

**Thesis title:** CPP MEDIATED DRUG DELIVERY FOR OCULAR DISEASE  
MANAGEMENT

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**Abstract:**

Blindness and vision impairment remains a major health concern in developing countries with more than 2 billion people visually compromised globally. In India, corneal opacities (8.2%) are the second leading cause of blindness among people of age 50 years and above. Major risk factors of corneal opacities include infectious and non-infectious keratitis, xerophthalmia, keratoconus, trauma, and few inherited and inflammatory disorders. Out of total visually compromised population, atleast 1 billion have preventable blindness owing to accurate diagnosis and timely management of ocular disorders. Present study aims at developing alternate strategies for efficacious management of fungal keratitis and keratoconus than the existing therapeutic regimes and also reduction in treatment duration as compared to the standard treatment protocols.

One of the approaches to increase therapeutic efficiency of any treatment protocol is to enhance the bioavailability of active drug molecules that will subsequently lead to reduced treatment duration and increased patient compliance. In past few decades, several nanocarrier mediated drug delivery strategies have been investigated. Cell penetrating peptides (CPPs), with an ability to translocate themselves across the cell membranes, have emerged as promising drug delivery vehicles for the management of various diseases including ocular disorders. Since the path breaking discovery of TAT, numerous CPPs have been designed and developed for their potential therapeutic applications in vitro and in vivo. The present

study aims at assessing the potential of an established cell penetrating peptide, Tat<sub>2</sub>, for efficacious management of fungal keratitis and keratoconus.

First part of the study investigates tissue penetration ability as well as antifungal efficacy of a CPP (Tat<sub>2</sub>) conjugated standard drug (Natamycin) used for the treatment of fungal keratitis. Natamycin is the only FDA approved drug that is used as a first line of treatment for fungal keratitis caused by filamentous fungi, however natamycin is known for poor corneal penetration. To address this drawback, natamycin has been successfully conjugated with a cell penetrating peptide, Tat<sub>2</sub>. Recently, we have shown the enhanced cellular uptake as well as antifungal efficacy of Tat<sub>2</sub> conjugated natamycin (Tat<sub>2</sub>natamycin) in vitro. In the present study, tissue penetration ability and antifungal efficacy of Tat<sub>2</sub>natamycin have been investigated and compared with natamycin alone in vivo. Results have shown that Tat<sub>2</sub>natamycin exhibited five- fold higher ocular penetration than natamycin alone when given topically. Complete resolution of fungal keratitis in 44% of the animals in Tat<sub>2</sub>natamycin treated group as compared to only 13% of the animals in natamycin treated group further highlighted its increased antifungal efficacy. Hence, this conjugate is a promising antifungal strategy with enhanced ocular penetration as well as efficacy against selected fungal species.

In the second part of the study, cell penetrating ability of Tat<sub>2</sub> conjugated riboflavin has been assessed in vitro. Dresden protocol, involving riboflavin as a photosensitizer, is used as a standard therapy for the management of keratoconus. However, less ocular permeability of riboflavin across corneal epithelial layer necessitates the removal of corneal epithelial layer prior to the initiation of protocol. As a result of this, severe post-op complications including pain, infections, sterile infiltrates, corneal haze etc., have been reported. In the current study, we have conjugated a cell penetrating peptide, Tat<sub>2</sub>, with riboflavin to enhance its cellular penetration across human corneal epithelial cells in vitro. Significantly enhanced cellular

uptake of Tat<sub>2</sub>riboflavin, in addition to negligible cytotoxicity, was observed in human corneal epithelial cell line. Upon quantification, Tat<sub>2</sub>riboflavin was found to enter more than 80 % of the cell population as compared to approximately 0.1 % of the cells treated with riboflavin alone. The study demonstrates the potential of Tat<sub>2</sub>riboflavin in modifying the standard Dresden protocol with respect to the epithelial debridement step, paving way for the *in vivo* studies.