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About the impact of water movement on the permeation behaviour of nanoparticles in mucus

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ABSTRACT

The purpose of this study was to establish a method to evaluate the diffusion behaviour of nanoparticles (NP) in mucus taking also the water movement into account. For this purpose, NP based on different chitosan derivatives, either thiolated or not, and marked with fluorescein diacetate were prepared by ionotropic gelation with hyaluronan. NP size and polydispersity were in the respective intervals 363.5 ± 33.3–385.7 ± 36.5 nm, and 0.35 ± 0.11–0.39 ± 0.10. An in vitro study of water-assisted NP transport through mucus was realized by filling the barrel of a syringe kept in vertical position, tip down, with mucus. Then a bottom-to-surface PBS flow across the mucus layer was realized by connecting the tip of the syringe to the bottom of a vertical cylindrical vessel by a flexible tubing, filling the vessel with PBS, level with the surface of the mucus layer in the syringe, and dripping PBS into the vessel without causing any phase mixing. Although the mucoadhesive NP interact more strongly with the mucus, yet they are able to overcome this barrier with the aid of the water movement from lumen to epithelium. This new method promises to be more predictive of in vivo NP transport across the mucus than already reported methods, as it takes into account the water movement and regulates its contribution to the physiologic value.

1. Introduction

Mucoadhesive polymeric nanoparticulate systems (NP) have raised interest as vehicles for drug delivery by the oral route for their potential ability to improve the bioavailability of drugs with low mucosal permeability and/or poor chemical stability in the gastrointestinal environment (Dünnhaupt et al., 2011; Ponchel et al., 1997; Sakuma et al., 2002; Sarmento et al., 2007; Takeuchi et al., 2001). To be absorbed across the intestinal epithelium into the systemic circulation, however, the NP will have to cross the layer of stagnant mucus adjacent to the intestinal membrane. Mucus is a viscoelastic gel layer primarily composed of crosslinked and entangled mucin fibers, continuously secreted and renewed by goblet cells and submucosal glands. The viscoelastic properties of GI mucus are essential for its protective and lubricating properties. In this light, mucus represents a barrier that can affect the transit of most orally administered particles. This layer is also crossed by the electrolytes and water that are absorbed by it (Hastewell et al., 1991), thereby generating pores that can be travelled by NP. These are thus given the possibility to reach the intestinal epithelium and cross it by transcytosis. In this context the tendency of NP to adhere to the mucus gel plays an important role since on the one hand it opposes the physiologic transit of the delivery system through the GI tract away from the absorption site, thus favouring drug absorption, whereas, on the other hand, it hampers the water-driven transport of NP from luminal to epithelial side of the mucus layer, thus slowing down absorption (Grießinger et al., 2015). Indeed, Maisel et al. (2015) on the basis of their results obtained in vivo with mice, stated that water absorption by the intestine increases in the fed state, and non-mucoadhesive NP can take advantage of this, for advective transport and hence, efficient and uniform drug delivery. In contrast mucoadhesive NP stick to the outer layers of mucus and aggregate in clumps far from the epithelial surface. In the light of the above information, it could be hypothesized that the water movement in the intestinal mucus could influence the distribution of mucoadhesive NP in the GI tract. For this reason, the purpose of the present work has been to develop and evaluate a simple in vitro system allowing the investigation of the movement of NP, either mucoadhesive or not, across a layer of porcine mucus concurrently crossed by a flow of

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phosphate buffered saline pH 6.8, 0.13 M (isotonic) (PBS), that could be adjusted to the physiologic flux value calculated from literature data for the human intestine (Lennernäs et al., 1994). Water-soluble quaternary ammonium-chitosan conjugates were used to prepare the NP (Zambito et al., 2008). To differentiate between more and less mucoadhesive NP these were prepared from chitosan derivatives containing thiol groups on their chains (more mucoadhesive) or thiol-free ones (less mucoadhesive) (Zambito et al., 2013).

2. Materials and methods

2.1. Materials

The following materials were used, along with other usual reagent grade solvents and chemicals: fluorescein diacetate (FDA), D-(+)-trehalose dehydrate, thioglycolic acid, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride, cellulose membrane tubing MW cut-off 12.5 kDa (all from Sigma); 2-diethylaminoethyl chloride hydrochloride (Fluka); hyaluronic acid (HA), MW 950 kDa (Contipro, Dolní Dobrouč, Czech Republic); chitosan minimum 90% deacetylated from shrimp shell (Ch) (Chitoclear FG90, Primex, Drammen, Norway). The above materials were used, each where appropriate, to prepare the following polymers and polymer derivatives according to the methods described in the cited references:

Reduced-MW chitosan (rCh, viscometric MW 32 kDa) and hyaluronic acid (rHA, viscometric MW 470 kDa) (Zambito et al., 2013); quaternary ammonium-rCh conjugates (QA-rCh), synthesized at 50 °C or 60 °C to give structurally different derivatives, coded QA-rCh50 and QA-rCh60, respectively (Zambito et al., 2006, 2008); thiolated derivatives of QA-rCh, coded QA-rCh50-SH and QA-rCh60-SH, respectively (Zambito et al., 2013). All aqueous solutions/dispersions were prepared with freshly distilled water.

