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Synthesis of thiolated, PEGylated and POZylated silica nanoparticles and evaluation of their retention on rat intestinal mucosa in vitro

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ABSTRACT

In this study, we synthesised thiolated silica nanoparticles using 3-mercaptopropyltrimethoxysilane and functionalised them with either 5 kDa methoxy polyethylene glycol maleimide (PEG) or 5 kDa alkyne-terminated poly(2-ethyl-2-oxazoline) (POZ). The main objectives of this study are to investigate the effects of pH on the size and ξ-potential of these nanoparticles and evaluate their mucoadhesive properties ex vivo using rat intestinal mucosa. The sizes of thiolated, PEGylated and POZylated silica nanoparticles were 53 ± 1, 68 ± 1 and 59 ± 1 nm, respectively. The size of both thiolated and POZylated nanoparticles significantly increased at pH ≤ 2, whereas no size change was observed at pH 2.5–9 for both these two types of nanoparticles. On the other hand, the size of PEGylated nanoparticles did not change over the studied pH range (1.5–9). Moreover, thiolated nanoparticles were more mucoadhesive in the rat small intestine than both PEGylated and POZylated nanoparticles. After 12 cycles of washing (with a total of 20 mL of phosphate buffer solution pH 6.8), a significantly greater amount of thiolated nanoparticles remained on the intestinal mucosa than FITC-dextran (non-mucoadhesive polymer, $p < 0.005$) and both PEGylated and POZylated nanoparticles ($p < 0.05$ both). However, both PEGylated and POZylated nanoparticles showed similar retention to FITC-dextran ($p > 0.1$ for both). Thus, this study indicates that thiolated nanoparticles are mucoadhesive, whereas PEGylated and POZylated nanoparticles are non-mucoadhesive in the ex vivo rat intestinal mucosa model. Each of these nanoparticles has potential applications in mucosal drug delivery.

1. Introduction

Oral drug delivery is the preferred administration route for most drugs, as it has several advantages over other routes, including better patient adherence (especially for chronic diseases) and possibilities for flexible dosing (Date et al., 2016). In addition, oral dosage forms generally cost less to manufacture than other formulations (e.g. injectables, eye drops and inhalators) as they do not require sterilisation (Yun et al., 2013; Date et al., 2016) or use of complex delivery devices. However, about 70% of new drugs do not reach pre-clinical development due to low bioavailability resulting from poor oral absorption (Gao et al., 2013). Drug absorption in the gastrointestinal tract (GIT) is hampered by a number of physiological barriers, including the mucus, the harsh pH and digestive environment of the GIT, tight junctions, epithelial cells and sub-epithelial tissues (Lundquist and Artursson, 2016).

Mucus is a viscous gel secreted by goblet cells, which are found in various organs, including the eye (Kessler and Dartt, 1994), the GIT (Deplancke and Gaskins, 2001) and the respiratory tract (Spicer et al., 1983). It consists mainly of water (~ 95%), alongside cross-linked and entangled mucin fibres, lipids, proteins, salts, cellular debris and bacteria (Moghissi et al., 1960; Bansil and Turner, 2006; Johansson et al., 2011; Leal et al., 2017). Mucus can be targeted using mucoadhesive drug delivery systems that adhere to this layer to prolong the residence time of the dosage forms, leading to sustained release of the loaded drugs and enhanced bioavailability compared to conventional non-mucoadhesive formulations (Bernkop-Schnürch, 2005; Khutoryanskiy, 2011).

Several types of nanoparticles have been shown to have great potential as drug delivery systems (Nguyen et al., 2016; Hu et al., 2017; Davoudi et al., 2018). Siew et al. (2012) developed nanoparticles based on mucoadhesive quaternary ammonium palmitoyl glycol chitosan. These nanoparticles enhanced the oral absorption of both hydrophilic (ranitidine) and hydrophobic drugs (griseofulvin and cyclosporine A). Bernkop-Schnürch and co-workers have developed thiolated polymers and shown their potential in the design of mucoadhesive nanoparticulate drug delivery systems (Dünnhaupt et al., 2011; Bonengel and

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Bernkop-Schnürch, 2014). Prego et al. (2006) designed chitosan nanocapsules to enhance the absorption of salmon calcitonin from intestine and prolong its action as a result of their mucoadhesive properties and strong interaction with the intestinal mucous membranes.

Previously, Khutoryanskiy et al. have developed thiolated silica nanoparticles and demonstrated their mucoadhesive properties on ocular (Irmukhametova et al., 2011) and urinary bladder mucosa (Mun et al., 2016). They also demonstrated that these nanoparticles could be easily functionalised via fluorescent labelling, PEGylation and POZylation (introduction of polyethylene glycols and polyoxazolines, respectively) (Irmukhametova et al., 2011; Irmukhametova et al., 2012; Mun et al., 2014a; Mansfield et al., 2015; Mansfield et al., 2016). PEGylation of thiolated silica nanoparticles was found to reduce their retention on the ocular (Irmukhametova et al., 2011) and urinary bladder mucosal surfaces (Mun et al., 2016). More recently, Mansfield et al. (2015, 2016) demonstrated that POZylation of these nanoparticles could enhance their penetration into porcine gastric mucosa.

Clearly, the nature of the adhesion between two surfaces (here, the nanoparticles and the mucous membrane) is highly dependent on the properties of both (Smart, 2005; Varum et al., 2010; Khutoryanskiy, 2011). For example, we previously showed that thiolated and PEGylated silica nanoparticles were less retentive on the ocular surface compared to the urinary bladder mucosal surface (Irmukhametova et al., 2011; Mun et al., 2016). This difference in retention might be due to the rougher structure of the latter resulting in an increased contact area (Irmukhametova et al., 2011; Khutoryanskiy, 2011; Mun et al., 2016). The mucoadhesion of POZylated silica nanoparticles has never been studied previously. Therefore, in the present work, we sought to investigate the retentive properties of these silica nanoparticles in the rat intestinal mucosa. We further analysed the physicochemical properties of these nanoparticles, particularly their pH-stability profiles.

