Mucus Penetrating Nanoparticles Improve Drug and Gene Delivery to the Gastrointestinal Tract

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ABSTRACT SUMMARY
We have previously shown that mucus penetrating nanoparticles (MPP) have improved distribution and retention in the cervicovaginal tract. Here, we demonstrate that nanoparticles that rapidly penetrate gastrointestinal (GI) mucus are also able to enhance gastrointestinal delivery. MPP have improved distribution on healthy and ulcerative colitis (UC) colorectal tissues, and more evenly coat the epithelial surface of the small intestine after oral administration compared to conventional mucoadhesive nanoparticles (CP). MPP have the potential to significantly improve drug delivery to the GI tract, leading to better treatments of GI tract diseases and systemic absorption of drugs.

INTRODUCTION
The GI tract is a common target for drug delivery. It presents over 300 m² of surface area for drug absorption, and large numbers of gut-associated lymphoid tissues that sample the lumen. Many investigators have used micro- and nanoparticles to prevent early break-down and metabolism of drugs. However, these particles also have to face the GI mucus barrier. Mucus is a highly viscoelastic substance that serves as first line of defense on various epithelial surfaces such as the GI tract, respiratory tract, and cervicovaginal tract by efficiently trapping pathogens and particulates [1].

Our previous work has shown that nanoparticles densely coated with polyethylene glycol (PEG) can efficiently penetrate mucus [2]. We have shown that MPP up to 120 nm in size are able to diffuse through mouse colorectal mucus [3]. We have also previously shown that MPP are able to take advantage of advective fluid flow, leading to rapid homogeneous distribution of nanoparticles on the highly folded epithelial surface of the cervicovaginal tract, whereas CP are stuck in the luminal mucus layer [4]. Here, we investigate the use of MPP administered in a hypotonic vehicle for improved drug delivery to the small and large intestine by oral and local colorectal delivery via enema.

EXPERIMENTAL METHODS
MPP were prepared as previously described. Briefly, carboxylated polystyrene beads (PS-COOH, Invitrogen) were obtained, 5 kDa methoxy-PEG-amine (Creative PEGworks), N-Hydroxysulfosuccinimide (Sigma), and 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC, Invitrogen) were suspended in 200 mM borate buffer together with the PS-COOH and reacted for 4 h [5]. Nanoparticles were washed and analyzed for size and ζ-potential using dynamic light scattering and laser Doppler anemometry.

To induce ulcerative colitis, animals were anesthetized with isoflurane, 0.125 mg/g of 2,4,6-trinitrobenzenesulfonic acid (TNBS, Sigma-Aldrich) in 50% ethanol were administered, and mice with 5% body weight loss were used for experiments, as previously described [6].

For colorectal distribution, animals were anesthetized and 20 or 50 µL of hypotonic enema fluid containing MPP or CP were administered. For distribution in the small intestine, 50 µL of hypotonic fluid containing MPP or CP were administered via 24G gavage needle. Tissues were excised after 5-10 minutes for colorectal distribution and after 2 h for small intestine distribution. For intestinal loop models, mice were anesthetized with avertin via intraperitoneal injection, ileum sections were exposed, 2 cm of tissue were tied off using sutures, and 200 µL of fluid were injected into the loop. Tissues were excised after 5 minutes.

For cryo distribution, tissues were flash-frozen in optimal cutting temperature compound (OCT™), cut into 6 µm sections, and nuclei were stained with DAPI. For quantified distribution, tissues were sliced open longitudinally and flattened between two glass slides. Six images were taken along the tissue and quantified using ImageJ™. All images were taken using an inverted epifluorescence microscopy (Zeiss Axio Observer).

RESULTS AND DISCUSSION
We have previously demonstrated that diffusion of MPP in ex vivo mucus samples leads to improved distribution over the vaginal mucosal epithelium in mice, which occurs in only minutes when MPP are administered in a hypotonic vehicle that causes fluid absorption by the epithelium [7]. Here, we found that hypotonically administered MPP up to 200 nm in size have significantly improved distribution on mouse colorectal tissue compared to CP, covering >80% and <40% of the tissue surface, respectively (not shown). CP aggregated in the sloppy mucus
layer and are unable to reach all the folds of the epithelial surface, unlike MPP that evenly coat the epithelium (Fig 1).

Inflammatory bowel disease affects 1-2 million Americans every year, 50% of who are diagnosed with ulcerative colitis (UC). It is known that mucus barrier properties and secretions are altered in patients with UC, so it is important to understand how changes in mucus properties influence local drug delivery with nanoparticles. We used multiple particle tracking to observe the transport of nanoparticles in mucus layers on freshly excised ex vivo colorectal tissue [3]. We found that, unlike in healthy colorectal mucus, a significant fraction of 200 nm MPP diffused in UC colorectal mucus. It has been previously shown that small nanoparticles preferentially adhere to ulcerated regions on IBD tissues [8], likely due to trapping in the mucus layer coating inflamed tissues. In contrast, MPP distribute over and penetrate into ulcerated portions of the UC coloectums (Fig 2), potentially leading to improved treatment of the disease.

To demonstrate the broad applicability of MPP for drug delivery in the GI tract, we investigated the distribution of MPP on small intestine tissues after oral administration. We found that MPP coat >70% of the jejunal surface area, whereas CP coat <40%, similar to what we found in the colorectum (not shown).

In addition, when nanoparticles were administered directly into the ileum, as done in intestinal loop models, CP and MPP distribution was indistinguishable, suggesting that this model is not representative of normal bowel transit (Fig 3). Because of their ability to reach a larger fraction of the epithelial surface, MPP are likely to improve drug delivery to the GI tract, potentially including delivery to the systemic circulation, leading to improved treatment and prevention of a variety of diseases.

CONCLUSION
MPP are able to take advantage of advective transport, resulting in near-complete coverage of the epithelial surface of the colorectum. In addition, MPP distribute evenly over and penetrate into ulcerated regions of UC tissues, whereas CP aggregate in the outer mucus layers and tissue surfaces. When administered orally, MPP distribute more evenly on the epithelial surface than CP. However, when MPP and CP were administered in large volumes directly to the intestines, as done in intestinal loop models, there was no distinguishable difference in distribution, indicating that these models are not representative of normal GI tract transit. Improved distribution with advective absorption suggests that MPP can significantly improve local and potentially systemic drug delivery for the prevention and treatment of GI tract diseases.

REFERENCES

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