UNION UNIVERSITY

OCULAR DELIVERY OF PEPTIDES AND PROTEINS

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OUTLINE

- Structure of eye and different pathways of ocular administration
- Challenges for ocular delivery of proteins/peptides
- Formulation considerations
- Peptide transport systems in the eye
- Ocular administration for topical delivery of proteins/peptides
- Ocular administration for systemic delivery of proteins/peptides
- Strategies for ocular delivery of proteins/peptides

STRUCTURE OF THE EYE

Outermost coat: Clear, transparent cornea and white, opaque sclera

Middle layer: iris anteriorly, choroid posteriorly and intermediate ciliary body

Inner layer: retina

Topical administration with trans-corneal permeation

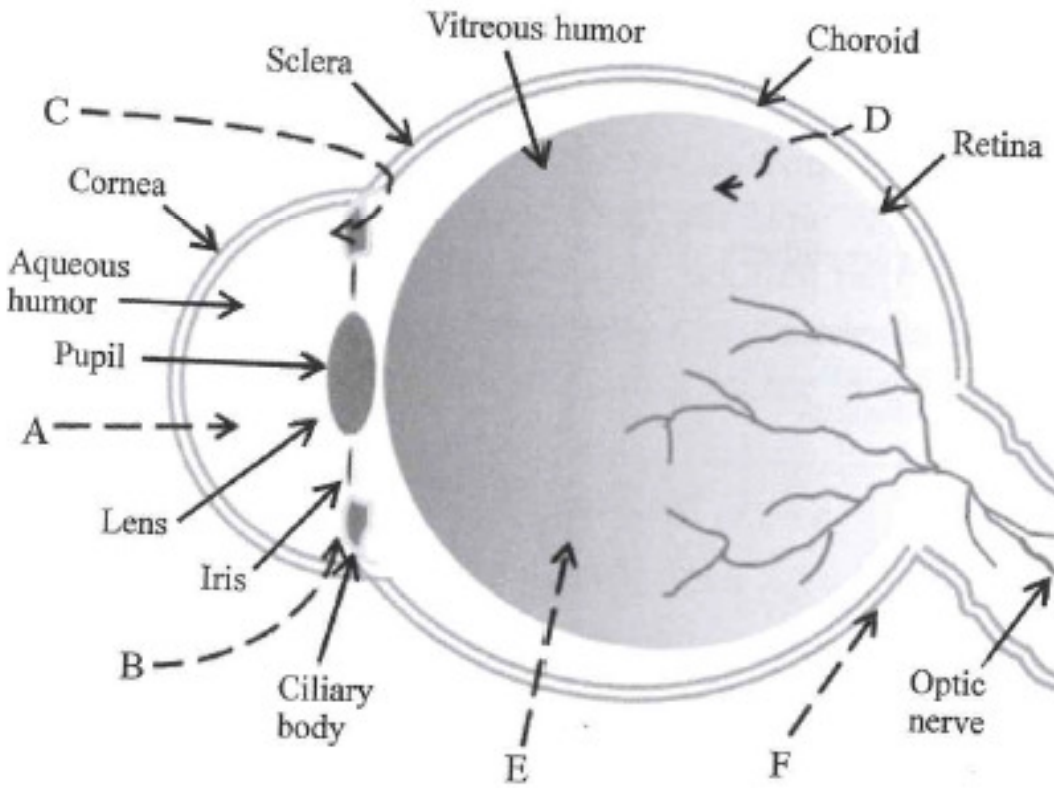
Topical administration with non-corneal permeation across the conjunctiva and sclera

Drug distribution from the blood through the blood-aqueous barrier into the anterior chamber

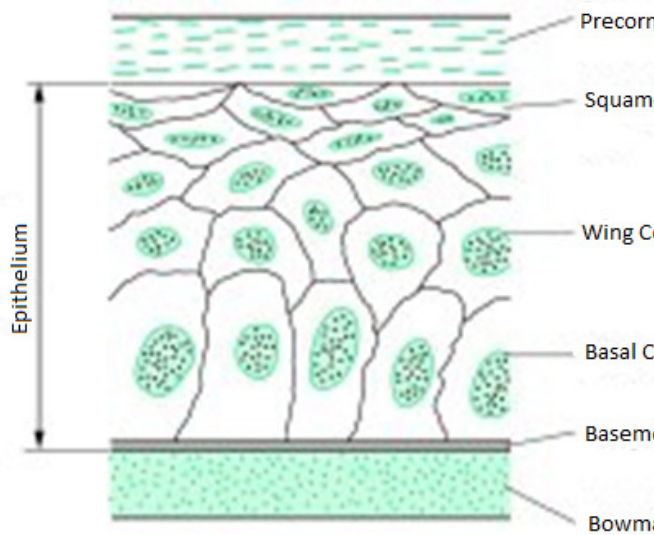
Drug distribution from the blood-retina barrier into the posterior chamber

