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BIOMATERIALS FOR DRY EYE SYNDROME
Master of Science Thesis

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Kuivasilmäisyys on sairaus, jossa silmän sarveiskalvon pinnalla oleva kyynelkalvo rikkoutuu. Sarveis- ja sidekalvon kosteutus ja voitelu häiriintyvät kyynelkalvo epätasapainosta. Kuivasilmäisyys on ikääntyvien ihmisten sairaus, mutta sitä tavataan myös sarveiskalvovaurio- ja reumapotilailla. Joidenkin lääkkeiden käyttöön liittyy sivuoireina kuivasilmäisyyttä. Kuivasilmäisyyteen on saatavilla kaupallisia oireita lievittäviä silmätippoja, jotka kosteuttavat ja voitelevat silmän pintaa, mutta eivät hoida sairautta. Kuivan silmän tipat on valmistettu luonnon polymeereistä tai synteettisistä polymeereistä, joita annostellaan vähintään kahdesti päivässä. Terapeuttisen vaikutuksen kesto perustuu viskositeettiin tai tarttumiseen silmän pintaan heikoilla sidoksilla. *In situ* -geeliytyminen lämpötilan, pH:n tai suolapitoisuuden muutoksesta on ollut tutkijoiden mielenkiinnon kohteena viime vuosina. Myös polymeerien tarttumista limakalvon pintaan (mukoadheesio) heikoilla vuorovaikutuksilla tai vahvemmilla kovalenttisilla sidoksilla on tutkittu. Amfifiliset polymeerit ovat myös vallanneet alaa, sillä niiden oletetaan sopivan kyynelkalvon pinnan öljykerrokseen ja edelleen sen alla olevaan vesikerrokseen. Amfifilisella polymeerillä on myös kyky kantaa hydrofobista lääkemolekyyliä. Polymeerien tiolointiin ja sen tutkimiseen on panostettu viime aikoina paljon. Tiolointi on menetelmä, jolla parannetaan polymeerin mukoadheesiota. Erityistä mielenkiintoa on herättänyt kitosaanin ja hyaluronihapon tiolointi, jolloin myös terapeuttinen molekyyli, kuten trehaloosi tai albumiini voitaisiin ladata polymeerimatriisiin. Tässä kirjallisuusselvityksessä käsitellään silmän anatomiaa erityisesti kyynelkalvon, sarveiskalvon ja sidekalvon osalta. Tässä opinnäytetyössä on käsitelty kuivasilmäisyyteen johtavia syitä ja nykyisiä sekä kehityksen kohteena olevia hoitomenetelmiä. Lopuksi esitellään sarveiskalvon simulointimenetelmiä, joita on käytetty laboratorio-olosuhteissa.

ABSTRACT

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Dry eye syndrome (DES) is disease of the eye surface, where tear film is ruptured and lubrication or moistness of the corneal and conjunctival epithelia do not work properly. DES is most common in older people, but also people with rheumatoid diseases, damaged corneal epithelia and certain medications are more likely to have DES. There are lubricating and moisturizing eye drops to relieve the symptoms of DES, administered at least twice a day depending on the severity, but they are only palliative. Eye drops for DES are composed of natural and synthetic origin polymers and their blends. Retaining methods are mainly viscosity and adherence to surfaces by weak interactions. *In situ* gelation by temperature, pH and osmotic change have been under research in recent years. Many studies have concentrated on ambiphilic polymer conjugates, which fit to the oily and aqueous layer of the tear film and have a better ability to carry a hydrophobic therapeutic drug molecule. Interest has also been pointed to mucoadhesive polymers, which are adhering to mucin by covalent bond. Thiolation of the polymer has been seen as a very promising method to achieve better muchoadhesion and thus has been extensively studied. A chitosan and hyaluronan are natural origin polymers, which have been thiolated and these polymer matrixes can be loaded with trehalose or albumin as therapeutic agent. In this thesis the anatomy of the eye, and especially the corneal and precorneal area and the conjunctiva is discussed. Ethiology of DES and commercial available therapies are covered in this thesis, but also therapies under research and corneal simulation methods *in vitro*.

Foreword

My educational career started in 1985 after graduation from the secondary school. After that I qualified as a laboratory worker in Helsinki in 1987. After a couple of working years as laboratory technician I started further studies in a technical college in Turku and I qualified as Bachelor of Science in Food Engineering in 1993. Working life, family and motherhood took after graduation over ten years and enthusiasm to further education evolved in 2005. For the first two years (2005-2007) I was a full-time student, but the rest of the studies I have done along my full-time job. This literature survey was carried out during 2008 - 2011, while I was at Santen Oy as a full time hygienist at the microbiological research and quality assurance laboratory. Therefore this thesis was done mainly at my own free time although my employer had shown interest for this subject. This two years writing of the thesis have been challenging especially because of timetable adjustments, but little by little the writing proceeded.

Co-operation of the Tampere University of Technology and Santen Oy enabled me to write this interesting Master of Science thesis. I am grateful to Professor Minna Kellomäki for advice and reviewing my thesis. Scientific Affairs Director Olli Oksala and the Information Assistant Anne Tuhkanen from Santen Oy are worthy of my thanks. I am grateful to Linguistic Support Specialist Sari Mättö, who read my writings and helped me with the grammar. I also thank my colleagues Pirjo Sainio and Katja Niemelä for encouraging and supporting me during these laborious years. The greatest thanks I want to give to my dear husband Veli and my lovely sons Eetu and Leevi for their understanding and support. I hope that I can make up for the missed family time somehow and focus on our shared hobby, orientating, in the future.

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Glossary

blepharitis	inflammation of the eyelid
bradytrophic	receives nutrients by diffusion or by capillaries
catifloxacin	antibacterial fluoroquinolone (drug information 2009)
cevimeline	muscarinic agonist used in treatment of Sjögren's syndrome
conjunctivitis	inflammation of the conjunctiva
filopodia	as lamellipodia, but forms membrane prostrusion (Alberts et al. 2003)
hanks solution	artificial tear flow solution used in eye simulations
hemidesmosome	junctions that binds epithelial cell to the basal lamina (Alberts et al. 2003)
hypoesthesia	impaired sense of touch
hyperosmolality	osmotic pressure is higher than blood plasma
hypoosmolality	osmotic pressure is lower than blood plasma, synonym to hypotonic
keratinisation	become impregnated with keratin
keratoconjunctivitis sicca	dryness of the cornea and conjunctiva, dry eye syndrome
lamellipodia	dence meschwork of actin filaments oriented to close to plasma membrane (Alberts et al. 2003)
lectin	proteins that are specialized to recognize particular oligosaccharide side chains (Alberts et al. 2003)
mucin	mucoproteins, occurring in saliva, tears and produce a very viscous solution in water
mucus	slimy protective secretion of mucous membrane, consisting mainly of mucin, salts, cells and water
neovascularisation	formation of new blood vessels
ofloxacin	fluoroquinolone antibiotic drug
pilocarpine	muscarinic alkaloid used in treatment of glaucoma
zeta potential (ZP)	magnitude of the ZP gives an indication of the stability of colloidal system. Large negative or positive ZP means stabile form (silver colloids 2009)
pseudoplastic	liquid whose viscosity decreases with increasing shear rate (BeMiller et al. 2005)
Sjögren's syndrome	autoimmune disease
squamous	consisting of one or more layer of flat plate like cells
squamous metaplasia	conversion of wettable nonkeratinized epithelia into nonwettable keratinized epithelia
thioxtropic	liquid whose viscosity decreases over time at constant shear rate
work of adhesion	work done on the system, when two condensed phases forming an interface of unit area are separated reversibly to form unit areas. Sometimes called work of separation. (Iupac goldbook 2009)

