Pharm. 70:235-40 (1991). Mucin fibers contain highly glycosylated segments with high affinity for positively-charged particles, as well as periodic hydrophobic domains that can bind hydrophobic materials with high avidity, including many commonly used drug delivery materials such as poly-(lactic-co-glycolic acid) (PLGA). These complex charge interactions coupled with steric hindrance have proven difficult for nanoparticle drug delivery forms to overcome without some form of shielding. Conversely, however, conventional shielding approaches often yield a nanoparticle having greatly reduced transfection capability.

[0006] Polyethylene glycol (PEG) has emerged as the primary constituent in a wide range of shielding strategies, based at least initially on its demonstrated ability to *increase* mucoadhesion via interpenetrating network effects, thereby prolonging drug delivery. *See, e.g.* Huckaby and Lai, *Advanced Drug Delivery Reviews* 124:125-39 (2018). Unfortunately, however, these mucoadhesive strategies do not work for therapeutics requiring intracellular delivery such as nucleic acids, since enhanced mucoadhesion prevents the particles from reaching the underlying epithelium or other associated target tissues. Paradoxically, perhaps, PEG coatings have also found use in muco-inert approaches attempting to enhance particle diffusion. Notably, however, recent findings and conclusions with these types of systems have underscored the importance of high PEG grafting density on minimizing mucin affinity and enhancing mucus penetration. *Id.*, Wang and Lai, *Angew Chem* 47:9726-9729 (2008). These exceptionally dense and covalently-bound PEGylation strategies are problematic for efficient gene transfection.

[0007] Thus, there remains a need for new compositions and methods for gene transfer *in vivo* with improved mucosal penetration properties.

SUMMARY

[0007a] In one aspect, provided herein is a composition comprising: (a) a complex comprising a chitosan-derivative nanoparticle comprising amino-functionalized chitosan and at least one nucleic acid molecule, wherein the at least one nucleic acid molecule is non-covalently bound to the chitosan-derivative nanoparticle at an amino to phosphorous (N:P) molar ratio of greater than 3:1, thereby forming a derivatized chitosan nucleic acid complex having a positive charge; and (b) a plurality of linear block copolymers non-covalently bound to the chitosan-derivative nanoparticle, wherein said linear block copolymers comprise at least one polyanionic (PA) anchor region and at least one polyethylene glycol (PEG) tail region,

wherein the PEG-PA molecules are non-covalently bound to the chitosan-derivative nanoparticle, and wherein the composition comprises an amino to anion (N:A) molar ratio that is greater than 1:100 and less than 10:1.

[0007b] In another aspect, provided herein is a method for making any one of the compositions of claims 1 to 26, the method comprising: (a) providing a complex comprising the chitosan-derivative nanoparticle comprising amino-functionalized chitosan and the at least one nucleic acid; and (b) mixing the complex with a solution comprising PEG-PA molecules, thereby forming a reaction mixture comprising the composition.

[0007c] In another aspect, provided herein is a method for making any one of the compositions of claims 1 to 26, the method comprising simultaneously or sequentially admixing amino-functionalized chitosan, at least one nucleic acid, and PEG-PA molecules, thereby forming a reaction mixture comprising the composition.

[0007d] In another aspect, provided herein is use of a composition according to any one of claims 1 to 26, or a composition produced by any of the methods of claims 27 to 33 for the manufacture of a formulation for transfecting a cell with a nucleic acid.

[0008] Provided herein are compositions and methods for reducing mucoadhesion and enhancing mucus penetration of chitosan-nucleic acid nanoparticles, employing a reversible (*i.e.* non-covalently bound) polymeric coating comprising a plurality of linear block copolymers having a negatively charged anchor region and at least one (*e.g.*, hydrophilic) non-charged tail region. In preferred embodiments, the linear block copolymer is a diblock or triblock copolymer comprising at least one polyanion anchor region and at least one PEG tail region. In some embodiments, the compositions described herein surprisingly provide reduced mucoadhesion and enhanced mucus penetration at a lower surface coating density than