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Penetration of Fluorescent Nanoparticles into the Cornea

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Nanoparticles-based drug/gene delivery have been reported for potential therapeutic management of various ocular surface and corneal disorders [Kompella UB, et al., Nanomedicines for back of the eye drug delivery, gene delivery, and imaging. Prog Retin Eye Res. 2013;36:172-98]. In this study, we have examined penetration of mono-dispersed silica nanoparticles stained with Rhodamine B (RhB) and FITC at the microscopic level. Specifically, we have employed a custom-built confocal scanning microfluorometer (CSMF).

Our custom-built CSMF is designed for recording depth-resolved fluorescence across the cornea repeatedly over long periods (several hours) [Srinivas SP, Maurice DM., A microfluorometer for measuring diffusion of fluorophores across the cornea. IEEE Trans Biomed Eng. 1992 Dec;39(12):1283-91]. Depth resolution of the CSMF is ˜ 7 µm using a 40x water immersion objective of 1.2 mm working distance; Zeiss) at 2.66 µM of fluorescein. Excitation, obtained from blue/green LEDs, is < 2 µW at the focal plane. This limits the potential for photobleaching. Scanning speed > 40 µm/sec. Simultaneous trans-corneal fluorescence and scatter can be accomplished.

Mono-dispersed silica of 6 nm (Sigma Inc; Cat # S5130) were stained with RhB (Sigma Inc; Cat #83690) by overnight exposure of the nanoparticles to 0.1 mg/mL of the dye. The particles were washed in PBS 2-3x and then used next day. Excitation from a blue LED was filtered through an interference filter (470 + 10 nm). RhB fluorescence (> 530 nm) and scattered light collected through the exit slit, which is held parfocal to excitation slit, were detected by the PMTs (Fig. 1). Experiments were performed with excised porcine eye. As a lipophilic dye, RhB partitions into epithelium and accumulates over time and eventually diffuses into stroma as observed. We found significant uptake of the nanoparticiles into the epithelium. But lack of significant fluorescence at anterior stroma following FITC-stained nanoparticles (Fig. 3C) indicates that although the nanoparticles are taken up by the epithelium, not much is released into the stroma. When RhB-Si or FITC stained chitosan-dextran sulphate (CDNs; 400 nm) nanosuspension were administered on bare stroma, the particles penetrated significantly.

Our data is insufficient to explain the penetration of the Si nanoparticles across the cornea with and without epithelium. The collagen fibrils in the stroma, which lie in the lamellae and are parallel to the surface of the tissue, can be expected to offer steric hindrance to the movement of the particles in conjunction with the charged glycosaminoglycans surrounding each fibril. Moreover, we recall that the fibrils in a given lamella are parallel to one another with each lamella oriented at a finite angle with respect to the neighboring lamellae. How this intricate ultrastructure permits movement of nanoparticles (6-50 nm), with and without an inwardly-directed water movement, remains to be explored.

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