Anionic nanoparticles enable oral peptide delivery by enhancing intestinal permeability

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Oral Delivery via the Intestinal Epithelium

Oral delivery of macromolecular drugs is highly sought after due to its ease of administration and superior patient compliance. However, successful oral formulations must overcome poor transport of large molecules from the intestines to the bloodstream. Some intestinal permeation enhancers have been identified, but effective species tend to induce toxicity or invoke an immune response. Further, orally administered drugs have previously been loaded into or onto nanoparticles for improved bioavailability, but not co-administered as chemically separate entities.

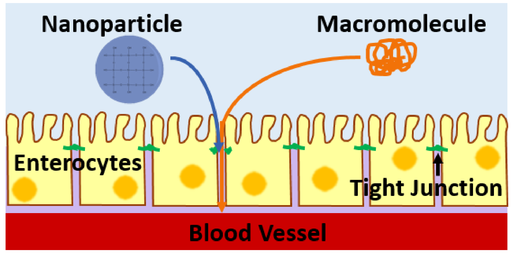

Figure 1: The intestines are lined with an epithelial layer of enterocyte cells, which form tight junctions between one another to act as a barrier to mass transport. Paracellular permeation enhancers, such as the nanoparticles employed here, decrease the resistance of this barrier to macromolecule transport.

Our approach utilizes orally co-administered nanoparticles to improve paracellular (between epithelial cells) absorption of macromolecules, including exenatide and insulin, without inducing intestinal toxicity.

Nanoparticle Characteristics

Table 1: DLS size and zeta potential data for nanoparticles in water at neutral pH. Values for particle number and surface area per mass were obtained from specification sheets provided by the supplier. n.f. = non-functionalized; PVP = 40,000 MW polyvinylpyrrolidone capping agent.

Core		Surface chemistry	Nomenclature	Z-average diameter		Zeta potential	Particles per mass	Surface area
Size (nm)	Material			d.nm	PDI	(mV)	#/mg	cm^2/mg
200	silica	n.f.	200 nm SiO^-	209	0.02	-57.6	1.09E+11	137
100	silica	n.f.	100 nm SiO^-	90	0.05	-41.2	9.61E+11	280
50	silica	n.f.	50 nm SiO^-	49	0.04	-41.4	7.81E+12	554
20	silica	n.f.	20 nm SiO^-	26	0.05	-57.6	7.89E+13	1212
50	silica	COOH	50 nm $SiO\text{-}COO^-$	46	0.15	-27.3	n/a	n/a
50	silver	PVP	50 nm Ag^-	54	0.14	-21.4	1.47E+12	109
50	gold	PVP	50 nm Au^-	61	0.09	-16.3	7.64E+11	59
50	polystyrene	n.f.	50 nm PS	58	0.06	0.2	n/a	n/a
50	silica	NH_2	50 nm $SiO\text{-}NH_3^+$	49	0.44	15.6	7.24E+12	547

50 nm Silica Most Effectively Permeabilizes the Intestines

Previously, we showed in Caco-2 epithelial monolayers that treatment with decreasing particle size and increasing anionic charge correlate with greater epithelial permeability. To test this relationship in mice, we delivered the model drug 4 kDa FITC-labelled dextran (FITX-DX4). As predicted, greater negative surface charge on the particles correlated with increasing efficacy. However, 50 nm particles induced greatest FITC-DX4 uptake, with 20 nm particles showing surprisingly little effect. This discrepancy is due to 20 nm particles more effectively binding and sticking to intestinal mucus (data not shown).

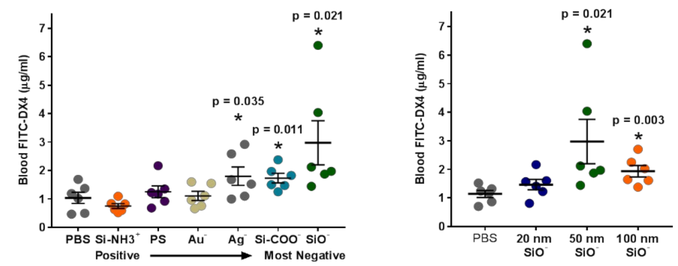

Figure 2: Oral treatment with silica nanoparticles improves absorption of 4 kDa FITC-dextran. Mice were orally administered 100 mg/kg nanoparticles in aqueous suspension, or PBS for control animals, then gavaged two hours later with 600 mg/kg FITC-DX4. Their serum was collected three hours later and examined for FITC-DX4 concentration. Serum FITC levels show that negatively charged particles are effective permeability increasers, while neutral and positive particles have little effect. Similarly, 50 nm silica particles were significantly more effective than differently sized particles of the same chemistry. Error bars display s.e.m. (n = 5). * $p < 0.05$ w.r.t. PBS control.

