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World Eye and Vision Congress

December 09-10, 2019 Dubai, UAE



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Early events of *Pseudomonas aeruginosa* microbial keratitis: An electron microscopic study

Purpose: To study the behavior of *Pseudomonas aeruginosa* bacteria inside the corneal stroma after corneal infection in human microbial keratitis.

Methods: Human whole donor corneas obtained from the eye bank were used in this project. Bacterial suspensions of wild type and mCherry expressing *P. aeruginosa* PAO-1L were prepared to a concentration of 107 cfu/ml. Fifty microliters of bacterial suspension was injected into the middle of the corneal stroma from the endothelial using a 25G hypodermic needle. The corneas were then incubated in medium for 24 hours, after which they were either examined live under a Laser Confocal Fluorescence Microscopy (CFLM) or fixed and processed for Transmission Electron Microscopy (TEM).

Results: After 24 hours of infection, *P. aeruginosa* bacteria were found to internalize into Corneal Stromal Keratocytes (CSK). Using TEM, PAO-1L bacteria were found clustered inside CSK with much fewer bacteria scattered in the stroma outside cells. With CFLM live imaging, mCherry-expressing PAO-1L bacteria were seen inside CSK, also with much fewer extracellular bacteria.

Conclusion: We have established *ex vivo* models of bacterial keratitis and demonstrated that *Pseudomonas aeruginosa* internalize early into stromal keratocytes, possibly to avoid contact with resident immune cells.

Biography

Elsahn is a fully trained, practicing ophthalmic surgeon. He graduated with honours from Egypt's prestigious Alexandria University, and completed an academic Ophthalmology residency. He then completed a cornea and refractive surgery fellowship at the renowned Wills Eye Institute in the United States of America. On returning to Egypt, Dr Elsahn completed a Master degree in laser eye surgery and customized corneal ablation, graduating with honours. In 2009, after attaining after attaining Fellowship of the Royal College of Surgeons in Ophthalmology, Dr Elsahn relocated to the United Kingdom, where he started a second cornea and refractive surgery fellowship in University Hospitals Southampton NHS Foundation Trust.