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

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ABSTRACT

Drug delivery *via* the eye, nose, gastrointestinal tract and lung are 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 & 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.

Key words

Mucin, silica, aluminium coated silica, poly (lactic-co-glycolic acid) (PLGA), PEGylated PLGA, dynamic light scattering, rheology.

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 seromucincious 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 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) (8-11,12,13). 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 characterized (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

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 were also adjusted to pH = 1, 3, 5 and 7.

Preparation of Silica Ludox® LS Samples (silica)

The stock silica dispersion was diluted to a concentration of 0.3wt% 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/100ml.

Preparation of Silica Ludox® CL Samples (Aluminium Coated Silica)

The stock aluminium coated silica dispersion (Al silica) was diluted to a concentration of 0.15wt% in 0, 1, 5, 10, 25 and 100 mM NaCl. The pH of 0 mM silica sample was adjusted to1and 3.

Preparation of PLGA Samples

0.1 gm of PLGA of ratio 50:50 was dissolved in ethyl acetate. 0.2 gm(s) of PVA were dissolved in H_2O. 6mls of this PVA solution were 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.

Preparation of PEGylated PLGA Samples

To prepare PEG-PLGA nanoparticles, 10 mgs of PEG-PLGA (Mwt of PEG: PLGA was 2000:5000, 5000:10000 & 5000:55000 g mol^{-1}) were dissolved in 1 ml of acetone. 100 µl 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.

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 is 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 figure 1.

Figure 1 here

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 addition of mucin indicating no interaction with the nanoparticle surface. In the nanoparticle/mucin mixture, mucin component to the combined particle size distribution is also evident, *and its intensity and position is invariant*. Changes in pH has no effect on this behaviour, reflecting the similar and strong charges on both the nanoparticle surface and the mucin. Further, there were little changes in the measured viscosity of the system.

As a "positive" control, the equivalent experiment was undertaken for a positively charged silica nanoparticle (alumina coated), of comparable size, figure 2.

Figure 2 here

Now the apparent dimension of the positively charged silica nanoparticles is significantly shifted to larger sizes upon addition of mucin reflecting the electrostatically driven adsorption of the negatively charged mucin and to the positively charged silica surface (1,2,10). The

intensity of the characteristic mucin peak is significantly increased but not its *position*, whereas the *position and intensity* of the silica peak are both altered. This behaviour was rather sensitive to pH, as the system at pH = 1 did not show this behaviour, easily interpreted in terms of the charge on the two components. In fact, depending on the relative charge ratios, the system may be sufficiently destabilised that coagulation of mucin/particles occurs, a process enabled by the addition of salt (32). Further, there were substantial increases in the measured viscosity of the system. Clearly, this has implications for the formulation of any mucin penetrating (nano) particle based drug delivery platform.

Figure 3 here

Having established that dynamic light scattering may be used to probe these systems, the study was extended to quantify the interaction between PLGA and mucin, as functions of pH and ionic strength. PLGA is a hydrophobic nanoparticle that is gaining interesting as a drug carrier for several drugs (33). The nanoparticle size distribution was recorded for PLGA and mucin mixtures over a range of pH values, the representative pH = 7 data is presented in figure 3.

Figure 4 here

The nanoparticle size distributions of PLGA/mucin mixture were virtually superimposed with that of the mucin alone, at all pHs examined ($1 < pH < 7$) reflecting a strong hydrophobic interaction, irrespective of the magnitude of the electrostatic component to the interaction. No residual component of the PLGA particle size distribution is evident, indicating significant differences to the silica cases, and a more pronounced interaction. Such a strong hydrophobic interaction will provide a basis for mucoadhesive nanoparticles, but not for mucus penetrating (nano) particles. One strategy for overcoming this adhesive property, is to tailor the nanoparticle surface, and a number of strategies are being explored and discussed in this special edition. Here, we demonstrate how PEGylation and light scattering may be used to demonstrate this phenomenon.

PEGylation of the nanoparticle surface should impart an hydrophilic nature to that surface (34), and this beneficial character is likely to be dependent on the PEG molecular weight and the PEG:PLGA ratio, figures 4-6.

Figures 5-7 here

The nanoparticle size distribution for the PEG-PLGA/mucin mixture is in the same size range as that of PEG-PLGA alone, indicating the absence of interaction due to PEGylation of the hydrophobic PLGA core. Changes in the PEG:PLGA ratio seems to have little effect on the interaction of the nanoparticle with mucin, consistent with what was observed previously - PEGylated PLGA nanoparticles that are well coated with PEG of 2kDa and 5KDa showed little interaction with mucin (35).

There are however, subtle changes in the *intensity* of the peak at particular sizes, implying that there may be a small fraction of nanoparticles bound up in the mucin. Looking at the peak ratios of these two environments – free nanoparticles and mucin – it is clear that the 5,000/55,000 PEG-PLGA nanoparticle seems to "entangle" with the mucin to a greater extent than the other two case presented, 2,000/5,000 and 5,000/10,000 PEG-PLGA cases. Further work is underway to elaborate these subtleties in the data.

4. Perspective and Context

Examining the interaction of nanoparticles with mucin - the key polymeric component within mucus – by dynamic light scattering provides informative insights into how the nanoparticles bind or otherwise with the biogel. Studies on mucin are however, only meaningful when extended to mucus, the "complete" biogel. Those experiments are rather more complex given the inherent opacity and heterogeneity of the sample. Physicochemical techniques such as multiple particle tracking (MPT), rheology, small-angle neutron scattering (SANS) and pulsed-gradient NMR (PGSE-NMR) – all discussed elsewhere in this Special Edition – have an important role to play here. To reinforce the necessity of considering different insights from several techniques, compare the rheology of mucin/nanoparticle systems and mucus/nanoparticle systems.