Oral Delivery of Exenatide in Mice

To evaluate true oral delivery of a peptide drug, exenatide-loaded capsules (1 mg/kg dose) were enterically coated and washed down the esophagus with either silica nanoparticle suspension (200 mg/kg), or saline for a negative control. Particle treatments greatly improved exenatide uptake compared to the peptide capsules without particle treatment. Compared to the same dose of subcutaneously administered exenatide, the silica-assisted, orally delivered peptide achieved 10% bioavailability.

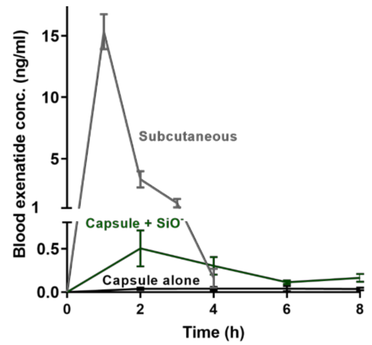

Figure 3: Silica nanoparticles enabled oral exenatide delivery in mice. Particle treatments enabled systemic uptake of exenatide administered orally in capsules. Error bars display s.e.m. (n = 5).

Efficacy of Orally Administered Insulin in Diabetic Mice

Silica-enabled, orally delivered protein drug maintains pharmacodynamic effect to treat disease. In this case, type 1 diabetic were administered insulin capsules washed down the esophagus with nanoparticle suspension or saline. Oral insulin sustained reductions in blood sugar for at least four hours, far longer than the current standard of subcutaneous injection. Further, the silica nanoparticle treatment achieved an impressive 23% relative bioactivity (dose-adjusted).

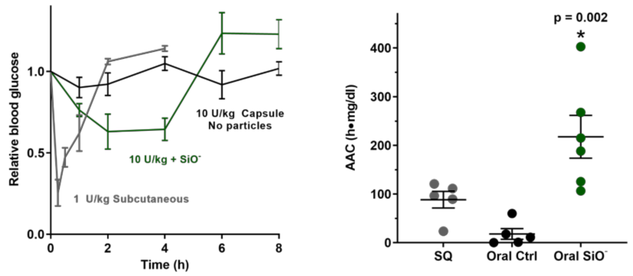

Figure 4: Silica nanoparticles enabled oral insulin delivery in mice. Orally administered insulin induced pronounced and sustained when co-administered with silica nanoparticles. Oral insulin without particles produced no effect compared to the control protein BSA. Further, particle treatments resulted in multiple-fold increases in the area above the blood glucose curve. Error bars display s.e.m. (n = 5). * $p < 0.05$

Safety Aspects of Intestinal Silica Nanoparticles

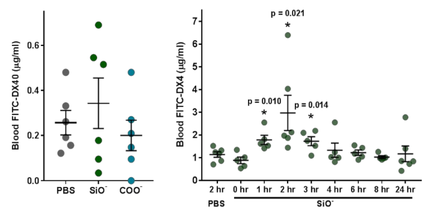

Figure 5. Intestinal permeation enhancement by silica nanoparticles is reversible and size selective. Treatment with silica particles did not significantly increase uptake of 40 kDa FITC-dextran. Further, permeability to 4 kDa FITC-dxtran returned to untreated levels within 4 hours after particle exposure. Together, these data demonstrate that the particles are unlikely to permanently damage the epithelium or to allow infiltration of bacteria. Error bars display s.e.m. (n = 5-6), * $p < 0.05$.

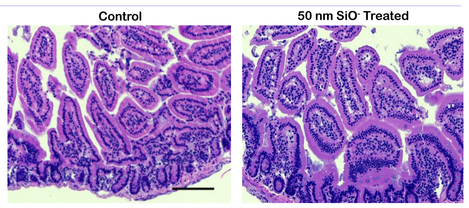

Figure 6. Histology indicated no particle-induced changes in intestinal health. Mice were gavaged with silica nanoparticles, or PBS as a control, then sacrificed five hours later for collection of intestinal tissue. H & E staining, as well as examination by a certified pathologist, confirmed no notable differences in tissue architecture, health, or immune cell infiltration between the control and treated intestines. n = 3. Scale bars = 100 µm.

