), DNA (6 wt%), and other unidentified materials. The sialic acid and sulphate content are very high in most of the moist mucosal epithelial interfaces, thereby imparting a pronounced negative charge, responsible for the rigidity of the structure *via* charge repulsion [4].

Mucus has various functions, notably in the case of exposed surfaces, to act as a barrier to prevent the access of foreign bodies to tissues and blood. Nanoparticles are therefore "trapped" by mucus due to hydrophobic, electrostatic and hydrogen bonding interactions [2] or by physical entrapment of the larger nanoparticles in the mucin network [2,5]. Nanoparticles adhering to mucus are then cleared along with the mucus [6].

In the design of a putative drug delivery nanoparticle, one may expect the nanoparticles to be able to traverse the mucus barrier if its size is smaller than the mesh size of the network (10–250 nm), and should not experience strong hydrophobic, electrostatic or hydrogen bond interactions with the mucin. Further, the nanoparticles will need to penetrate the mucus faster than its characteristic clearance rate [7]. Conventional nanoparticles commonly fail in one or more of these points and thus, to overcome this problem, to achieve longer residence times for drugs at absorption sites, there is a need to design mucus penetrating (nano)particles (MPP) [1].

Here, we show how dynamic light scattering in conjunction with – both very established colloid chemistry methodologies – may be used to quantify the interaction between mucin and

* Corresponding author. Tel.: +44 208 331 9927.
E-mail address: p.griffiths@gre.ac.uk (P.C. Griffiths).

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nanoparticles with different surface chemistries. The study will provide a better understanding of the complex nature of mucin/-particle interactions in terms of the surface characteristics of the nanoparticles.

A range of nanoparticles has been adopted – conventional nanoparticles such as anionic and cationic silica and the synthetic biodegradable polymer poly (lactic-co-glycolic acid) (PLGA) and its PEGylated derivative, (PEG–PLGA) [8–13]. PLGA is a copolymer of poly (lactic acid) (PLA) and poly (glycolic acid) (PGA) [14–17]. Various drugs have been loaded in PLGA nanoparticles such as paclitaxel [18], curcumin [19], clarithromycin [20], praziquantel [21], doxorubicin [22,23], streptomycin [24] and siRNA [25]. PEG–PLGA nanoparticles have been characterised [26–28] and tested for their loading capacity with various drugs as tumour necrosis factor alpha blocking peptide [29], isoniazid [30] and roxithromycin [31].

2. Materials and methods

2.1. Materials

Mucin type II porcine extracted from stomach, silica Ludox® CL, silica Ludox® LS, poly (lacto-co-glycolic acid) (PLGA), PEGylated PLGA (PEG 2000 and PLGA 10,000 g mol^{-1}), PEGylated PLGA (PEG 5000 and PLGA 10,000 g mol^{-1}) and PEGylated PLGA (PEG 5000 and PLGA 55,000 g mol^{-1}) were all received as supplied Sigma–Aldrich.

2.2. Methods

2.2.1. Preparation of mucin samples

A series of mucin solutions were prepared in 0, 1, 5, 10, 25 and 100 mM NaCl. The pH of 0 mM mucin samples was also adjusted to pH = 1, 3, 5 and 7.

2.2.2. Preparation of silica Ludox® LS samples (silica)

The stock silica dispersion was diluted to a concentration of 0.3 wt% in 0, 1, 5, 10, 25 and 100 mM NaCl. The pH of 0 mM silica sample was adjusted to pH = 1, 3 and 7. All concentrations are expressed in terms of wt% *i.e.* g/100 ml.

2.2.3. Preparation of silica Ludox® CL samples (aluminium coated silica)

The stock aluminium coated silica dispersion (Al silica) was diluted to a concentration of 0.15 wt% in 0, 1, 5, 10, 25 and 100 mM NaCl. The pH of 0 mM silica sample was adjusted to 1 and 3.

2.2.4. Preparation of PLGA samples

A total of 0.1 g PLGA of ratio 50:50 was dissolved in ethyl acetate. A total of 0.2 g(s) PVA was dissolved in H_2O. Six microlitres of this PVA solution was mixed with 0.5 ml of the PLGA solution with probe sonication for 30 min cooled by ice. The mixture was transferred to rounded bottom flask to evaporate the ethyl acetate on the rotary evaporator for 30 min. Excess PVA was removed through several centrifugation (45 min) and resuspension cycles. The resultant suspension was subsequently freeze dried.