Intra-vitreal drug administration route

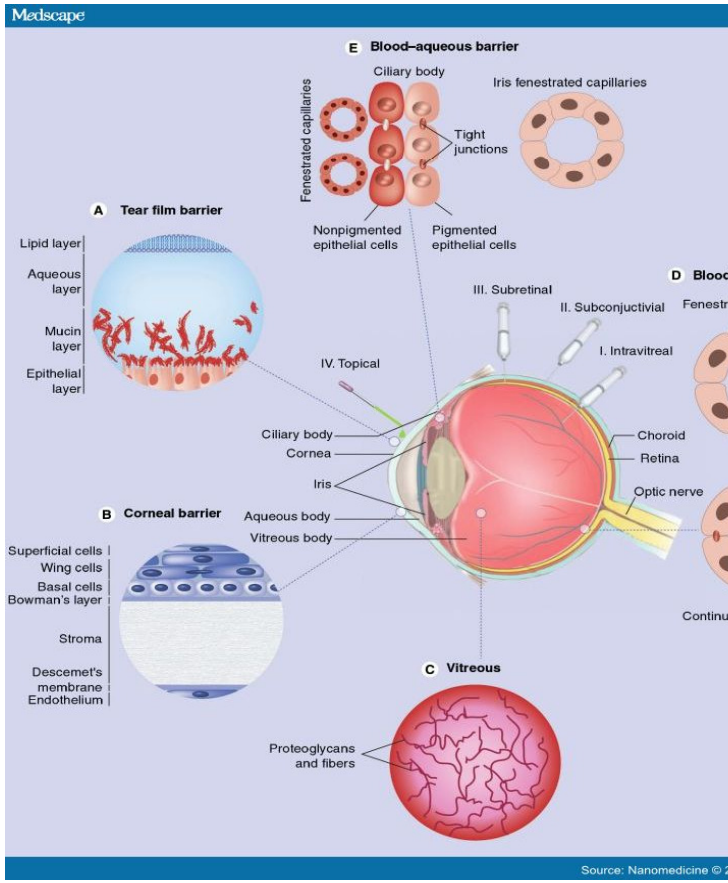
Sub-tenon injection



BARRIERS TO ABSORPTION



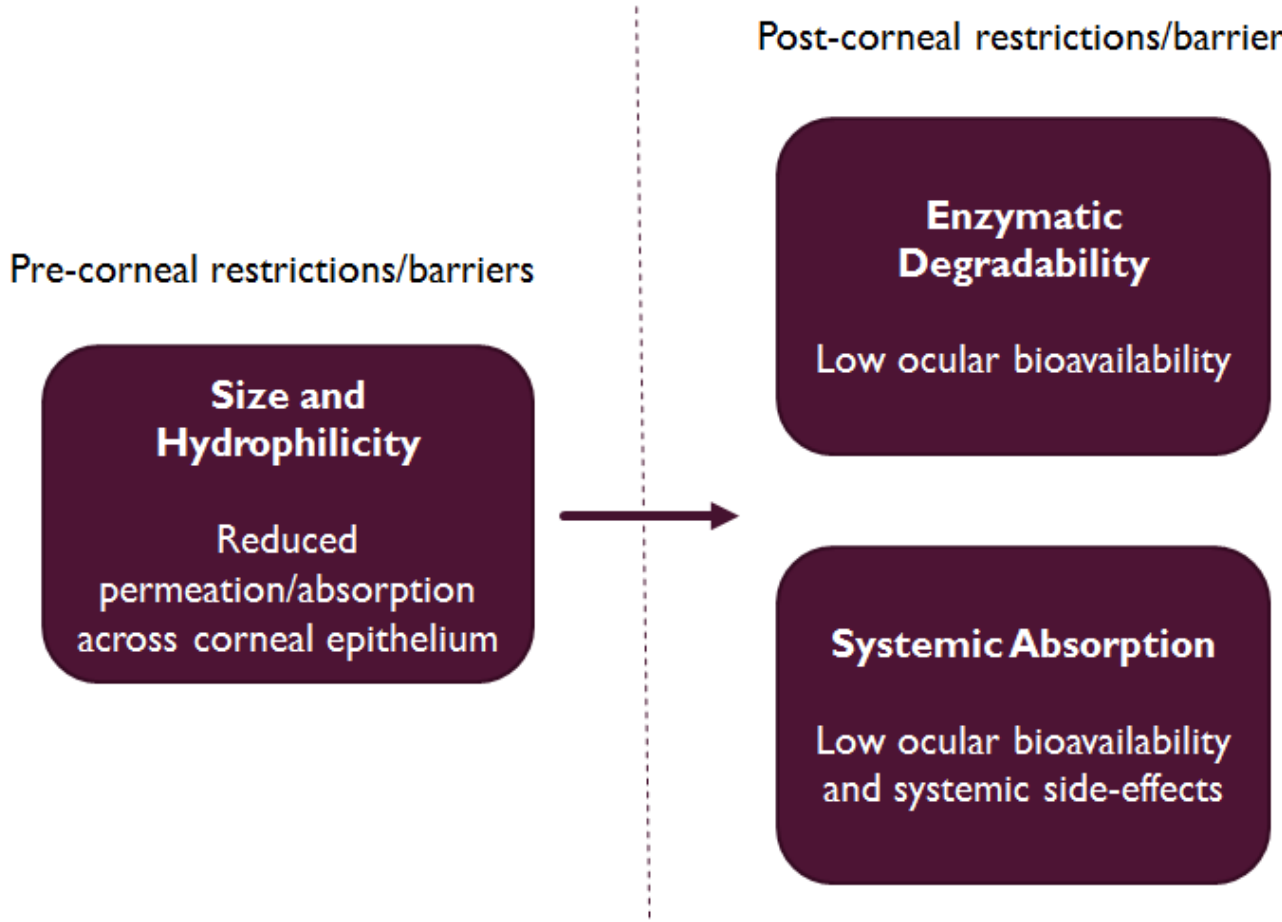
- Basal layer, 2-3 layers of wing cells and 1-2 outermost layers of squamous cells
- Outermost layers
  - Intercellular tight junctions surround the most superficial layers and restrict passage of peptides and proteins
  - Absorption relies on transcellular passage or strategies that can modulate the tight junctions
- Wing cells and basal cells
  - Intercellular spaces are wider and permit paracellular diffusion
- Negatively charged corneal epithelium offers greater resistance to negatively charged compounds as compared to positively charged ones



CHALLENGES TO OCULAR DELIVERY OF PROTEINS/PEPTIDES

- **Barriers for locally delivered drugs**
  - Loss of drug from ocular surface
  - Lacrimal-fluid barrier
  - Blood-ocular barrier

- Low drug contact time
- Tear production and turnover
- Consequent dilution

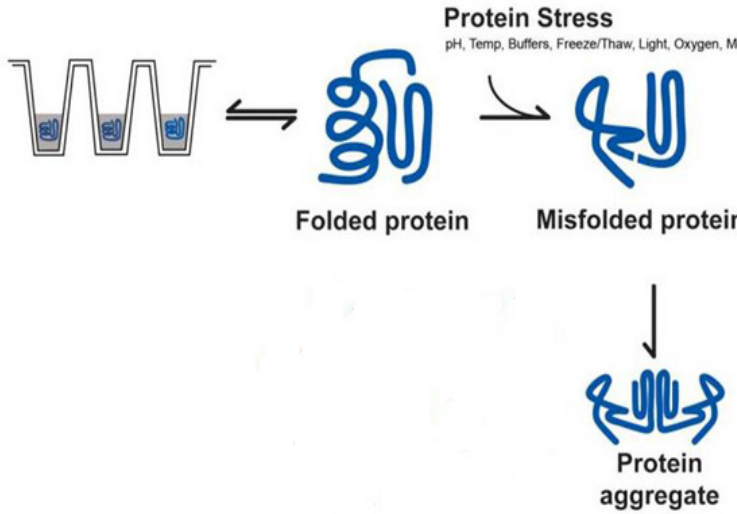


Schematic presentation of the different barriers for ocular delive
of proteins and peptides

FORMULATION CONSIDERATIONS

Aggregation

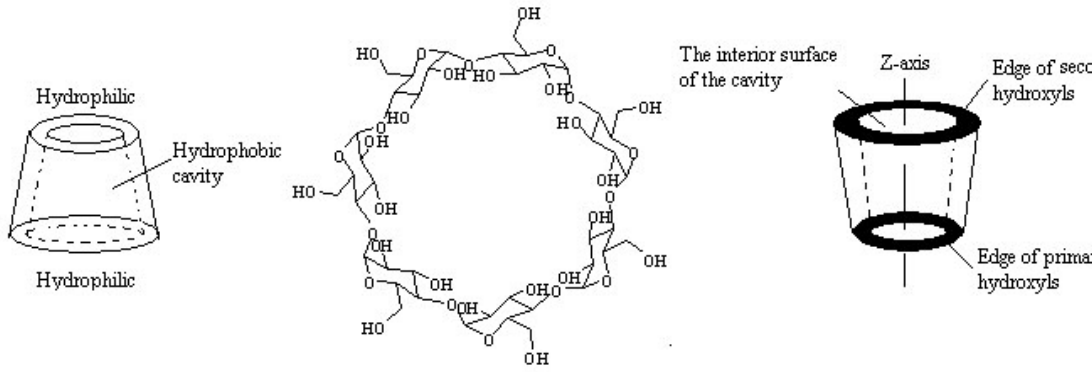
- Is induced by shaking, prolonged storage, heating, freezing, lyophilization
- Can lead to
  - Reduced bioactivity
  - Immunogenic reactions
  - Blockage of tubing, membranes or pumps in an infusion set
  - Unacceptable physical appearance such as opalescence
- Example:
  - Insulin can undergo self-association/aggregation due to the hydrophobic regions of the molecule
  - Human epidermal growth factor (hEGF) undergoes pH and concentration dependent aggregation
- Can be prevented by
  - Use of appropriate formulation excipients; example: mannitol, trehalose
  - Proper care in processing of formulation
  - Synthesizing a resistant derivative