Abbreviations

AA	acrylic acid
Ac	acetate
ADH	adipic acid dihydrazine
ALG	alginate
BMA	N-butyl methacrylate
BSA	bovine serum albumin
CARB	carbomer
CARR	carrageenan
CE	cellulose ether
CM	carboxy methyl
CMC	carboxymethyl cellulose
COL	collagen
CS	chitosan
Cys	cysteine
Da	dalton
DES	dry eye syndrome
DEX	dextran
DH	degree of hydrolysis
DiAc	diacrylate
EC	ethyl cellulose
ECM	extra cellular matrix
EGF	epithelial growth factor
EUD	eudragit L100
G	α-L-glucuronic acid
GEL	gelatin
GELG	gellan gum
Gly	glysine
Glyc	glycerate
GSH	glutatione, composed of glutaminic acid, cysteine and glycine
GAG	glycosaminoglycan
GFX	gatifloxacin
HA	hyaluronan
HAse	hyaluronase
HEMA	2-hydroxyethyl methacrylate
HGF	hepatocyte growth factor
HPMC	hydroxylpropyl-methylcellulose
ISH	imminothiolane
KCS	keratoconjunctivitis sicca
M	methyl
Ma	ß-D-mannuronic acid
MA	metacrylic anhydride
MAA	methacrylic acid

MC	methyl cellulose
MMA	methyl methacrylate
MUC	mucin gene
MUCAc	acetylated mucin gene
MW	molecular weight
Na-ALG	sodium alginate
NIPAM	N-isopropylacrylamide
OFL	ofloxacin
PAA	polyacrylic acid
PDMS	polydimethylsiloksane
PEG	polyethylene glycol
PEO	polyethylene oxide
POL	poloxamer
PPO	poly propylene oxide
PVA	polyvinyl alcohol
PVP	poly-*N*-vinyl-2-pyrrolidinone
SPR	surface plasmon resonance
TBA	4-thiobuthylamidine
TGF- β	transforming growth factor - beta
TPP	tripolyphosphate
TWA	total work of adhesion
XAN	xanthan
XYL	xyloglucan
ZP	zeta potential

1. Introduction

The eye is a sensitive organ for seeing, and the sense of sight is important property for a human being. Eye dryness is one of the diseases that may lead to poor sight or even blindness. Therapy of eye disorders is challenging because of the rapid tear flow rate and blood barriers in the cornea and retina. A therapeutic agent or lubricating polymer flows rapidly away from cornea and a drug molecule does not have much time to penetrate into the epithelium. The therapeutic effect of conventional eye drops goes mainly to nasal drainage and therefore administration must happen many times a day. (Johnson & Murphy 2004; Ludwig 2005; Seattone 2002) In this thesis, the ethiology and therapy of dry eye syndrome (DES), but also therapies under research, are discussed. The main focus is on polymers, which have mucoadhesive characters and are used or studied for DES. Other potential polymers which have mucoadhesive or drug carrier characters were also reviewed.

2. Anatomy and physiology of the eye

This thesis is focused on the cornea, conjunctiva, tear film and accessory structures such as the eyelids and the lacrimal apparatus. Other structures of the eye are discussed briefly.

2.1. Common structure of the eyeball

The ordinary organ of seeing is composed of eyeball (diameter about 2.5 cm) with the optic nerve and accessory structures including the eyelids, lacrimal apparatus and extraocular muscles. The globular eyeball is situated in the bony eye socket covered in fatty tissue. The eyeball is composed of three layers, which have different functions in front and back halves of the globe (Figure 1). Outer layer is the fibrous tunic, which forms the sclera posteriorly and the cornea and conjunctiva anteriorly. The vascular tunic or uvea is middle layer, which forms choroids in the posterior half of the eyeball. In the anterior half of the middle layer of the eyeball forms the iris and ciliary body. In the back part of the innermost layer of eyeball lies retina, which is composed of photoreceptor and pigment epithelium layers. The front part of the innermost layer forms the pigmented epithelium of the ciliary body and the iris. (Faller & Schuenke 2004)

There is in the eye three spaces, which are the anterior chamber, the posterior chamber and the vitreous body. The anterior chamber is filled with aqueous humour and it lies behind the cornea and front of the pupil and the iris. The meshwork of connective tissue so called trabeculae, lies at the confront space of the iris and the cornea. In the trabeculae space is the Schlemm canalis, which function is to circulate the aqueous humour to the vein. The aqueous humour is secreted from connection site of the anterior and posterior chamber of the pupil. The vitreous body is glassy gelatinous matrix containing 98 % water, collagen (COL) and hyaluronic acid (HA), but not blood vessels or nerves. The vitreous body is two thirds of the total volume of the eyeball and its function is to stabilize the ball. (Faller & Schuenke 2004)

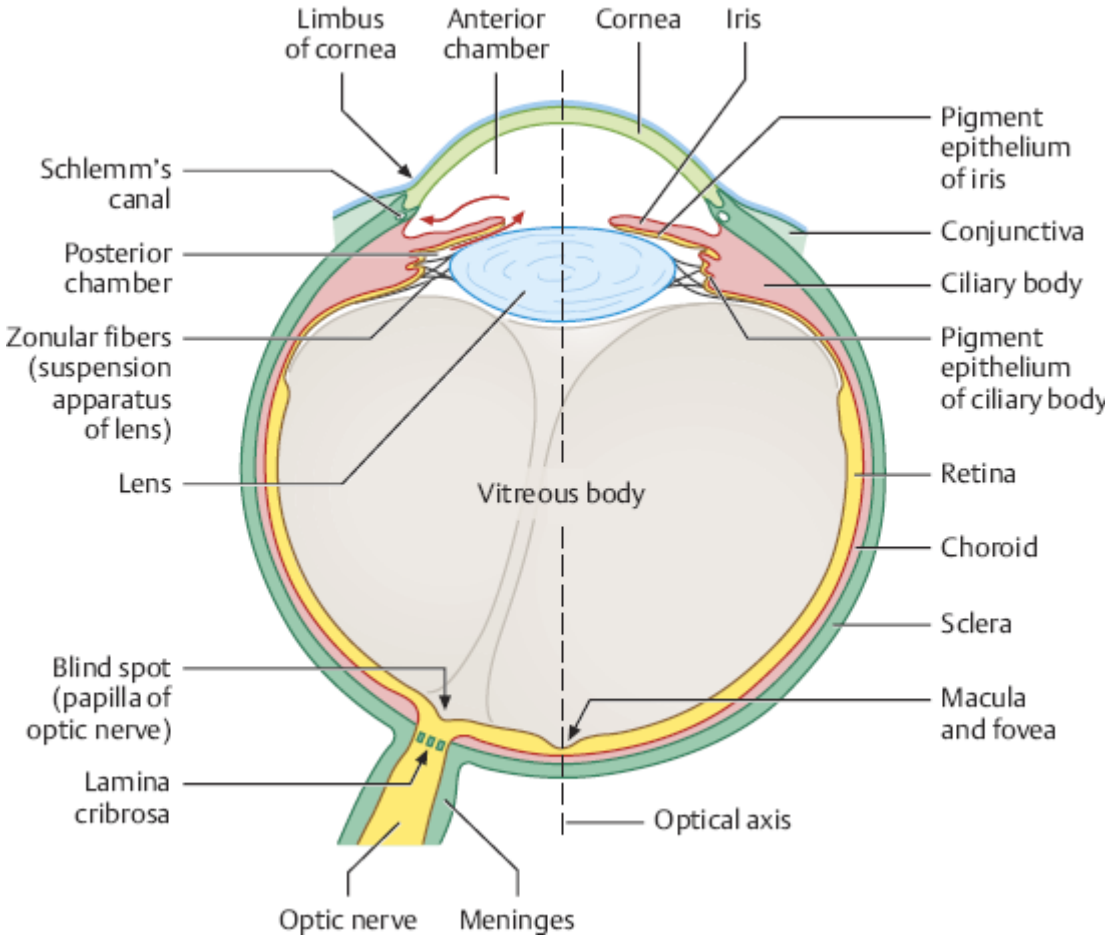


Figure 1. *Horizontal section through the eyeball. Different colors show the three tunics of the eyeball: internal (yellow) retina, pigmented ciliary body and iris, middle (red) choroids, iris and ciliary body, external (green) sclera and cornea. The egress of fluid from the anterior chamber is marked with red arrows (Faller & Schuenke 2004).*

