Mapping the Drainage Pathway from the Suprachoroidal Space: Evidence of Lymphatic Routing and Implications for Therapeutic Delivery in the Eye

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Introduction: The suprachoroidal space serves as a compartment for delivering biologics, nanoparticles, and genes to the posterior segment of the eye. However, the routes of drainage of solutes and fluid from this space remains elusive. The present study aims to map and characterize the drainage pathway from the suprachoroidal space by injecting a near-infrared fluorescent nanoparticle tracer injection.

Methods: A near-infrared fluorescent nanoparticle tracer, CF770 conjugated with bovine serum albumin (MW:70kDa, 0.5µL), was injected into the suprachoroidal space (10nl/s) of the right eye in adult mice (C57BL/6J; n=8). Sham-injected left eyes were utilized as controls. In vivo and ex vivo fluorescence images of the eye and neck lymph nodes were captured using a scanning laser ophthalmoscope at 10-, 15-, and 20-minute intervals post-injection. Mice were euthanized 20 minutes after injection, and their tissues were processed for histological validation. Sagittal sections of the orbit, 20µm thick, were double labeled with podoplanin and podocalyxin, marking lymphatic vessels and blood vessels' endothelial cells, respectively. Tissue sections without primary antibodies served as negative controls. Immunofluorescence-stained sections were imaged using a confocal scanning laser microscope and a near-infrared epifluorescence microscope at 20x and 63x magnifications.

Results: In vivo fluorescent imaging depicted the presence of a lymphatic network with a tendency for the nasal region in the orbit. Near-infrared epifluorescence microscopy revealed that the tracer drains through the sclera and orbit. Immunofluorescence analysis identified podoplanin-positive lymphatic vessels in the choroid with a central lumen, distinct from blood vessels. Furthermore, a near-infrared tracer was detected in the lumen of podoplanin-positive lymphatic channels in the conjunctiva. Ex vivo imaging demonstrated that the tracer injected into the right suprachoroidal space drains into the right accessory submandibular neck lymph node.

Conclusions: This study provides the first evidence that fluid and nanoparticles exit the eye through a nasal route into the sclera and orbit from the suprachoroidal space and subsequently, drain into the ipsilateral accessory submandibular lymph node. It presents evidence of lymphatic vessels in the choroid and demonstrates tracer draining into conjunctival lymphatic channels. A better understanding of this intricate pathway originating from the suprachoroidal space holds significant implications for designing new therapeutic modalities for glaucoma such as novel drainage devices or drug delivery strategies targeting the suprachoroidal space.