FORMULATION CONSIDERATIONS

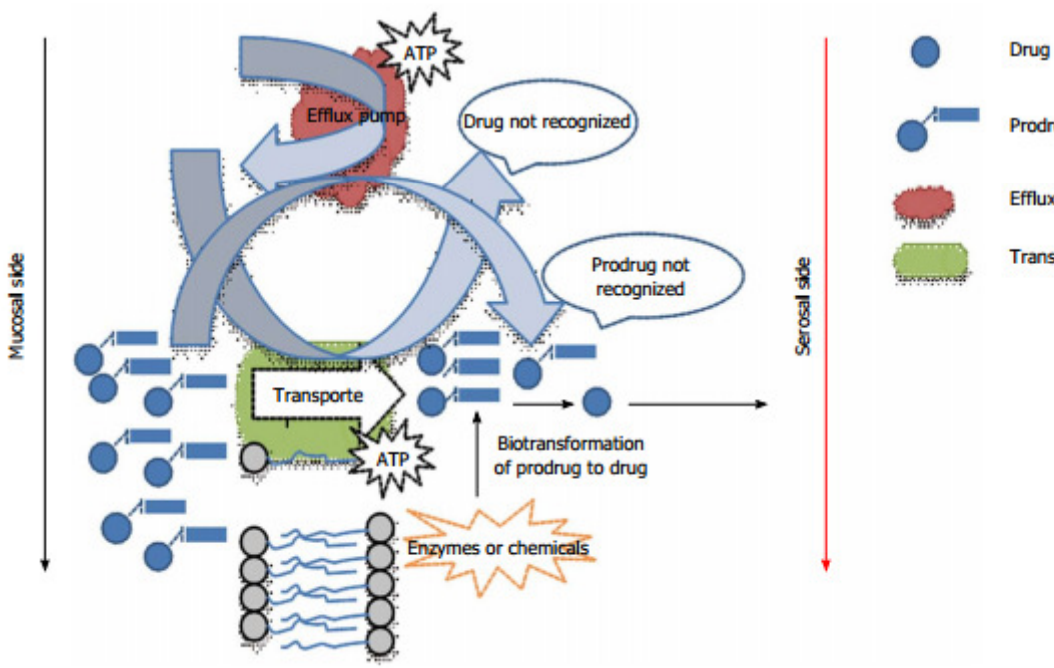
Formulation Additives

- **Protease Inhibitors:**
  - Used if the protein/peptide is likely to degrade upon ocular administration
  - Aminopeptidase inhibitors: bestatin, amastatin, puromycin, p-chloromercuribenzoate
- **Sugars:** Exert a protective effect on proteins by changing the solvent structure around the protein
- **Cyclodextrins:** Act by molecular encapsulation of amino acid chains thereby preventing hydrophobic interactions

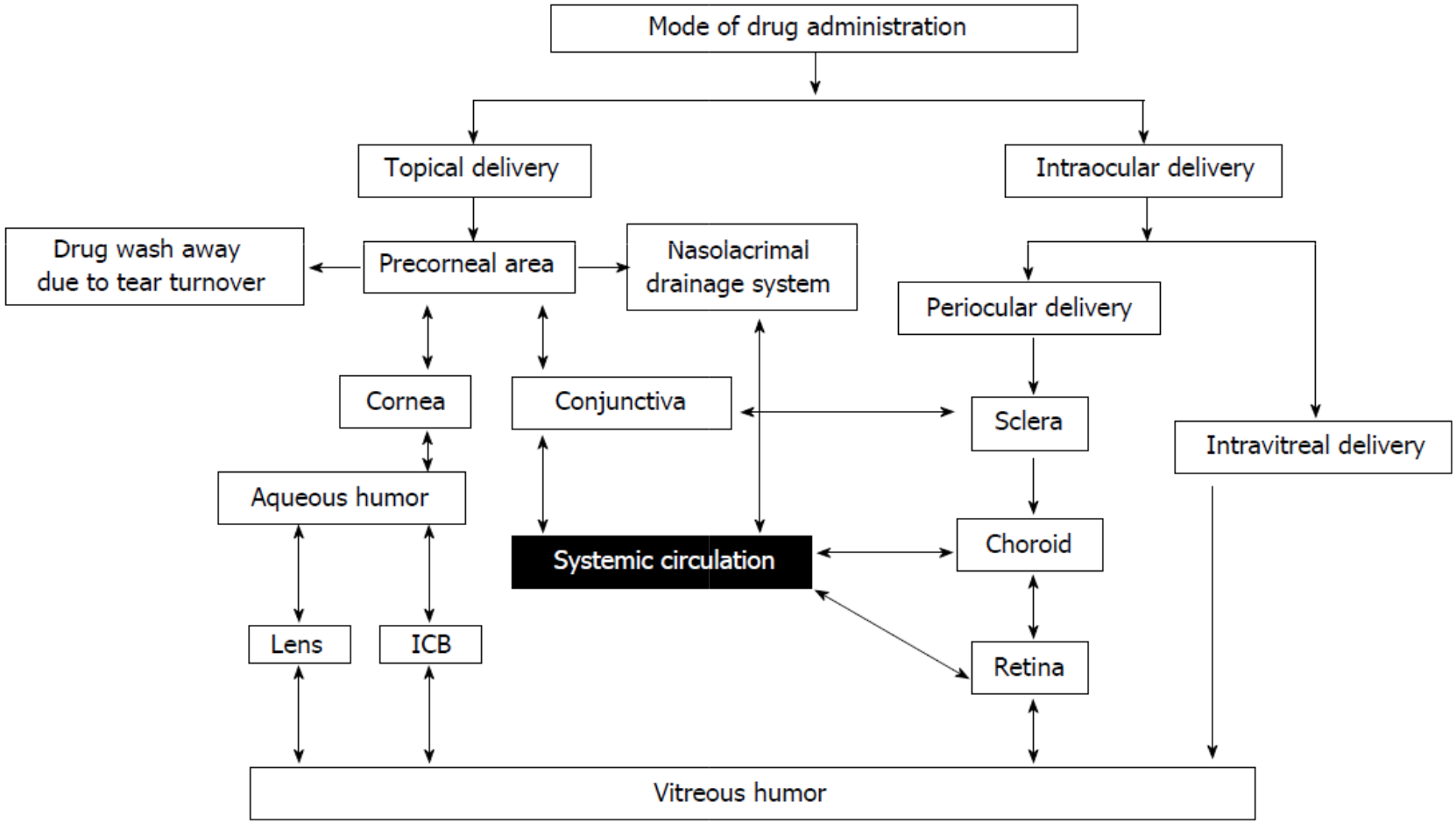


PEPTIDE TRANSPORT SYSTEMS IN EYE

- Epithelial cells express nutrient transporters and receptors on their surface which help the movement of vitamins and amino acids across cell membranes
- Proton coupled receptors help translocation of di- and tripeptides across the epithelium
- Transporters are classified as PepT1, PepT2 and peptide/histidine transporters (PHT1 and PHT2)
- Expression of PHT1 in bovine and human retinal pigment epithelial cells (BRPE and HRPE), ARPE-19 cells (human RPE cell type), bovine and human neural retina cells has been reported
- PepT2 and PHT 2 expression reported in bovine and human retina
- Drugs with poor ocular bioavailability can be suitably modified by design to facilitate recognition and uptake by peptide transporters



MODES OF OCULAR DRUG ADMINISTRATION



OCULAR ADMINISTRATION FOR TOPICAL DELIVERY

- Topical delivery is considered to be the best option for treatment of most ocular disorders
- Several peptides have been identified for treatment of ocular disorders like dry eye disease, age related macular degeneration, proliferative diabetic retinopathy, etc.
- Loss to systemic circulation must be minimized
  - Phenylephrine used as a vasoconstrictor to minimize systemic absorption
  - Use of mucoadhesive polymer to improve ocular absorption
- Adverse physicochemical properties or enzymatic degradation of peptides might render them less effective
  - Loading them in a carrier system like liposome or nanoparticle may limit some of these problems

OCULAR ADMINISTRATION FOR TOPICAL DELIVERY

Growth Factors

- Human Epidermal Growth Factor (hEGF) stimulates cell proliferation in the corneal epithelium thus causing epithelialization dur wound healing
- EGF can be produced biotechnologically in a commercially feasible manner
- It can thus be a suitable therapeutic agent for corneal trauma and during intraocular surgery

Tissue Plasminogen Activator

- tPA can be used to achieve clot lysis after surgery for cataract and/or glaucoma
- Since tPA is present in aqueous humor and other ocular tissues, its use is like a supplementation of body function