The optical refracting apparatus is composed of the anterior and posterior chambers of the eye, the lens and ciliary body, the iris with central opening (pupil), the cornea and the vitreous body. The lens is transparent and consists of a nucleus, epithelium and fibres. It is suspended by circular arrangement of zonular fibers (Figure 2). The lens can change its refraction by varying its shape. By this mechanism, which is also called accommodation, objects are brought into sharp focus from different distances. (Faller & Schuenke 2004)

2.2. Cornea

The cornea, the most anterior portion of the outer coat of the eye, is more strongly curved than the sclera. It is an optical window that makes it possible for humans to see. (Lang 2006).

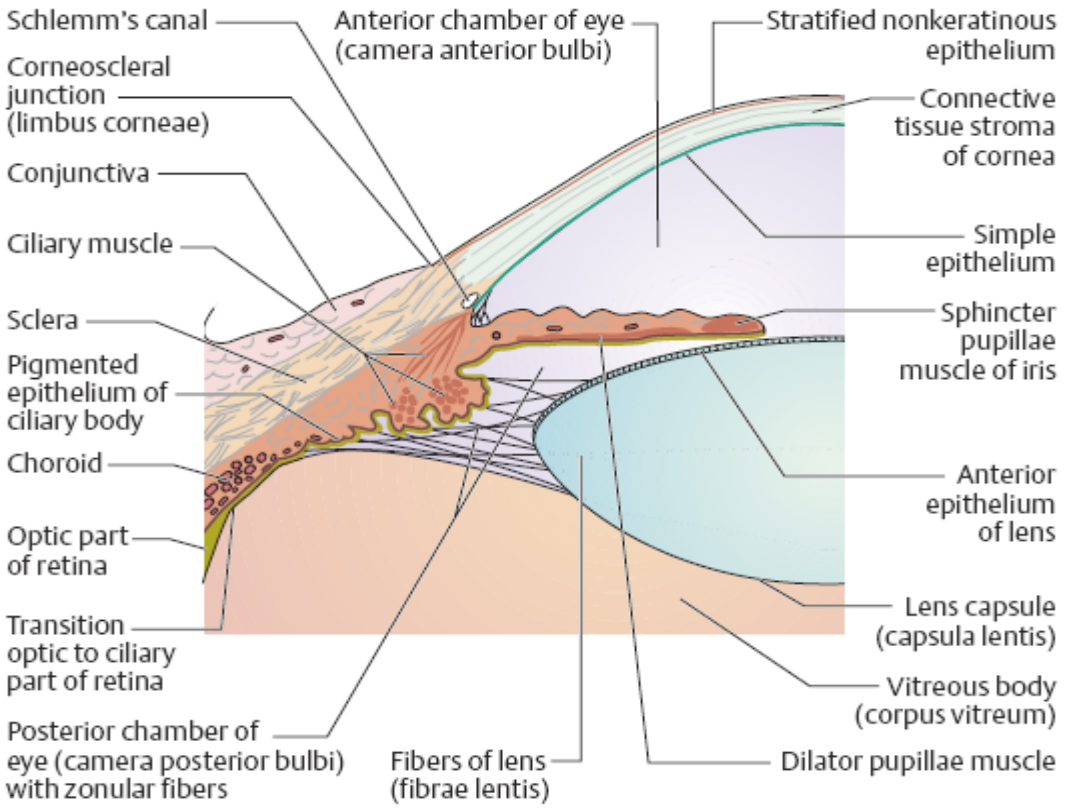


Figure 2. *Horizontal section through the anterior part of the eyeball. Lens accommodation takes place by relaxing or tensing the ciliary muscle. The corneal stratified nonkeratinous epithelium, connective tissue stroma and simple endothelium are depicted. (Faller & Schuenke 2004)*

The cornea is devoid of blood vessels and receives nutrients (amino acids and glucose) by diffusion from the aqueous humour, from tear film and by capillaries at its edge. Because of the bradytrophic nature of the cornea, the metabolism is slow and then also regeneration and healing is slow (Goodman 2003; Lang 2006). The cornea is sensitive and any injury to the cornea exposes sensory nerves and causes high-level pain with reflexive tearing and involuntary eye closing. (Lang 2006).

2.2.1. Layers of the cornea

The central thickness of the cornea is about 500 µm and it is composed of five layers, which are: epithelium, Bowman's membrane, stroma, Descement's membrane and endothelium (Figure 3). (Goodman 2003)

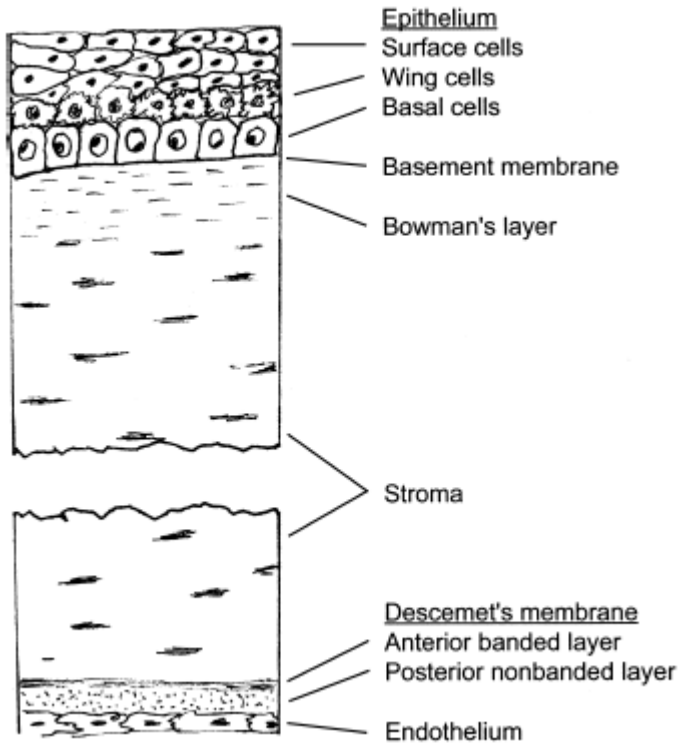


Figure 3. *Cross section of the cornea. Layers are starting from the outer part of the cornea: epithelium, Bowman's membrane, stroma, Descement's membrane and endothelium. (Goodman 2003).*