2.2. Preparation and characterization of NP

NP based on QA-rCh50 (NP-QA-rCh50), QA-rCh60 (NP-QA-rCh60), QA-rCh50-SH (NP-QA-rCh50-SH) or QA-rCh60-SH (NP-QA-rCh60-SH) self-assembled upon dropwise addition of a 450 μL volume of 0.0125 mg/mL of rHA (case of NP-QA-rCh50), or a 350 μL volume of 0.025 mg/mL of rHA (case of NP-QA-rCh60) in phosphate buffer pH 7.4, 0.13 M to 5 mL of 2 mg/mL QA-rCh50 or QA-rCh60, or 2 mg/mL QA-rCh50-SH or QA-rCh60-SH in the same buffer, under stirring at room temperature.

NP labeled with FDA were prepared according to Bernkop-Schnürch et al. (2006). Briefly, an ice-cooled 0.1% w/v FDA solution in acetonitrile was added to the NP in the 1:2 ratio, which prevented any marker precipitation, and the mixture was incubated 1 h at 25 °C under shaking. The final FDA concentration in the NP dispersion was 0.33 mg/mL. To prevent particle aggregation 3% w/v trehalose was added to each nanoparticle dispersion. Following preparation NP was checked for size and zeta potential (PSS NICOMP 380 ZLS). The evaluation of FDA amount entrapped in NP required the following alkaline treatment. NP dispersions were centrifuged (13,400 rpm for 30 min), the supernatant was mixed with an equal volume of sodium hydroxide 5 M and the mixture kept at 37 °C under shaking for 20 min. Under these conditions FDA was hydrolyzed to sodium fluorescein and could be quantified fluorometrically (excitation 485 nm, emission 520 nm). The marker amount entrapped in the NP (EE) was calculated as follows, using the appropriate calibration curve:

$$EE = (M_t - M_s)/M_t$$

where M_t is the total mass of FDA used for the preparation of labeled NP and M_s is the mass found in the supernatant.

2.3. Providing purified mucus

Porcine mucus was scraped off from the small intestinal tissue of a freshly slaughtered pig. The mucus was purified and homogenized by the addition of 5 mL of 0.1 M sodium chloride per gram of mucus, followed by stirring at 10 °C for 1 h. Subsequently the mixture was centrifuged (9000 rpm, 4 °C for 2 h), the supernatant discarded and the mucus separated for use from the granular material at the bottom of the centrifugation tube (Pereira de Sousa et al., 2015).

2.4. Rheological synergism of NP-mucus mixtures

Mucoadhesive properties were assessed by the rheological synergism method measuring the viscosity of NP-mucus mixtures at 37 ± 01 °C with a plate-plate viscometer (Haake Mars II, Thermo Corporation, Germany) (Hauptstein et al., 2014; Di Colo et al., 2009; Uccello Barretta et al., 2010). The gap was set at 0.5 mm, the shear rate at 50 s^{-1}. The mixtures were obtained by adding 1 mL of each FDA-labeled NP dispersion, prepared as described in Section 2.2, to 3 mL of cleaned mucus. As a reference, a 2 mg/mL solution of QA-rCh50 or QA-rCh60, or QA-rCh50-SH or QA-rCh60-SH in phosphate buffer pH 7.4 0.13 M (PB), containing the same concentration of FDA as that used to prepare the NP, and a 0.33 mg/mL FDA solution in the same buffer was mixed with 3 mL of cleaned mucus. Following their preparation the samples were allowed to equilibrate at 37 °C for 30 min, after which their viscosity was determined.

According to Hassan and Gallo (1990) the viscosity coefficient, η, of a hydrophilic dispersion of mucin and a mucoadhesive polymer results from the additive contributions of the viscosity coefficients of mucin, η_m, and polymer, η_p, and a viscosity component due to mucin-polymer interaction, η_{mp}. After measuring η, η_m and η_p the interactive component was calculated as:

$$\eta_{mp} = \eta - \eta_m - \eta_p \quad (1)$$

The contribution of the interactive component to the overall viscosity, i.e., the η_{mp}/η ratio was used for a comparative evaluation of polymer mucoadhesivity.

2.5. Transport through mucus studied by the rotating silicone tube method

The NP transport through a mucus layer was investigated using the method described by Dünnhaupt et al. (2011). Briefly, 300 μL of cleaned mucus was filled into silicone tubes of 30 mm length and 4 mm diameter and closed on one end with a cap. 50 μL of each NP dispersion or solution of constituent polymers were applied to the open end of a mucus-filled silicone tube and closed with another cap. All tubes were kept under horizontal rotation (≈50 rpm) for 5 h at 37 °C. At the end of each experiment the silicone tubes were frozen and, for analyzing the depth of NP diffusion into the mucus layer, the tubes were cut into slices of 2 mm length beginning with the extremity where the formulations were added. The marker in each slice was determined as described in Section 2.2.

2.6. In vitro study of water-assisted transport of NP through mucus

A mucus layer was realized by filling the barrel of a 1 mL plastic syringe with 350 μL of cleaned mucus. The barrel was kept, tip down, in vertical position and the mucus was prevented from escaping through the syringe tip by a 0.2 μm filter. 50 μL of each NP dispersion or solution of constituent polymers was placed on