Cyclosporin A

- It has immunosuppressive, anti-fungal and anti-inflammatory activity
- Primary use is inhibition of kidney graft rejection
- Instillation in eye can inhibit rejection of corneal grafts

STUDIES SHOWING OCULAR DELIVERY OF EGF

F Incorporated in Cationized Gelatin Hydrogel

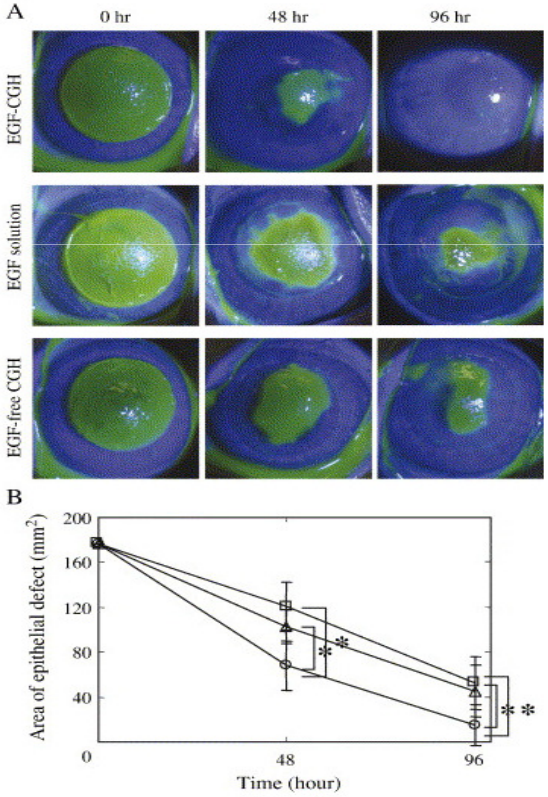


Fig. 5.

Enhanced wound healing in a rabbit corneal epithelial defect model. A. Fluorescein slit lamp micrograph of a representative corneal defect obtained 0, 48, and 96 h after the application of a CGH film with incorporated EGF (upper), EGF solution (middle), and an EGF-free CGH film (lower). B. Time-course of the closure of a corneal epithelial defect after the application of a CGH film with incorporated EGF (○), EGF solution (△), and an EGF-free CGH film (□). $^{*}p < 0.05$, significantly different from the control at the corresponding time point (Tukey–Kramer post-test).

EGF Incorporated in Beta Cyclodextrin Complex

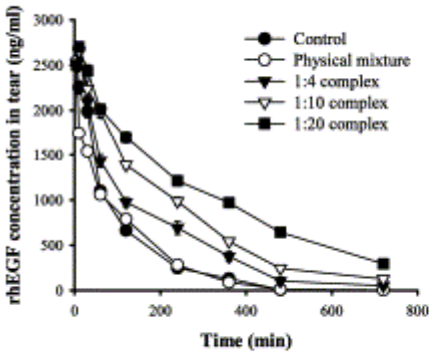


Fig. 6.

rhEGF concentrations in tears after ocular administration of poloxamer gels. The poloxamer gel was composed of P407/P188 (16/14) and rhEGF (0.5%) or rhEGF/HP-β-CD complex (0.5%). Each point represents the mean±S.E. (n=3).

Controlled-release of epidermal growth factor from cationized gelatin hydrogel enhances corneal epithelial wo healing, Hori K, Sotozono C, Hamuro J, Yamasaki K, Kimura Y, Ozeki M, Tabata Y, Kinoshita S, J Control Rele Apr 2;118(2):169-76.

rhEGF/HP-beta-CD complex in poloxamer gel for ophthalmic delivery, Kim EY, Gao ZG, Park JS, Li H, Ha Pharm. 2002 Feb 21;233(1-2):159-67.

OCULAR DELIVERY OF GANCICLOVIR

In vitro transcorneal permeation

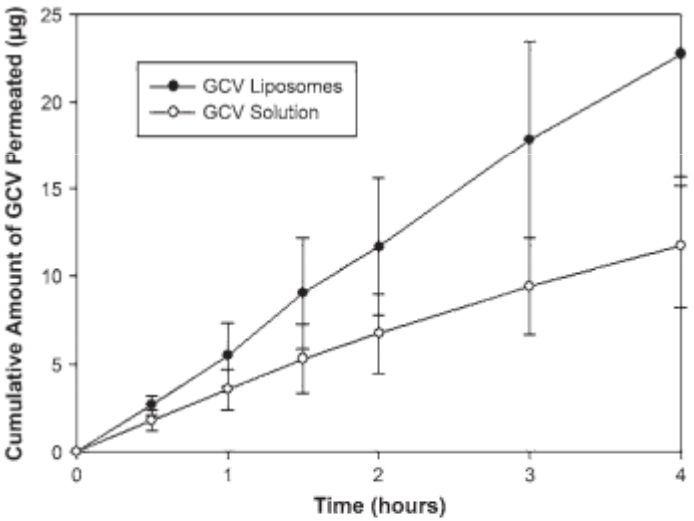


Figure 2. In vitro transcorneal permeation of GCV liposome preparation and solution ($\overline{X} \pm SD$, n = 5). GCV indicates ganciclovir.

Concentration in aqueous humor after instillation in rabbit eye

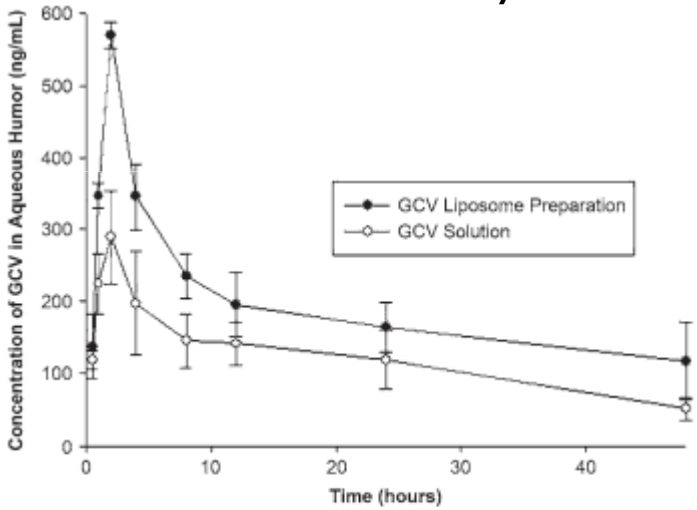


Figure 4. Concentration-time profiles of GCV in aqueous humor after instillation of 1.0 mg/mL GCV liposome preparation and GCV solution in rabbit (ng/mL, $\overline{X} \pm SD$, n = 5). GCV indicates ganciclovir.

ion and ocular pharmacokinetics of ganciclovir liposomes, Yan Shen, Jiasheng Tu, AAPS J. Sep 2007; 9(3): E371–E377.