2. Materials and methods

2.1. Materials

3-mercaptopropyltrimethoxysilane (MPTS), maleimide terminated methoxy poly(ethylene glycol) (PEG) 5 kDa, alkyne terminated poly(2-ethyl-2-oxazoline) (POZ) 5 kDa (polydispersity index, PDI ≤ 1.2), 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), fluorescein isothiocyanate (FITC), medium molecular weight chitosan (degree of acetylation 26.1%, 124 kDa), FITC-dextran (3.5–5 kDa) and triethyl amine (TEA) were purchased from Sigma-Aldrich (Gillingham, UK). FITC-chitosan was made in house (Symonds et al., 2016a). Fluorescein-O-methacrylate (FMA), dimethyl sulfoxide (DMSO) and NaOH were purchased from Fisher Scientific (UK). Dialysis membrane with molecular cut-off 12–14 kDa was purchased from Medicell International Ltd., UK.

2.2. Synthesis of thiolated silica nanoparticles

Thiolated silica nanoparticles were synthesised according to a previously published method (Irmukhametova et al., 2011). In brief, 20 mL DMSO and 0.5 mL of 0.5 M NaOH solution were added to 0.75 mL MPTS. The mixture was continuously stirred and aerated for 24 h at room temperature. Next, the nanoparticle suspensions were dialysed against deionised water (4 L, 8 changes of water over 2 days) using the dialysis membrane. The purified nanoparticle suspensions were refrigerated at 4 °C until use.

2.3. Synthesis of PEGylated and POZylated silica nanoparticles

Thiolated silica nanoparticles were functionalised using two different polymers; PEG and POZ. To synthesise PEGylated nanoparticles, 100 mg PEG was added to 10 mL thiolated nanoparticle suspension and the mixture was stirred for 24 h at room temperature. To synthesise POZylated particles, 5 mL of thiolated nanoparticle suspension was diluted with 5 mL DMSO and then 100 mg POZ was added to the diluted thiolated nanoparticles. To this mixture, 200 µL TEA was added to enhance the thiol-yne click reaction (Mansfield et al., 2015). The reaction mixture was left for 24 h with continuous stirring. The nanoparticles were purified by dialysis as described in Section 2.2 and refrigerated at 4 °C until use.

2.4. Fluorescent labelling of nanoparticles

Each of the thiolated, PEGylated and POZylated silica nanoparticle suspensions (5 mL) were diluted with DMSO (5 mL). To this, 2 mL (3.59 µmol) of 1.8 mM fluorescein-O-methacrylate solution (in 1:1 deionised water: ethanol) and 200 µL TEA was added. The reaction mixture was stirred in the dark for 24 h. Subsequently, the fluorescently labelled nanoparticles were purified (again in the dark) as described in Section 2.2.

2.5. Characterisation of nanoparticles

The size and ξ-potential of the nanoparticles were measured using Zetasizer Nano-ZS (Malvern, UK). For the size measurements, the samples were diluted 1:100 with ultrapure water before analysis. A refractive index of 1.475 and an absorbance of 0.1 were used for all measurements. Measurements were conducted in triplicate for 10 s per run, with 12 runs per reading at 25 °C. ξ-potential values were measured using DTS-1070 folded capillary tube cuvettes (Malvern, UK). Samples were measured using 3 repeats of 20 sub-runs per reading. At least 3 samples were measured and processed using the Smoluchowski model (Fκa = 1.50).

Transmission electron microscopy (TEM) was conducted using a JEM-2100 PLUS Electron Microscope (JEOL, USA) at an accelerating voltage of 200 kV. Three drops of nanoparticle suspensions were placed on a carbon-coated copper grid and left for 1 min before being loaded into the instrument. The morphology of the nanoparticles was investigated without any staining.

2.6. Determination of thiol content

Ellman's assay was used to quantify the free thiol groups available on the surface of the nanoparticles. Initially, the nanoparticles were lyophilised using the Heto Power Dry LL 3000 freeze-drier (Thermo Electron Corporation). The nanoparticles (3 mg) were suspended in 10 mL phosphate buffer solution (0.5 M, pH 8). Then, 0.5 mL aliquots of nanoparticle suspension were reacted with 0.5 mL DTNB (0.3 mg/mL) in the dark for 2 h. Next, the product was centrifuged for 10 min at 13000 rpm (Sanyo, Micro Centaur, UK) and 200 µL aliquots of supernatant were loaded into a 96 well-plate. The light absorbance was measured at 420 nm using an Epoch microplate reader (BioTek Instruments, Inc.). In order to obtain a calibration curve, serial solutions of L-cysteine HCl over the concentration range of 0.004 to 0.634 µmol/mL were prepared and reacted with DTNB under the same conditions as the nanoparticles. Phosphate buffer solution (pH 8) was used as the blank control. Finally, the amount of free thiols per gram of the particles was calculated.

2.7. FTIR spectroscopy

FTIR spectra of the freeze-dried nanoparticles were recorded using a Spectrum 100 FTIR spectrophotometer (Perkin-Elmer, UK). The spectra were collected from an average of 4 scans, with a resolution of 4 cm^{-1} over the range of 4000–650 cm^{-1}.

2.8. Thermogravimetric analysis (TGA)

Freeze-dried samples were analysed for all three types of silica nanoparticles using the Q50 thermogravimetric analyser (TA Instruments,