Epithelium

The epithelium is about 50 µm thick, having four or five cellular layers. The cell layers are: deep basal columnar layer, middle wing cells and then superficial squamous surface cells (Goodman 2003). Replacement of the epithelial cells (life time average 4-8 days) occurs by mitotic division of the limbal basal layer, where undifferentiated pluripotent cells are located. Cells migrate to the central cornea and are differentiated into transient amplifying cells and basal cells. The corneal epithelial cell layer mass is a complex phenomenon, where there are three stages: proliferation of the basal epithelial cells, centripetal mass movement of peripheral epithelial cells and cell loss resulting from death and desquamation. These three phenomena are not independent of each other, but

are rather controlled by complex interactive feedback mechanism that maintains the situation, cell density, cell distribution, polarity and layer thickness. (Farjo et al. 2008)

Bowman's membrane

The Bowman's membrane is a compressed acellular anterior stroma, which is about 15 µm thick and composed of randomly arranged of compact COL lamella binding certain growth factors. When the Bowman's membrane is disrupted a scar is formed and it does not heal or renew. (Goodman 2003)

Stroma

The third layer of the cornea is a stroma and it takes about 90% of corneal thickness. It is composed of about 250 regularly spaced COL lamellae that contain 600 Å fibrils that expand across the entire cornea. Keratocytes and glycosaminoglycans (GAG) are clear because the wavelength of visible light is about 5000 Å. The stroma is avascular and regenerates very slowly. (Goodman 2003)

Descement's membrane

The Descement's membrane is the basement membrane of the endothelium, whose thickness is 10 – 12 µm. It is divided into anterior banded and posterior nonbanded parts, where latter tends to grow with age. (Goodman 2003)

Endothelium layer

The endothelium is the most inner layer of the cornea. It is a cellular monolayer and non-renewable. Endothelial cells do not multiply and the cell density is about 3500 cells/mm^2 at birth and decreases through life at about 0.6% per year and about 10% by intraocular surgery. (Goodman 2003)

2.2.2. Swelling of the cornea

The epithelium to endothelium to electrolyte ratio of diffusion is about 1:200, which means that the epithelium is a good water barrier. Stromal GAG ground substance with anionic charges is like a dry sponge and has potential to absorb water. Stromal swelling and corneal hydration is balanced and it tries to remains constant (70 %) by endothelial ion pump and epithelium evaporation. The cornea's transparency rests on a certain fluid content and the degree of the swelling of the lamellar COL fibers of the stoma. The

cornea becomes cloudy and disturbs vision, if this specific degree of swelling changes. (Goodman 2003)

2.2.3. Corneal epithelial wound healing

After a small corneal epithelial injury, cells begin to cover the defect as rapidly as possible (within minutes) by a combination of cell migration and spreading. In larger defects this lag phase takes a few hours. The lag phase is necessary for preparatory cellular changes of an anatomical, physiological and biochemical nature to occur before rapid cell movement. Various cell membrane extensions, such as lamellipodia, filopodia and ruffles, develop at the leading edge of the wound. Anchoring hemidesmosomes disappear from basal cells. The early nonmiotic coverage phase is remarkable for its speed; cells have been measured to migrate at a rate 60 – 80µm/h (Matsuda et al. 1985). At 24-30 hours after medium-size epithelial injuries, mitosis or cell proliferation begins and restores the rarefied epithelial cell population. After large injuries, significant increases in cellular divisions occur as late as 94 hours. The basal cells, transient amplifiying cells and the limbal stem cells take part in this reconstitutive mitosis. (Farjo et al. 2008) Sufficient tear production and full eye lubrication is essential during epithelial wound healing, because the damaged area disturbs tear film stability and may cause further damage. Return to normal situation after injury lasts from months to years (Wilson et al. 2001) but can be accelerated with lubricates and therapeutic eye drops.

2.3. Conjunctiva

The tunica conjunctiva is a lining of the inner surface of the eyelids, which consists of two or more layers of nonkeratinized stratified columnar epithelium with goblet cells, wing cells and a loose, cell rich lamina propria containing multiple blood vessels. Conjuctival epithelium is 10 - 12 cells in thickness (Goodman 2003). Goblet cells in the conjunctival epithelium produce mucus to lubricate and moisten the corneal epithelia. The tunica extends around the fornix of the conjunctiva to the eyeball, which it covers with a layer of stratified squamous epithelium that extends up to the cornea margin. Figure 4 shows the palpebral conjunctiva, conjunctival fornix, bulbar conjunctiva and the cornea (Lang 2006).

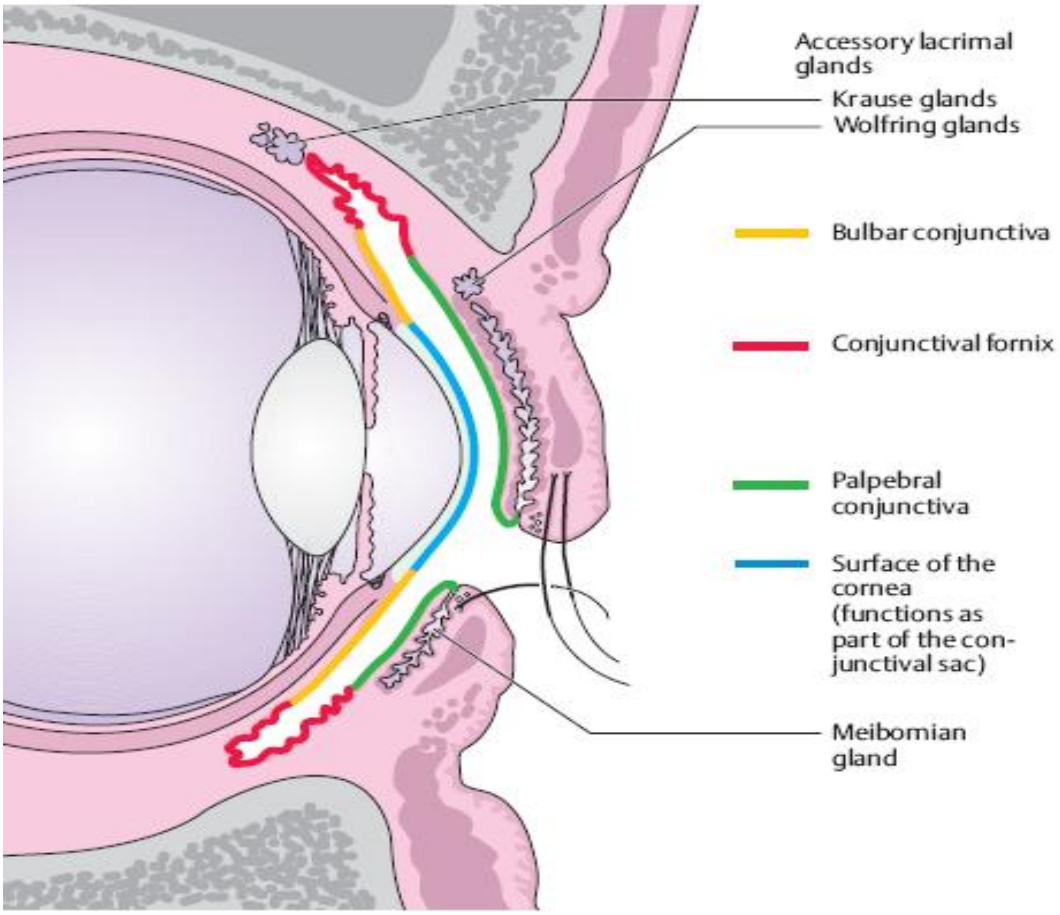


Figure 4. *Cross section of the anterior part of the eye, where the lining of the surface of the eyelids and the cornea is shown (Lang 2006).*

2.4. Accessory structures of the eye

Accessory structures of the eyeball are the eyelids, the lacrimal apparatus and the extraocular muscles. These structures have influence to the front eye status and function, so these structures are discussed in following chapters.