OCULAR ADMINISTRATION FOR SYSTEMIC DELIVERY

- Occurs because of contact of instilled solution with conjunctival and nasal mucosae
- **Advantages:**
  - Relative ease and low cost of formulating and administering eye drops (compared to injections)
  - Relative insensitivity of eye towards immunological reactions (compared to lung and gut)
  - Absence of first pass metabolism
- **Challenges:**
  - Reproducible delivery
  - Low bioavailability

OCULAR ADMINISTRATION FOR SYSTEMIC DELIVERY

sulin:

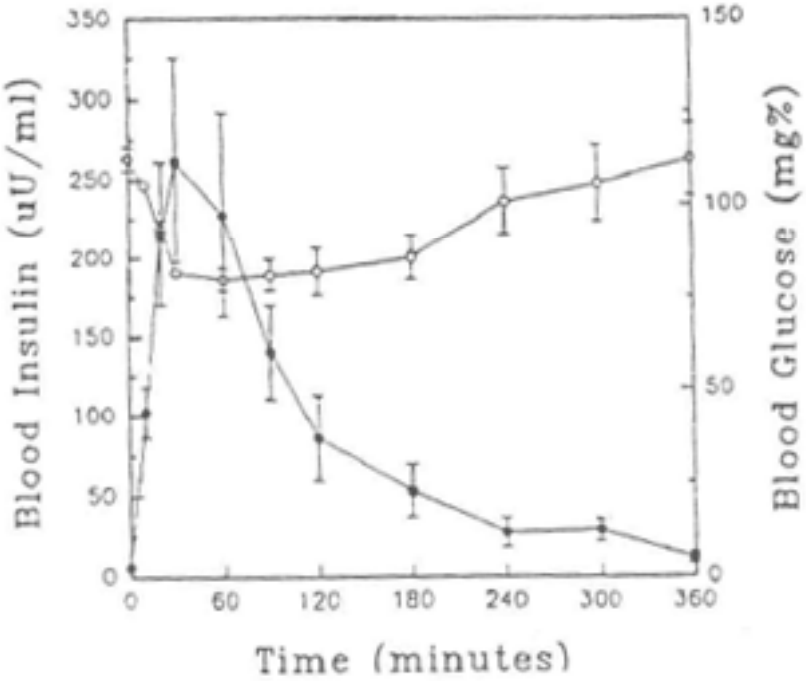
When administered to the eye, a sustained lowering of blood glucose was observed

Use of absorption enhancers may often be required to enhance absorption of peptides through the eye

Absorption enhancers must be safe and non-irritating to the eye

Order of efficacy: Saponin>Fusidic Acid>BL-9 = EDTA>Glycocholate>Decamethonium=Tween 20

Aminopeptidase inhibitors or peptide analogs that are resistant to enzymes also help to improve bioavailability



Systemic absorption of insulin (± SEM; n=5) following the ocular instillation of a 0.25% insulin solution containing Brij-78 as an enh Data generated following a b.i.d. administration of eyedrops over three-month period (• - blood insulin concentration; o – blood g levels)

EFFECT OF ABSORPTION ENHANCER (BRIJ 78) ON SYSTEMIC DELIVERY OF INSULIN FROM AN OCULAR INSERT DEVICE

Table 1—Summary of the Efficacy of 0.5-, 1-, and 2-mg Insulin Ocular Delivery Systems.

Formulation	Insulin (mg)	Brij-78 (μg)	Area Above the Curve (% h) mean ± SD	Duration of BGC <80% of Initial (h) mean ± SD
Eyedrop 1	0.5	20	54 ± 12	0.5 ± 0.1
Device 1	0.5	0	59 ± 59	0
Device 2	0.5	10	65 ± 83	0
Device 3	0.5	20	405 ± 25[a]	6.7 ± 0.8[a]
Device 4	0.5	30	425 ± 53	9.2 ± 1.9
Device 5	0.5	50	Hypoglycemia	
Eyedrop 2	1	20	81 ± 10[b]	0.9 ± 0.3[b]
Device 6	1	0	218 ± 92	0
Device 7	1	10	162 ± 90	0
Device 8	1	20	552 ± 93[a]	10.2 ± 0.4[a,c]
Device 9	1	30	Hypoglycemia	
Device 10	1	50	Hypoglycemia	
Device 11	2	0	182 ± 109	0
Device 12	2	10	174 ± 139	3.8
Device 13	2	20	Hypoglycemia	

[a] Significantly different from the corresponding eyedrop formulations ($p < 0.05$). [b] Significantly different from eyedrop 1 ($p < 0.05$). [c] Significantly different from device 3 ($p < 0.05$).

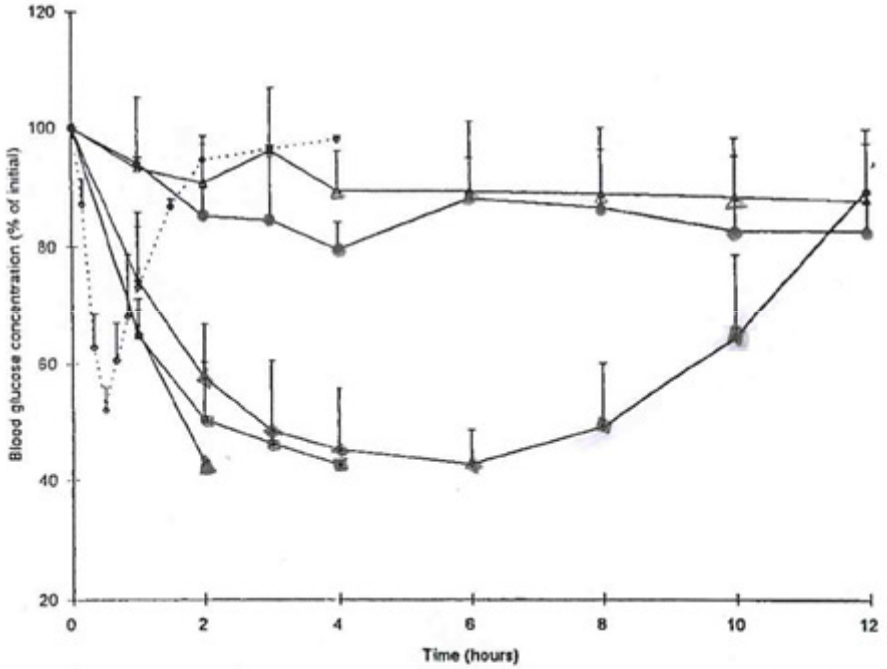


Figure 2—Mean blood glucose concentrations after ocular administration of 1-mg insulin delivery systems: eyedrop 2 (◇), device with no Brij-78 (△), device with 10 μg of Brij-78 (●), device with 20 μg of Brij-78 (◆), device with 30 μg of Brij-78 (■), and device with 50 μg of Brij-78 (▲). Each value represents the average ± SD of three rabbits, except the last formulation, that was carried out with two rabbits.

ect of Brij-78 on systemic delivery of insulin from an ocular device, Lee YC, Simamora P, Yalkowsky SH, J Pharm Sci. 1997 Apr;86(4):430-3.

OCULAR ADMINISTRATION FOR SYSTEMIC DELIVERY

- **Glucagon**
  - Used in treatment of hypoglycemia
  - Can be delivered by the ocular route and has been reported to increase blood glucose
  - Mol wt. is lower than insulin; may not need absorption enhancers
- **Calcitonin**
  - Long term administration required for treatment of hypercalcemia
  - Besides the ocular route, other alternative routes like nasal, rectal, transdermal have also been explored

TRANS-SCLERAL DELIVERY OF IgG TO THE RETINA

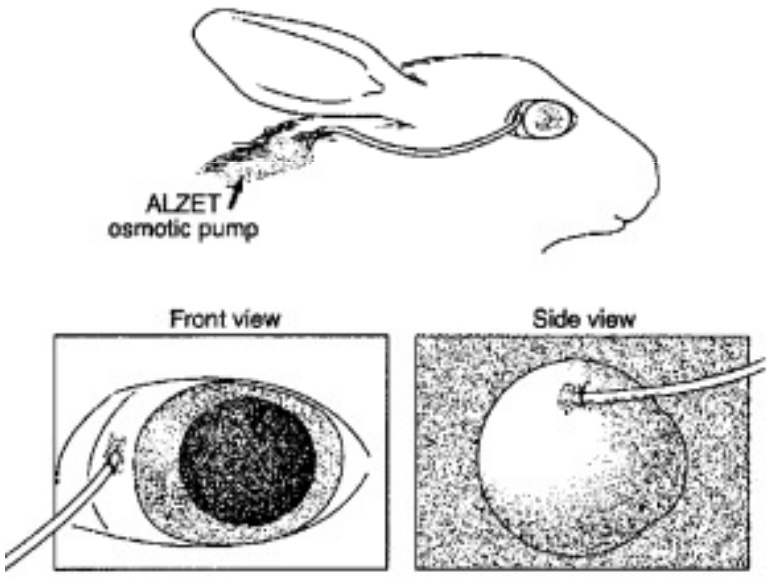


FIGURE 1. Schematic of osmotic pump placement.