2.2.5. Preparation of PEGylated PLGA samples

To prepare PEG–PLGA nanoparticles, 10 mg of PEG–PLGA (Mwt of PEG: PLGA was 2000:5000, 5000:10000 and 5000:55,000 g mol^{-1}) was dissolved in 1 ml of acetone. Hundred microlitres of this PEG–PLGA acetone solution was added dropwise to a vigorously stirred 2 ml sample of deionised water. The acetone is then evaporated on magnetic stirrer for 10 min. The final concentration of PEG–PLGA was 0.2 wt%, and its pH adjusted to pH values of 1, 3, 4.5 and 7.

2.2.6. Dynamic light scattering

An appropriate mucin stock solution was diluted with the various nanoparticle dispersions in a 50:50 ratio and examined using Malvern Zetasizer Nanoseries ZS. Measurements were carried out at temperature of 37 °C and scattering angle 173°. DLS was used to detect diffusion and size of the mucin, nanoparticles and nanoparticles/mucin mixtures.

3. Results and discussion

Dynamic light scattering is frequently used to calculate the size of colloidal nanoparticles. Under limiting conditions, the Stokes–Einstein equation is used to derive the size (often the diameter in commercial instruments) from the measured mutual diffusion coefficient. To this end, an accurate measure of the nanoparticle size is only obtained if the viscosity is known, and thus, experiments are usually conducted as close to infinite dilution as is possible. Frequently, that is not always possible, as interactions between nanoparticles and polymers often depend on concentration. Here, we have explored both experimental designs, dilute systems and those where a network of mucin will exist, as well as characterised the rheology of the system for comparison. The focus of this paper was the light scattering data, although representative rheology data will be discussed. Further, as background screening, a series of mucin solutions spanning $0 < C_{mucin} < 10$ wt% were prepared to explore the effects of pH, salt and mucin concentration on mucin diffusion (data not presented). In essence, the diffusion coefficient decreases with the increase in mucin concentration for $C_{mucin} > 0.5$ wt%, the critical overlap or "gel onset" concentration, but for $C_{mucin} < 0.5$ wt% was unaffected. The addition of salt or changes in pH had a negligible effect on the diffusion coefficient.

With this insight, the quantification of interaction between mucin and silica was undertaken as a function of ionic strength and pH. Initially, negatively charged and hydrophilic nanoparticulate silica of 10–15 nm size was chosen as a "negative" control. The nanoparticle size distribution (PSD) is presented in Fig. 1.

As might be expected, the silica nanoparticle component to the combined particle size distribution for the various replicate experiments/samples, shows no change *in position or intensity* upon

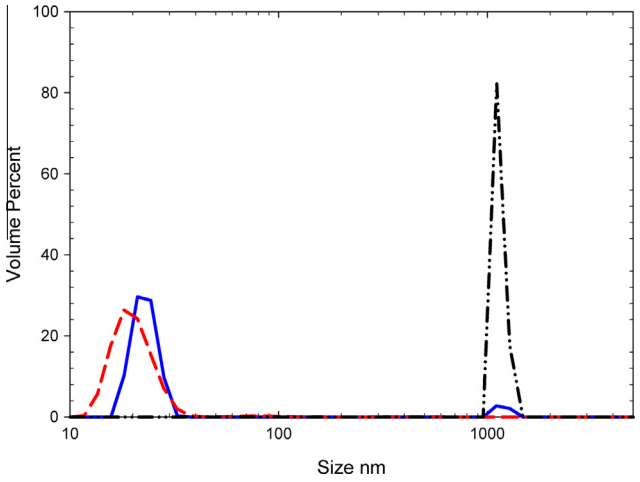

Fig. 1. Representative particle size distributions for negatively charged silica (Ludox® LS, 0.3 wt%) in the absence and presence of 0.025 wt% mucin, pH = 7 and added ionic strength = 0 mM; ___ negatively charged silica alone; ___ negatively charged silica mucin mixture; _.._ mucin alone.

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