2.4.1. Eyelids (Palpebrae)

In the front, the cover of the upper and lower eyelids protects the eyeball from injury. They are soft tissue and shaped such that the eyeball is completely covered when they are closed. Eyelids are actively closed by striated muscle fibers of the *orbicularis oculi* muscle and opened by the *levator palpebrae* muscle. This open and close movement is called eye blinking, which happens regularly (20 - 30 times/min). Blinking helps to distribute uniformly glandular tear film like a windshield wiper over the conjunctiva

and cornea, keeping them wet and lubricated. Strong mechanical, optical, and acoustic stimuli such as a foreign body, blinding light, or sudden loud noise automatically elicit an eye-closing reflex. The cornea is also protected by additional upward movement of the eyeball. (Lang 2006)

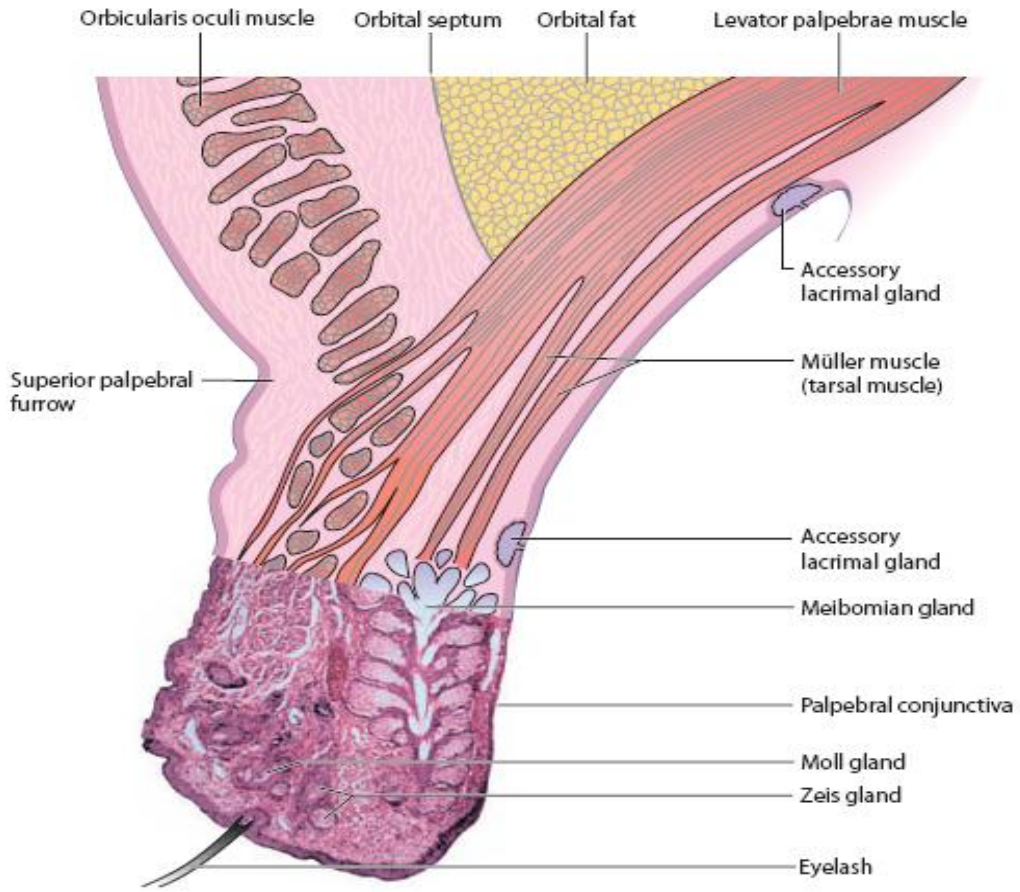


Figure 5. *Eyelid is composed of a superficial layer, including the skin, glands of Moll and Zeis and* orbicularis oculi *and palpebrae muscles. The deep layer of the eyelid consist of the tarsal plate, tarsal muscle, palperal conjunctiva and meibomian glands (Lang 2006).*

The eyelid consists of superficial and deep layers (Fgure 5). The superficial layer of the eyelid is the thinnest in the body (Goodman 2003), well-vascularized and there is sweat and sebaceous glands. The deep layer of the eyelid is composed of the tarsal plate, which gives firmness and shape. Smooth mucles of the *levator palpebrae* in the tarsal plate is equipped with sympathetic nervous system and regulates the width of the palpebral fissure. The palpebral conjunctiva forms an articular layer for the cornea and is firmly attached to the tarsal plate. (Lang 2006)

In the deep layer of the eyelid are sebaceous glands which lubricate the margin of the lid. Their function is to stop the escape of tear fluid and form the superficial oily layer over the tear aqueous film. Oily layer prevents tear fluid evaporating. The fibers of muscle at the inner site of these sebaceous glands (near the margin of the eyelid) squeeze out the ducts of the tarsal glands every time the eye blinks. (Lang 2006) Table 1 presents the secreting cells and glands of the eyelid, their function and mechanism.

Table 1. Secretion glands and cells of the eyelids and cornea (Goodman 2003; Lang 2006).

Gland	**location**	**secretion product (and its type)**	**secretion mechanism** [1)]	**note**
Epithelial cells	cornea and conjunctiva	sulfinated mucin	anchored and	-
Goblet cells	conjunctiva	carboxylated mucin (slime)	apocrine for a time, then holocrine	-
Lacrimal gland	conjunctiva	modified sweat (aqueous)	eccrine	20-42 glands in upper fornix and 6-8-lower
Accessory lacrimal gland: Glands of Krause	conjunctiva	modified sweat (aqueous)	eccrine	3 glands upper and 2 lower border of the tarsus
Accessory lacrimal gland: Glands of Wolfring	wall of the palpebral conjunctiva	modified sweat (aqueous)	eccrine	-
Sebaceous glands: Meibomian glands	tarsus	sebum (oil)	holocrine	not associated with eyelashes
Sebaceous glands: Zeis gland	lash follicle	sebum (oil)	holocrine	connected with eyelashes
Sweat glands: Moll gland	lash follicle	modified sweat (thicker solution than sweat)	apocrine	empties into a lash follicle

[1)] Secretion mechanism explanations: eccrine = secretion of the cell is liquid, holocrine = secretion of the cell is hole content of the cell, apocrine = secretion of the cell is hole content of the secretorium.

Near the sweat and sebaceous glands are eyelashes on the eyelids. The eyelashes function is to prevent dust and sweat from entering to the eye. On the upper eyelid, approximately 150 eyelashes are arranged in three or four rows; on the lower eyelid, there are about 75 in two rows. (Lang 2006)

2.4.2. Lacrimal apparatus

The lacrimal apparatus includes the lacrimal gland and the pathways of the tear drains. The lacrimal glands are about the size of a walnut and lie above the eyeball, and drain trough several ducts into the outer part of the fornix (Figure 6) (Faller & Schuenke 2004).