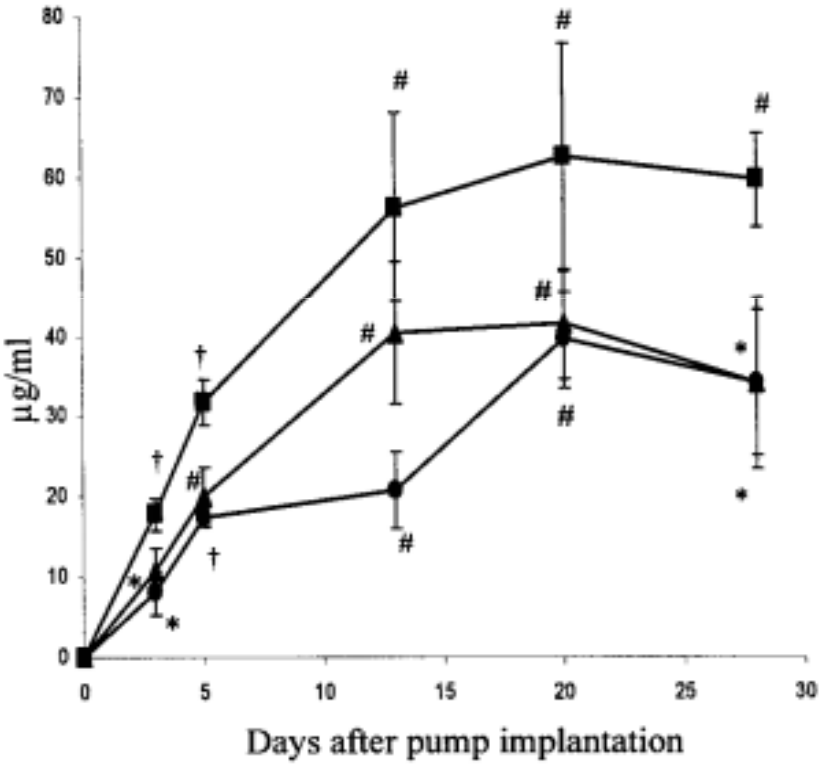


FIGURE 2. Concentration of FITC–IgG (1 mg/ml delivered at 2.5 μl/h) in the choroid (proximal hemisphere [■] and distal hemisphere [▲]) and the retina (●). *$P < 0.01$, #$P < 0.005$, †$P < 0.001$ versus day 0. $n = 4$ for all times.

Delivery of Bioactive Protein to the Choroid and Retina, Ambati J, Gragoudas ES, Miller JW, You TT, Miyamoto K, Delori FC, Adamis AP, Invest Ophthalmol Vis Sci. 2000 Apr;41

STRATEGIES FOR THE OCULAR DELIVERY OF PROTEINS/PEPTIDES

rugs

ange physicochemical properties of a drug to improve rmeation across cornea and enhance bioavailability

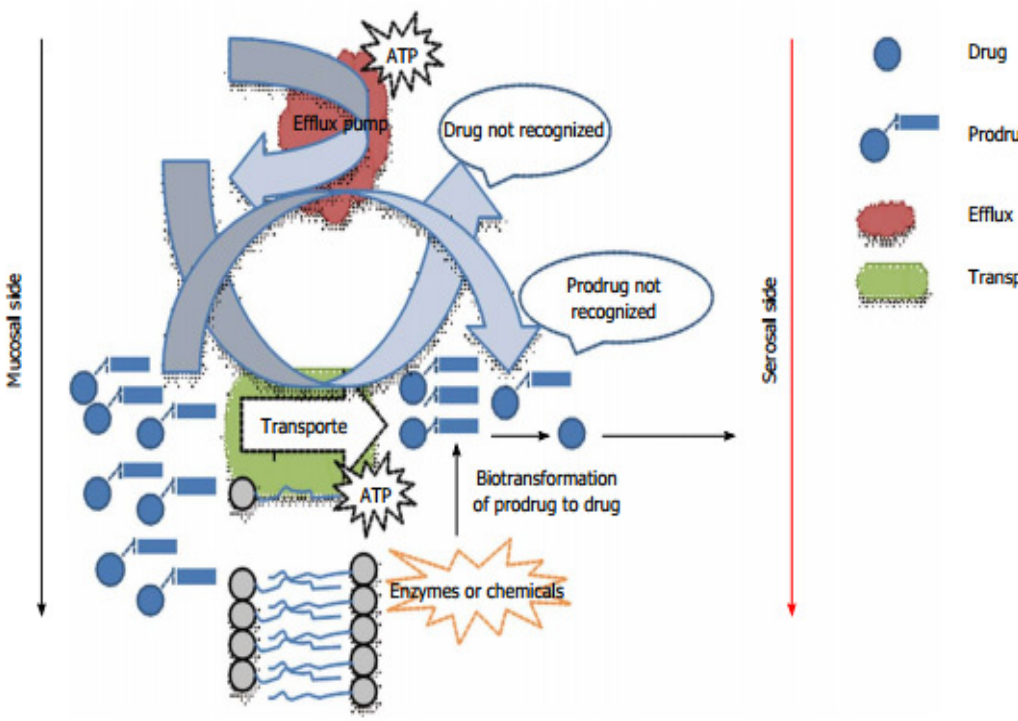
st prodrug for ocular delivery: Dipivefrin, prodrug of epinephrine ed to treat glaucoma

esirable properties

- Good stability
- High enzyme lability

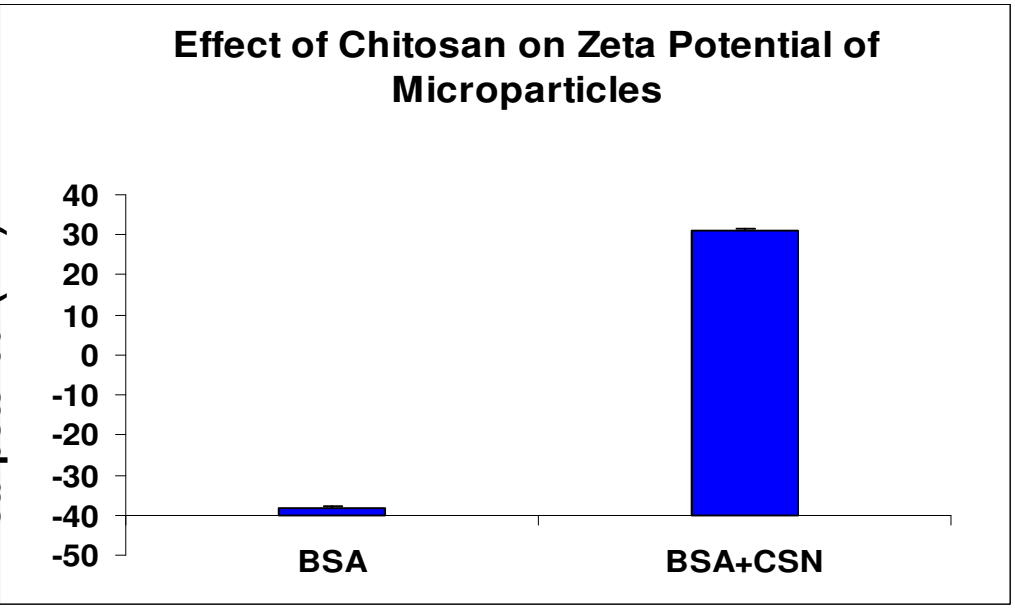
ost common barriers that can be overcome are