MLCK-Dependent Rearrangement of Tight Junctions

To investigate mechanisms of the particles' action, we used the Caco-2 cell model of the intestinal epithelium. In nanoparticle-treated samples, some cells form clusters not containing ZO-1 within their junctions, potentially creating an effective permeation pathway. Further, signaling pathway analysis indicates that the enzyme myosin light chain kinase is critical to this effect in the cells.

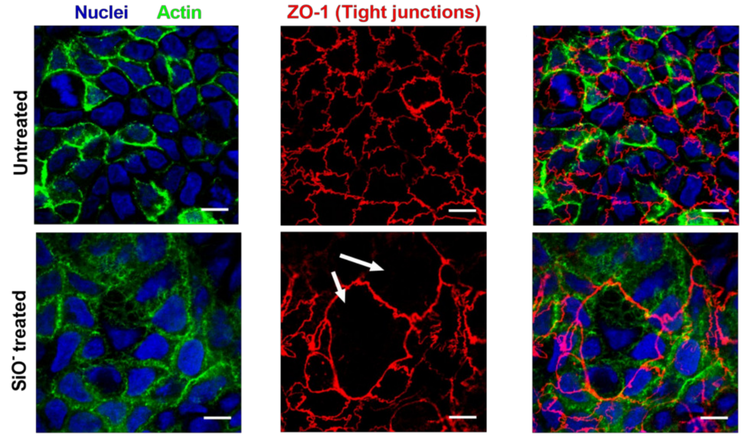

Figure 7: Caco-2 monolayers treated with silica nanoparticles exhibit repatterning of tight junctions. Caco-2 cells were imaged for the tight junction protein ZO-1 at the apical surface, as well as actin and nucleic acids approximately 2 µm below the apical surface. Compared to untreated monolayers, those treated with 50 nm silica nanoparticles exhibited communities of several cells inside which the tight junctions were not normally expressed (white arrows). Scale bars = 10 µm.

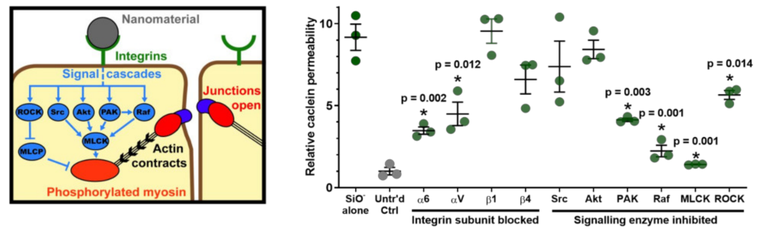

Fig. 8: Silica nanoparticles increased permeability by binding cell surface integrins and inducing tight junction rearrangement. There are several pathways through which integrin activation and myosin light chain phosphorylation are linked to intestinal permeability. The permeation enhancing effect of silica nanoparticles in vitro was reduced by blocking particle binding to integrin α-subunits or by inhibiting a subset of intracellular signalling cascade proteins. Error bars display s.e.m (n = 3). *$P < 0.05$ w.r.t. silica treatment alone. Akt = protein kinase B; MLCK = myosin light chain kinase; MLCP = myosin light chain phosphatase; PAK = p-21 activated kinase; Raf = rapidly accelerated fibrosarcoma kinase; ROCK = Rho-associated protein kinase; Src = protooncogene c-Src.

Conclusions and Future Work

Conclusions

- Anionic nanoparticles, especially 50 nm silica, are effective intestinal permeation enhancers that enable oral delivery of peptide and protein drugs.
- Silica nanoparticles increase the pharmacodynamics of orally administered insulin, achieving over 20% relative bioactivity with respect to the current gold standard of subcutaneous injection.
- Nanoparticle treatments interact with integrins and activate an MLCK-dependent pathway, increasing epithelial permeability in a size-selective and reversible manner.

Future Work

- Incorporate silica nanoparticles and peptide drugs into a single dosage form to better co-localize and improve delivery efficacy.

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