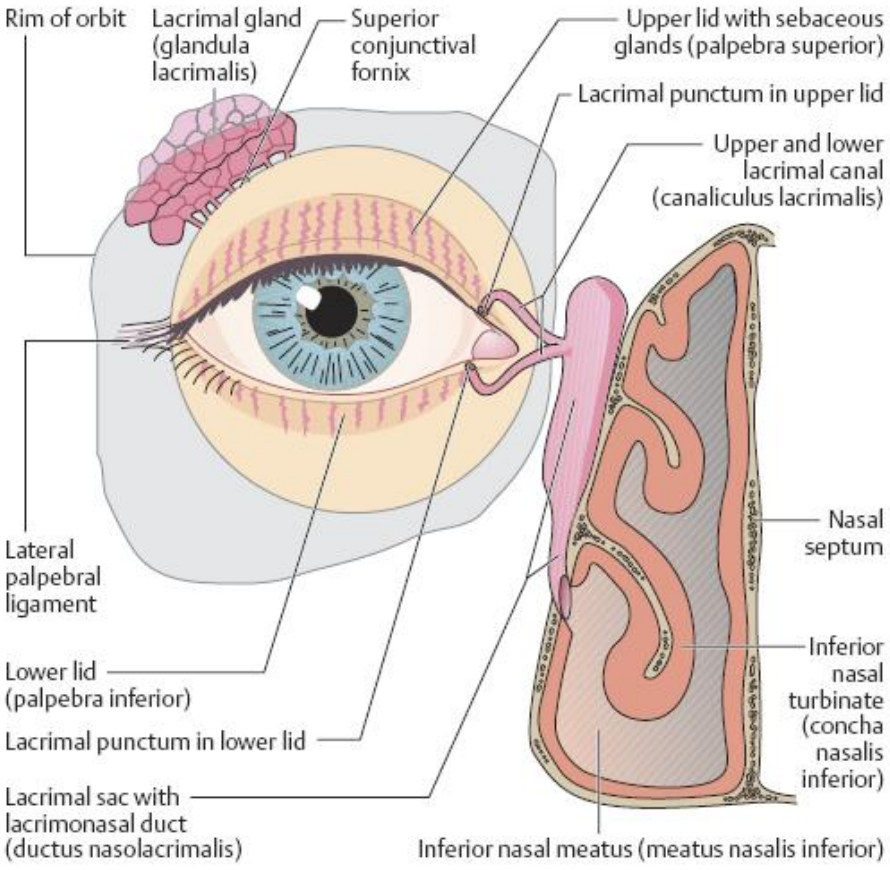


Figure 6. *The lacrimal apparatus. The lacrimal fluid is secreted by the lacrimal glands and channelled over the cornea to the lacrimal sac (Faller & Schuenke 2004).*

The lacrimal fluid moisture the anterior surface of the globe, cleans and nourishes the cornea. It is distributed evenly by blinking and accumulates in the inner angle of the eye. (Faller & Schuenke 2004) The lacrimal glands receive its sensory supply from the lacrimal parasympatic nerve. (Lang 2006)

3. Tear film

The preocular tear film has a trilaminar structure (Figure 7), generally consisting of an outer oily layer, a middle aqueous layer and an inner mucinous layer (Lang 2006).

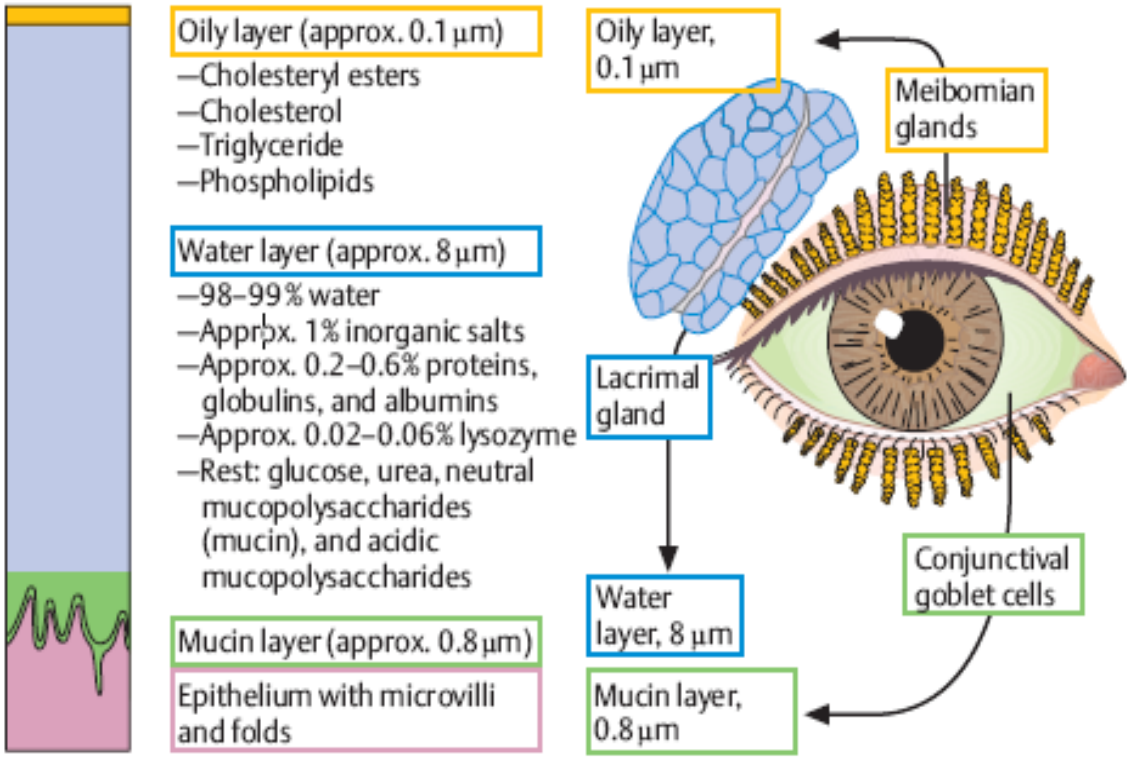


Figure 7. *The tear film is composed of three layers: an oily layer, a watery layer and a mucin layer. The oily layer prevents desiccation; the watery layer keeps the cornea clean and optically transparent, and the mucin layer stabilizes the tear film by transmenbrane and secreted glycogalyx (Lang 2006).*

The tear film has a variety of different functions, including maintenance of a smooth surface for optical clarity, lubrication to facilitate eyelid blink and protection against ocular infection. The functions also include providing O_2, other nutrients and growth factors to the cornea. Tear-specific proteins as lysozyme, beta-lysin, lactoferrin and gamma globulin give antimicrobial characteristics to the tear fluid (Goodman 2003). The tear film is vulnerable and that makes it difficult to study, because conventional

chemical fixation disturbs its morphology. Thickness of the tear film is measured by optical coherence tomography and it is in humans 3.30 ± 1.5 µm (Wang et al. 2003), hyposmolar 302 mOsm/l (Johnson & Murphy 2004), production rate 1.2 µl/minute, volume 6.5 µl, turnover every 5 minutes and pH 7.4 (Goodman 2003).