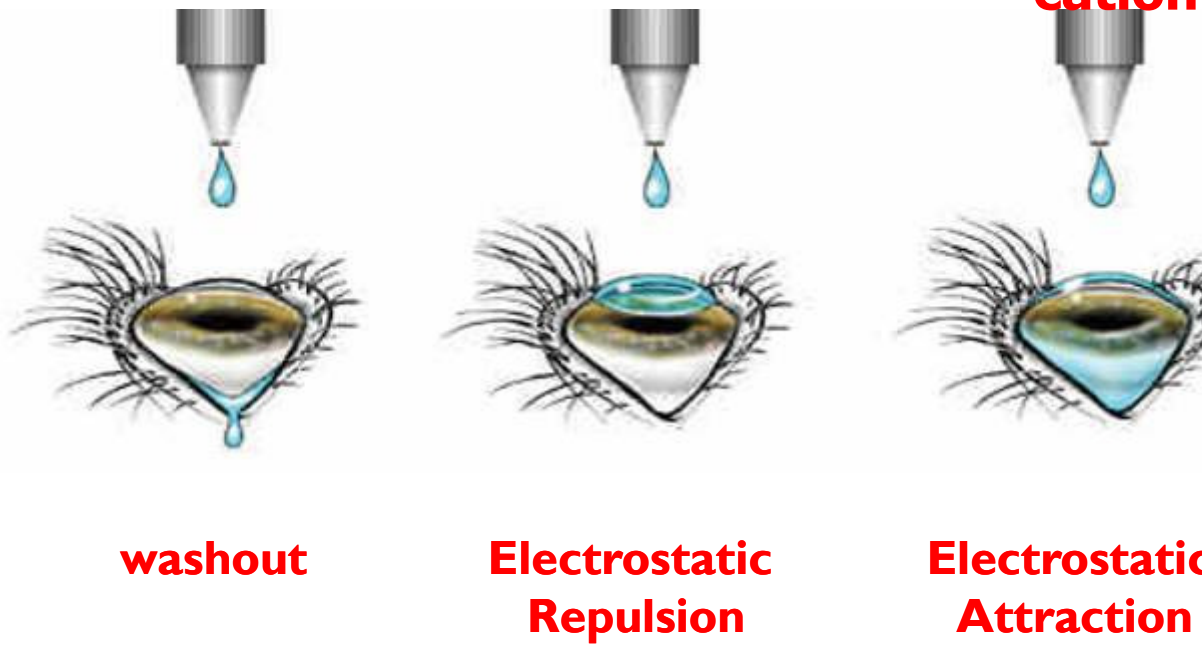
- A low aqueous solubility, which prevents the development of aqueous eyedrops
- A low lipid solubility, which results in low corneal permeation and low ophthalmic bioavailability
- A short duration of action due to rapid drug elimination from site of action
- Systemic side-effects, due to low corneal and high systemic absorption



STRATEGIES FOR THE OCULAR DELIVERY OF PROTEINS/PEPTIDES

- **Mucoadhesive Particulate Carriers**
  - Cornea and conjunctiva have a net negative charge
  - Cationic polymers help to increase the concentration and residence time of polymer-associated drug
  - Chitosan – biocompatible, biodegradable, enhances the paracellular transport of drugs

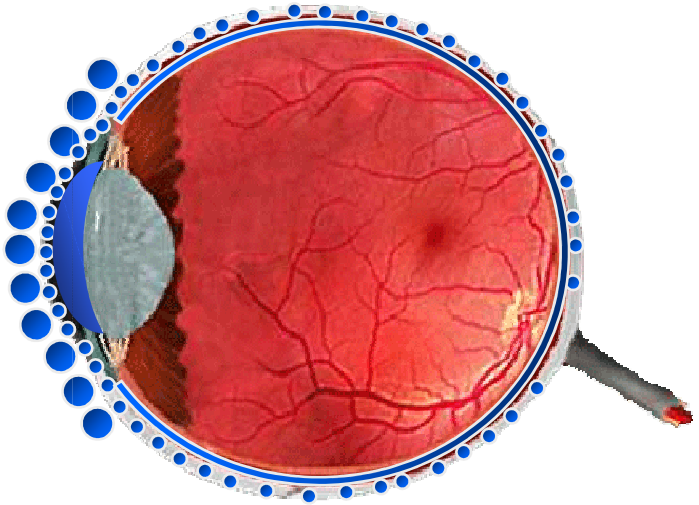




DELIVERY MECHANISM OF CATIONIC NANOPARTICLES

Electrostatic interaction leading to

- Retention at the surface
- Reservoir effect in :
  - Cornea
  - Conjunctiva
- Transcorneal Route
- Diffusion via the scleral route
  - Sustained release to the retina



CHITOSAN NANOPARTICLES FOR CYCLOSPORIN A DELIVERY

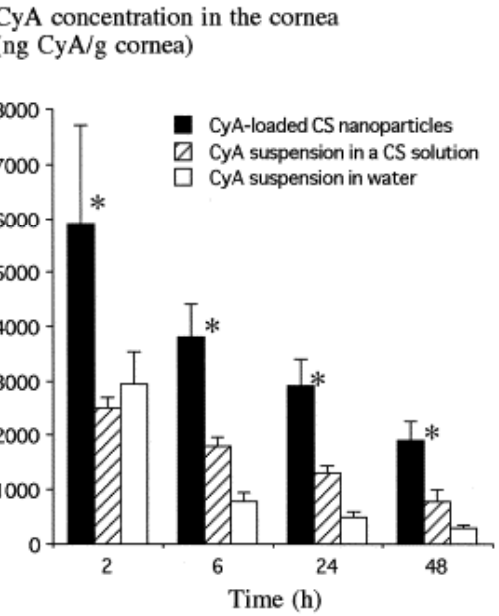


Fig. 3.

CyA concentration in the cornea after topical administration in rabbits of CyA-loaded CS nanoparticles and control formulations consisting of a CyA suspension in a CS aqueous solution and a CyA suspension in water (* denotes statistically significant differences, $P<0.05$).

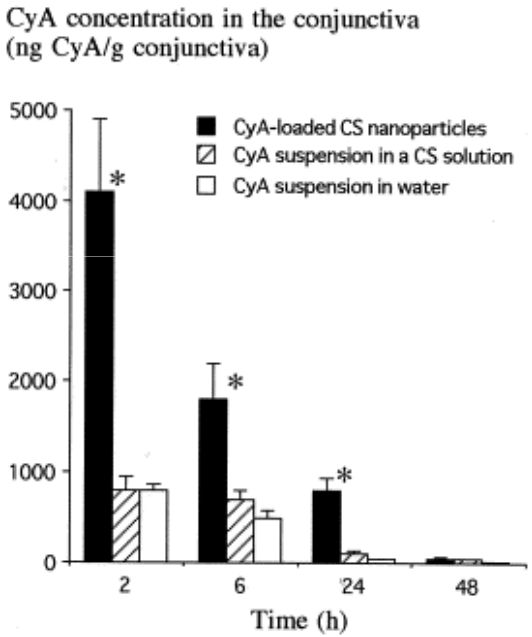


Fig. 4.

CyA concentration in the conjunctiva after topical administration in rabbits of CyA-loaded CS nanoparticles and control formulations consisting of a CyA suspension in a CS aqueous solution and a CyA suspension in water (* denotes statistically significant differences, $P<0.05$).