The viscosity of mucin solutions increased on addition the nanoparticles, the increase being considerably greater in the case of nanoparticles (or surfactant micelles) with cationic or hydrophobic surfaces. Interestingly, the addition of non-ionic Pluronic copolymer micelles led to a decrease in viscosity (unpublished results). Similar behaviour - significant increases in viscosity, though the magnitudes of the viscosity were quite different – were observed

when these same nanoparticles were added to mucus. Hence, one may argue that the use of mucin is a useful screening tool to probe the strength of an interaction with candidate nanoparticles.

In contrast, dynamic oscillation tests showed that both mucin and mucus are shear-thinning viscoelastic gels, in which the elastic modulus dominates the loss modulus. The gel nature, which is strongly dependent on ionic strength and pH, leads to high resistance to deformation at low shears ("rest"), but is easily broken down with increasing shear. Accordingly, modification of this shear profile by the addition of nanoparticles could disrupt this crucially important function. For the sort of nanoparticles employed here, whilst increases in viscosity were indeed observed, both the elastic and storage moduli were increased. The elastic character of the gel was however retained.

Therefore, the interaction of nanoparticles with biogels, and the varied components within that biogel, are complex, leading to changes in behaviour over different length- and timescales. A full appreciation of the implications of those interactions is necessary, one that is only accessible by comparing insights from a range of techniques.

5. Conclusion

Mucin is the main solid component of mucus, responsible for most of its gel-like properties and structure. Mucin undergoes electrostatic, hydrophobic and hydrogen bonding interactions, and thus, the presence of any non-endogenous materials such as drug delivery nanoparticles will undergo a myriad of interactions if it is to traverse this barrier. Here, it is shown that dynamic light scattering in conjunction with rheology, provide convenient and accessible methodologies for probing the interactions between nanoparticles and mucin. Electrostatic attraction is a driving force when the nanoparticle/mucin bear opposite charges leading to mucoadhesive properties and a substantial increase in the viscosity of the system, whereas negatively charged hydrophilic nanoparticles are unperturbed by the presence of the mucin. The viscosity of the mucin solution increases only weakly. PEGylating hydrophobic surfaces, as evidenced by a series of PLGA nanoparticles, imparts a hydrophilic nature to the nanoparticle surfaces eliminating the hydrophobic "entrapment". Surprisingly, the "slippery nature" of the PEGylated PLGA nanoparticles was not dependant on the molecular weight or thickness of PEG layer, provided the nanoparticles are well coated with PEG. One successful

strategy to design mucus penetrating particles would be to ensure the particle surface is hydrophilic and weakly negatively charged.

Figure legends

Figure 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 = 0mM; _ _ _ negatively charged silica alone; ___ negatively charged silica mucin mixture; _.._ mucin alone.

Figure 2: A representative particle size distributions for positively charged silica (Ludox® CL, 0.3 wt %) in the absence and presence of 0.025 wt% mucin, pH = 7 and added ionic strength = 0mM; _ _ _ positively charged silica alone; ___ positively charged silica mucin mixture; _.._ mucin alone.

Figure 3: A representative particle size distributions for positively charged silica (Ludox® CL, 0.3 wt %) in the absence and presence of 0.025 wt% mucin, pH = 1 and added ionic strength = 0mM; _ _ _ positively charged silica alone; ___ positively charged silica mucin mixture; _.._ mucin alone.

Figure 4: A representative particle size distributions for hydrophobic PLGA nanoparticles (0.3wt%) in the absence and presence of 0.025 wt% mucin,pH = 7 and added ionic strength = 0mM; _ _ _ PLGA alone; ___ PLGA mucin mixture; _.._ mucin alone.

Figure 5: A representative particle size distributions for PEG-PLGA nanoparticles (PEG: PLGA 2000:5000 g mol^{-1}) (0.2 wt %) in the absence and presence of 0.025 wt% mucin, pH = 1 and added ionic strength = 0mM; _ _ _ PEG-PLGA alone; ___ PEG-PLGA mucin mixture; _.._ mucin alone.

Figure 6: A representative particle size distributions for PEG-PLGA nanoparticles (PEG: PLGA 5000:10000 g mol^{-1}) (0.2 wt %) in the absence and presence of 0.025 wt% mucin, pH = 3 and added ionic strength = 0mM; _ _ _ PEG-PLGA alone; ___ PEG-PLGA mucin mixture; _.._ mucin alone.

Figure 7: A representative Particle size distributions for PEG-PLGA nanoparticles (PEG:PLGA 5000:55000 g mol^{-1}) (0.2 wt %) in the absence and presence of 0.025 wt% mucin, pH =1 and added ionic strength = 0mM; _ _ _ PEG-PLGA alone; ___ PEG-PLGA mucin mixture _.._ mucin alone.

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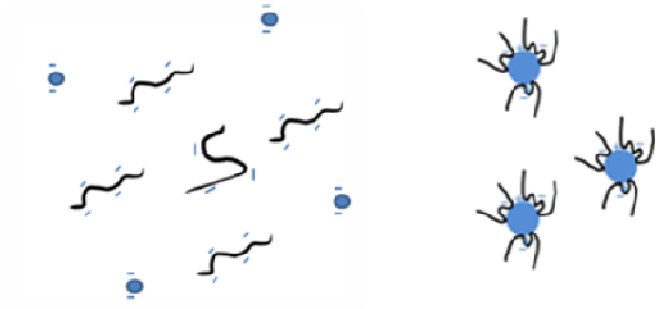

The non-interaction of like-charged particles and mucin fibres (left) and the charge-induced binding of the same (right) illustrating the dynamic environments observed by DLS in this study