3.1. Lipid layer

The primary function of the lipid layer is to stabilize the tear film by increasing surface tension and slow down evaporation of the aqueous layer of the tear film. It prevents the spill over the tears to the skin and has antimicrobial activity (Craig & Tomlinson 1997). The lipid layer is composed of two phases (McGulley & Shine 1997): a relatively thick non-polar outer layer and thin polar inner layer. The outer layer contains nonpolar lipids such as wax esters, sterol esters, hydrocarbons and triglycerides. The inner polar layer is mainly composed of phospholipids and it owns surfactant properties by its amphipathic phospholipids which lie between the polar aqueous layer and the nonpolar oily stabilizing interactions. Evaporation of the tear film is inhibited by the outer nonpolar layer while the inner polar layer stabilizes the oily phase by acting as surfactant to allowing interface with the aqueous layer (Figure 8). The phospholipids, sphingomyelin and phosphaditylethanoamine are important elements of the polar phase architecture,

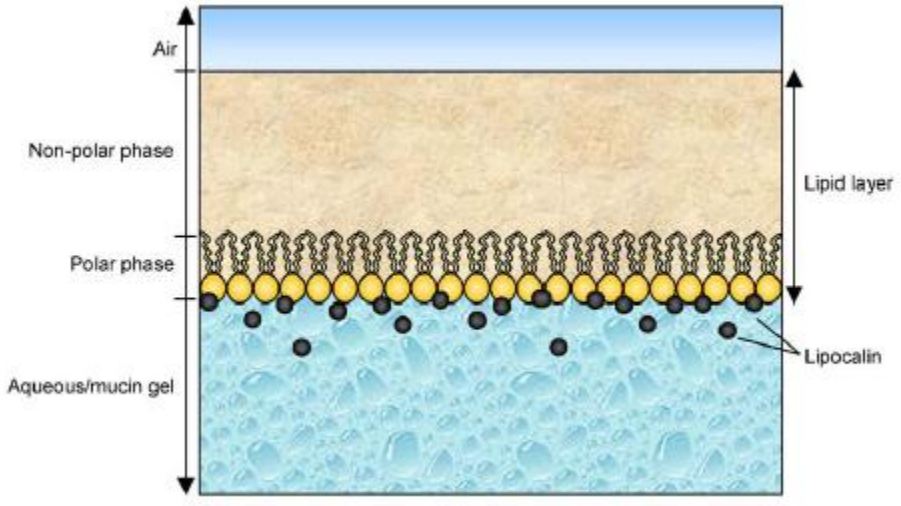


Figure 8. *Interface of the lipid and aqueous layer of the tear film. The outer oil layer consists of nonpolar lipids and the inner layer contains polar phospholipids. The outer layer retards aqueous evaporation, while the inner layer takes care of interactions with aqueous layer. Protein lipocalin stabilizes by forming complexes with polar lipids. (Johnson & Murphy 2004)*

because they are able to form ionic and highly directional hydrogen bonds. The tear protein lipocalin forms complexes with polar lipids, decreases surface tension of the aqueous layer, and helps the lipid layer to spread and form a stable membrane (Nagyová & Tiffany 1999). The tear film has a highly regulated structure, which spontaneously forms due to intermolecular forces (Virtanen et al. 1998). The quality of the lipids in the lipid layer of the tear film is important for its proper function (Johnson & Murphy 2004).

3.2. Aqueous layer

The middle aqueous layer is approximately 8 µm thick (Lang 2006) and is produced by the lacrimal glands and the accessory lacrimal glands of Krause and Wolfring (Goodman 2003). It consists primarily of water, but also electrolytes and plenty of proteins. The composition of the tear film is a highly dynamic flux, its specific content varying depending on the environmental and individual conditions. The task of the aqueous is to clean the surface of the cornea and ensure mobility of the palpebral conjunctiva over the cornea and a smooth corneal surface for high-quality optical images. (Johnson & Murphy 2004)

3.2.1. Electrolytes

Electrolytes present in tear film include sodium, potassium, magnesium, calcium, chloride, bicarbonate and phosphate ions (Barton et al. 1997). The electrolytes act as a buffer and maintain constant pH, are responsible for osmolality of the tears and have an important role in ocular surface integrity. An increased electrolyte concentration in the tear film damages the ocular surface directly and indirectly by triggering inflammation. The electrolyte composition in the tear film is thus critical in maintaining ocular surface health. (Gilbard & Rossi 1992)

3.2.2. Proteins

In the tear film, over 60 different proteins are identified and these are mainly secreted by the acinar cells of the lacrimal glands (Gachon et al. 1979). The protein composition of the tear film fluctuates with tear flow rate, conjunctival simulation, eye closure, age and ocular surface diseases (McGill et al. 1984). The primary regulated proteins in the tear film are lysozyme, lactoferrin and lipocalin, which are secreted in response to an

intracellular stimulus with nearly same rate as tear flow. A small proportion of proteins are derived trough the leakage of plasma components trough ocular surface capillaries without tear stimulation. These plasma origin proteins include serum albumin, immunoglobulin, ceruloplasmin, transferin and monomeric immunoglobulin A. The concentration of these proteins decrease in nontraumatically stimulated tears, but increase following mechanical irritation or inflammation of the ocular surface. In tear deficient disease the amount of the regulated protein decreases and serum derived proteins increase causing chronic leakage from inflamed surface capillaries. (Fullard & Snyder 1990)

3.2.3. Growth factors

Peptide growth factors and vitamin A are essential to the health of ocular surface epithelial cells (Sommer 1983; Fox et al. 1984). Those molecules regulate epithelial proliferation, motility and differentiation by acting via autocrine and paracrine mechanisms. Also involving in wound healing and immune modulation are important functions. Numerous growth factor, including epithelial growth factor (EGF) transforming growth factor-beta (TGF-β) and hepatocyte growth factor (HFG) have been found in tears (Li et al. 1996). These growth factors and vitamin A present at the ocular surface are mainly produced endogenously with an additional amount delivered by conjunctival blood vessels. The majority of the growth factors and vitamin A in the tear fluid are secreted by the lacrimal glands, but EGF and TGF-β are mainly synthesized in corneal epithelial cells. It is reported that the concentration of the EGF is decreased under tear deficiency and it seems reasonable to presume that secretion of the other supportive factors are similarly affected. (Johnson & Murphy 2004)

3.3. Mucus layer

In general, mucus is composed of mucins and organic salts suspended in water. Mucins are a family of high-molecular-weight glycoproteins that have at least half of their mass as O-linked carbohydrate. Mucins are classified as transmembrane or secretory mucins (Figure 9). Secreted mucins are further sub-classified as gel-forming or soluble, based on their ability to form polymers. Human genome mapping has identified 21 mucin genes and nine (9) of them (MUC1, MUC2, MUC4, MUC5AC, MUC7, MUC13, MUC15, MUC16 and MUC17) have been found in the eye. The mucin genes have been

numbered chronologically in the order they have been reported. (Johnson & Murphy 2004)

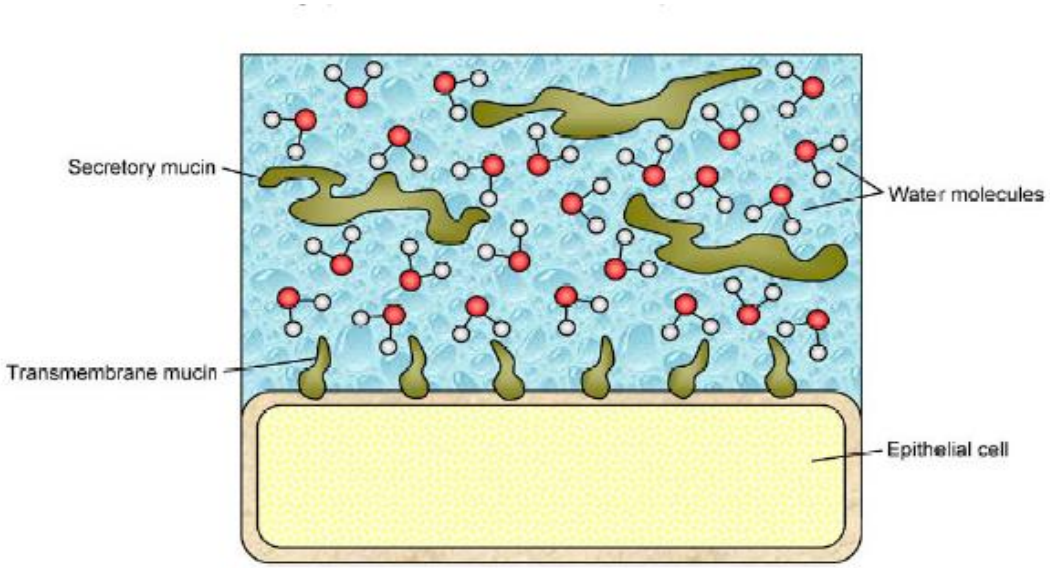


Figure 9. *Water molecules, transmembrane and secretory mucins in mucinous layer of the tear film. Transmenbrane mucins are anchored to the epithelial cells, while secretory mucins interact with water molecules enhancing adhesion to transmenbrane mucins. (Johnson & Murphy 2004)*

