

Thesis title: APPLICATION OF CELL PENETRATING PEPTIDES IN THE MANAGEMENT OF ANTERIOR SEGMENT DISEASES OF THE EYE

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ABSTRACT:

Human eye is a highly complex organ that provides one of the crucial senses, the sense of sight, essential for interacting with the surrounding environment. It's complexity is justified by the accurate arrangement of tissues creating various anatomical and physiological barriers at multiple levels to restrict the entry of any exogenous substances thereby protecting the inner ocular tissues. In addition to the protective nature these barriers, they also limit entry of therapeutic molecules consequently leading to reduced bioavailability of the drugs at the site of action. The primary barrier encountered by any topically applied therapeutics is the cornea. As an avascular clear tissue, cornea allows the passage of light to retina in the eye. According to WHO, 2.2 billion people have visual impairment or blindness out of which 4.2 million account for corneal opacities. Multiple attempts have been made to provide effective and efficient treatment strategies for corneal diseases. One such approach is to enhance the penetration of the drugs to attain clinically relevant concentrations at the desired therapeutic site. A class of peptides known as cell penetrating peptides (CPPs) can traverse the plasma membrane carrying cargo molecules attached to it. Usually ranging from 5 to 40 amino acids in length, it has the potential to carry along different types of cargoes such as therapeutic agents, proteins, nucleic acids, plasmids and even nano particles such as liposomes. This thesis presents novel strategies for therapeutic interventions for anterior segment disease management.

The first part introduces a novel CPP, designed to target the corneal tissue for drug delivery in corneal diseases. Corneal Targeting Sequence 1 (CorTS 1) has been developed by modifying a conserved leucine rich repeat (LRR) motif present in corneal proteins. The novel CorTS 1 peptide exhibits a promising cell penetrating activity with no notable cytotoxicity and an increased accumulation in corneal stroma than in aqueous humor *in vitro* and *in vivo*

conditions, respectively. The peptide also delivers protein cargo (beta galactosidase) in its active biological form inside human corneal epithelial cell (HCE) line. Interestingly, antimicrobial activity has been also noted against MRSA and *Fusarium dimerum*. CorTS 1 also possesses anticollagenolytic activity thereby holding a promising potential in treatment of microbial keratitis and stromal melts.

The second part of the study demonstrates *in vivo* efficacy of a novel CPP drug conjugate based strategy for the treatment of Keratoconus (KC). KC is a common corneal disorder characterised by progressive thinning leading to cone shaped cornea with irregular astigmatism and impaired vision. Corneal collagen crosslinking (CXL) is the standard treatment employed to halt the disease progression. A concerning step of this procedure is epithelial debridement carried out to facilitate the entry of poorly permeable riboflavin (Rb). In this study the Rb is covalently conjugated with the dimer of a well known CPP (Tat dimer – Tat₂) resulting in Tat₂riboflavin or RiTe conjugate to enhance the penetration and thereby improve the efficiency of the CXL protocol. Approximately a 2 fold increase in tissue penetration was achieved in rabbit corneas upon conjugation with the CPP. The comparative analysis of RiTe conjugate mediated and standard CXL exhibited an equivalent extent in crosslinking in both types of CXLs as observed in enzymatic digestion of corneas. No endothelial damage and keratocyte loss in case of RiTe conjugate mediated CXL in contrast to standard CXL further establishes the safety of the proposed protocols along with the efficacy. The two above-mentioned interventions highlight the potential application of CPPs and CPP drug conjugates for treating anterior segment diseases or disorders of the eye.