Gemini Surfactant-Phospholipid Nanoparticles as Gene Carriers for Glaucoma Gene Therapy
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Purpose
The current standard of care for glaucoma treatment is intraocular pressure management, which does not fully prevent or reverse vision loss. Thus, there is a strong incentive to pursue alternative treatment strategies that address the glaucomatous neurodegenerative mechanisms. The current study examines physiochemical, toxicological and ocular biodistribution parameters of gemini surfactant-phospholipid nanoparticles (GL-NPs) after topical and intravitreal administration in mice.

Methods
Nanoparticles were constructed from gemini surfactant, 12-7NH-12 with two neutral helper lipids, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine and Cy5-labelled plasmid (GL-NPs). The transfection efficiency and toxicity of GL-NPs with varying GL-NP charge ratio, lipid composition, mode of assembly, and manufacturing technique were screened in the retinal neuronal cell line RGC-5, using flow cytometry and PrestoBlue™ assays. Particle size and zeta potential were determined by dynamic light scattering. Toxicity and permeation of GL-NPs was studied in a human corneal epithelial model (Mattek) by MTT assay and confocal microscopy. GL-NP biodistribution and gene transfer capacity was assessed in C57BL/6N mice, following topical and intravitreal administration by confocal microscopy of ocular sections.

Results
Particle size of GL-NPs ranged between 150-180 nm, and their zeta potential ranged between +9.2 ± 2.1 and +48.7 ± 2.2 mV. GL-NPs were highly biocompatible with retinal RGC-5 and corneal HCE models, with >95% viability. The optimum GL-NP had a transfection efficiency of 14.5±1.4%. GL-NPs were able to bind to the corneal epithelial surface and achieve a moderate permeation depth (35-40 μm), following topical application in the HCE model. GL-NPs localized in the nerve fibre layer of the retina following intravitreal injection. After topical administration, GL-NPs localized in several anterior chamber tissues, including limbus, iris and conjunctiva. In each case, GL-NPs were trafficked as single, non-aggregated particles, indicating a thermodynamically stable distribution in the targeted ocular tissues.

Conclusion
GL-NPs are suitable gene carriers for both intravitreal and topical ocular administration to the retina and anterior chamber of the eye.