Chitosan nanoparticles: a new vehicle for the improvement of the delivery of drugs to the ocular surface. Application to cyclosporin A, De Campos AM, Sánchez A, Alonso MJ. Int J Pharm. 2001 Aug 14;224(1-2):159-68

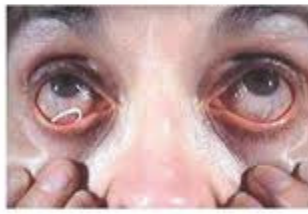
STRATEGIES FOR THE OCULAR DELIVERY OF PROTEINS/PEPTIDES

ydrogel Delivery Systems

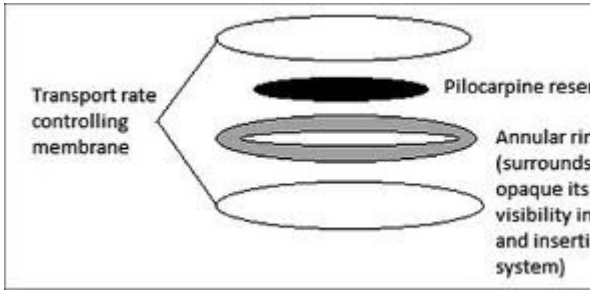
Allows slow release of drug from a hydrogel inserted beneath the eyelid

Ocusert: First such device

- Non-erodible ocular insert
- Pilocarpine alginate core sandwiched between two transparent, rate controlling membranes



Photograph of patient with Ocusert (piloca
place in lower cul-de-sac of right ey



PLGA MICROSPHERES FOR DELIVERY OF VANCOMYCIN

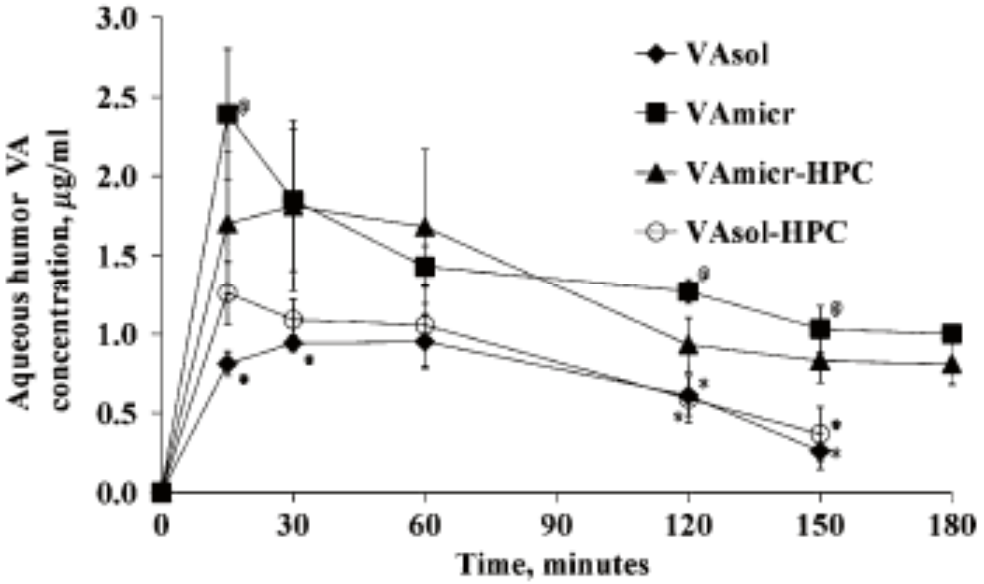


Fig. 4. VA concentration profiles in the aqueous humor of rabbits after administration of VA_{micr} and VA_{micr}–HPC microsphere suspensions and of the reference solutions (VA_{sol} and VA_{sol}–HPC) (Mean ± SE, $n = 6$; *significantly different from the VA_{micr} and the VA_{micr}–HPC formulations, $P < 0.05$, #significantly different from the VA_{micr}–HPC formulation, $P < 0.05$).

Table 2
Pharmacokinetic parameters in aqueous humor after in vivo administration in rabbits of the preparations under study

Preparation	C_{max} (μg/ml ± SE)	T_{max} (min)	AUC (min μg/ml ± SE)	AUC relative
VA_{micr}	2.47 ± 0.49	15	248.2 ± 35.2	2.31
VA_{micr}–HPC	1.80 ± 0.54	30	206.6 ± 52.8	1.92
VA_{sol}	0.94 ± 0.05	30	107.4 ± 17.3	1.00
VA_{sol}–HPC	1.26 ± 0.21	15	122.9 ± 26.7	1.14

spheres for the ocular delivery of a peptide drug, vancomycin using emulsification/spray-drying as the preparation method: in vitro/in vivo studies, Gavini E, Chetoni P, Cossu M, Alvarez MG, Saettone MF, Giunchedi P, Eur J Pharm Biopharm. 2004 Mar;57(2):20

STRATEGIES FOR THE OCULAR DELIVERY OF PROTEINS/PEPTIDES

- **Absorption Enhancers**
  - Promote penetration of drugs through corneal barrier by changing integrity of epithelial cell layer
  - Examples: EDTA, sodium glycocholate and related cholates, tween-20, saponin
- **Miscellaneous Approaches**
  - **Cell penetrating peptides:** TAT (Trans-activating transcription factor from human immunodeficiency virus) exhibit efficient penetration to the retina after topical delivery
  - **Intravitreal injections**
    - Can cause several complications like hemorrhage and retinal displacement
    - Bevacizumab (Avastin): Used for the treatment of ocular vascularization

TABLE 5.1 List of disorders/indications where therapeutic peptides could be delivered through ocular route

Disorder/Indication	Therapeutic peptide
Antiallergic, antiinflammatory	ACTH
Analgesic	β Endorphin, Leu-enkephalin
Antiscarring agent in glaucoma filtration surgery	Integrin-binding peptide
Attenuate miotic response	Somatostatin
Choroidal or retinal neovascularization	Octreotide, Urokinase derived peptide, Cyclic integrin-binding peptide
Corneal epithelial wound	Insulin-like growth factor derived peptide Substance P derived peptide
Diabetes mellitus	Insulin
Diabetes insipidus	Vasopressin
Diagnosis of thyroid cancer	TSH
Dry eye disease	Cyclosporine A
Hypoglycemic crisis	Glucagon
Immunostimulant	Met-enkephalin
Induction of uterine contractions	Oxytocin
Induction of vitreous detachment in vitretomy	Integrin-binding peptide
Paget's disease	Calcitonin
Secretion of insulin	VIP
Uveal melanoma and retinal blastoma	Apoptosis inducing peptide

TABLE 5.2 Reported literature related to ocular delivery of proteins and peptides

Protein/ peptide	Delivery strategies	Concluding remarks
Insulin	Penetration enhancer	The insulin bioavailability was 5.7 to 12.6% with polyoxyethylene-9-lauryl ether, 4.9 to 7.9% with GC, 3.6 to 7.8% with Na taurocholate and 8.2 to 8.3% with Na deoxycholate, as compared to 0.7 to 1.3% in the absence of absorption promoters.
Cyclosporine A	Azone penetration enhancer	Cyclosporine-treated grafts contained significantly fewer infiltrating T-lymphocytes than did the drug/solvent-treated allografts, indicating that the topical application of cyclosporine actively inhibited the entry of T-cells into the grafts.
IgG protein	Transscleral delivery	IgG protein delivered to the retina and choroid in an optimum concentration for the treatment of chorio-retinal disorders with negligible systemic absorption.
Vancomycin (peptide)	PLGA microparticles	PLGA microparticles loaded with peptide drug showed high and prolonged concentration of vancomycin and increased level of AUC (2-fold) as compared to aqueous solutions.
Ganciclovir (GCV)	Prodrug	Glycine-valine-GCV is the effective and lead candidate for the treatment of Human Cytomegalovirus (HCMV).
Vasoactive intestinal peptide (VIP)	Liposome	Treatment of ocular inflammation by modulation of macrophage and T-cell activation of the immune system.
VIP	Liposomes	For the treatment of endotoxin induced uveitis (EIU), liposomal delivery increased VIP efficiency and bioavailability.
Ganciclovir (GCV)	Prodrug	Diester GCV prodrugs demonstrated excellent chemical stability, high aqueous solubility and markedly enhanced antiviral potency against the herpes viruses without any increase in cytotoxicity.

Adapted from Peptide and Protein Delivery by Chris Van Der Walle