3.3.1. Transmembrane mucins

Transmembrane mucins (MUC1, MUC4, MUC13, MUC15, MUC16 and MUC17) contain hydrophobic, cell membrane-spanning domains in their carboxyl-terminal region, which anchor them to the apical surface of the conjunctival and corneal epithelial cells, facilitating formation of the ocular surface glycogalyx (Johnson & Murphy 2004). Ocular surface glycogalyx acts as a barrier to pathogens by sustaining bacterial flora (Corfield 1997) and its wettability stabilizes the overlying tear film (Sharma 1998a). Moreover, transmembrane mucins have been implicated in cellular signalling. For this purpose, they have short cytoplasmic tails, which can be extensively phosphorylated and hence potentially participate in intracellular signal transduction cascades. (Zrihan-Licht et al. 1994)

3.3.2. Secretory mucins

The major gel-forming mucin MUC5AC and MUC2 of the tears is secreted by the holocrine conjunctival goblet cells (Goodman 2003). Secreted gel-forming mucins from

entangled linear polymers are responsible for the non-Newtonian thixotropic or viscoelastic properties of mucin gels that avoid shearing damage during movements of the lids and globe (Corfield et al. 1997). MUC7 is a small monomeric and soluble mucin produced by lacrimal glands and conjunctiva. Polymerization occurs through covalent inter- and intramolecular disulphide bonds between cysteine (Cys)-rich globular regions and lectin-like protein-to-carbohydrate bonds (Bansil et al. 1995; Johnson & Murphy 2004).

3.3.3. Interactions in mucin

Glycogalyx forming transmembrane and secretory mucins are sited to the border of the corneal surface and tear film aqueous layer, but they do not firmly adhere to the corneal epithelium. Loose interactions such as hydrogen bonds are due to unevenly distributed polar regions of the mucin molecules. Charged mucin molecules have a tendency to produce linkage with themselves, but the linkage formation is inhibited by competing with water molecules. These polar regions arise from the extensive glycosylation of the serine and threonine residues within their nucleotide tandem repeat domains. A lack of adhesion allows spreading of the tear film, disabling the aqueous layer from forming beads and also prevents microtrauma to the ocular surface by reducing transmitted shear during blinking. The mucinous coating of the hydrophobic epithelial cell surface provides smooth distribution of the overlying aqueous layer (Gipson & Inatomi 1997; Sharma 1993; Bansil 1995)

Mucus layer acts as a barrier to potential pathogens. The diversity of the ocular mucin linked to oligosaccharide epitopes creates an enormous repertoire of potential binding sites for harmful contaminants and acts as a debris removal system (Thomsson et al. 2002). Apolar and weakly polar contaminants, such as cell debris, lipids, gram-negative bacteria and many airborne particulates, bind to the hydrated mucin because the free energy of adhesion is greater for apolar particles and hydrated mucins in an aquenous medium than for apolar particles and epithelium in mucus. In the healthy eye tear film (Figure 10) acts as described above, but problems occur when normal movement of mucus is diminished. Contaminants can adhere to the surface of the cornea and apolar molecules bind epithelium rather than mucin increasing the risk of infection. (Sharma 1998a)

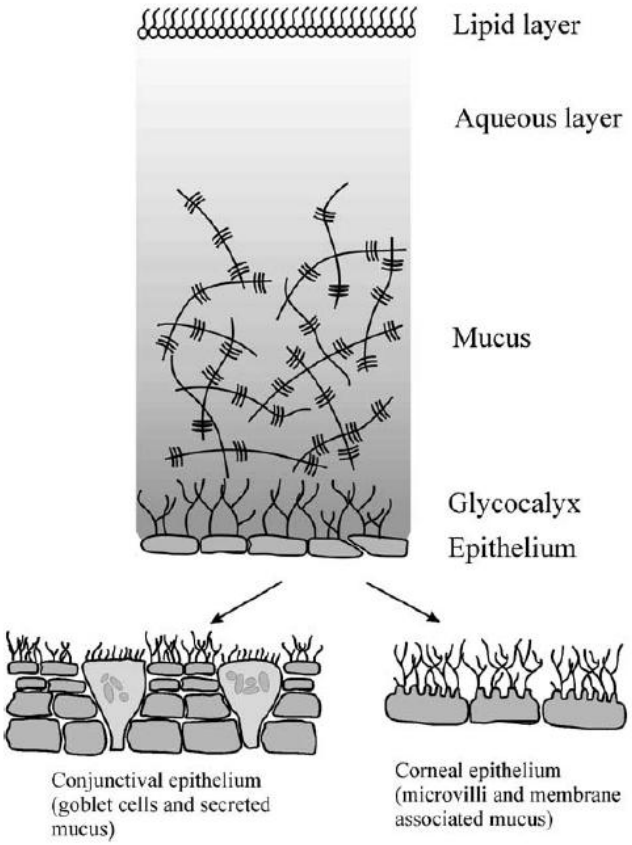


Figure 10. *Corneal and conjunctival epithelium of the eye, which is protected by tear film. Secretory mucin is produced by conjunctival goblet cells and membrane associated mucus is produced by corneal epithelium cells (Ludwig 2005).*

3.4. Mucoadhesion

Muchoadhesion is a capability of the macromolecules to retain on the inner mucus layer of the tear film not only by viscosity effects, but also physicochemical interaction with the mucin layer covering the cornea. Hydrogen bonding, van der Waals forces, and electrostatic and hydrophobic interactions are physicochemical intermolecular reactions with mucous substrate. For many polymers hydrogen bonding appears to play a significant role in mucoadhesion, thus the presence of the water seems to be a prerequisite for a majority of the mucoadhesion phenomena. Muchoadhesive polymers, which can establish interaction with mucin layer, are usually hydrocolloids with numerous hydrophilic functional groups such as carboxyl, hydroxyl, amine and sulphate. (Saettone 2002)

4. Dry eye

The National Eye Institute of the USA/Industry Workshop adopted a definition of the dry eye, which is as follows: “Dry eye is a disorder of tear film due to tear deficiency or excessive tear evaporation which causes damage to the interpalpebral ocular surface and is associated with symptoms of ocular discomfort” (Lemp 1995).

Dry eye can be characterized by the severity of the conditions. In the mild case of dry eye, no damage of the cornea or conjunctiva is found, but some disorders in the tear film are found. In the moderate case of dry eye, mild damage of the cornea, such as superficial punctuate keratopathy, is found. In the severe case of dry eye, keratinisation of the conjunctiva, corneal damage, epithelial defects and subsequently higher risk of secondary infections are found. (Calonge 2001) Keratoconjunctivitis sicca (KCS) is a severe case of dry eye, which is non-infectious keratopathy by reduced moistening of the conjunctiva and cornea. KCS is also called dry eye syndrome (DES) and it is a result of dry eyes. Most forms of dry eye, such as interpalpebral surface damage, tear instability, and tear hyperosmolarity, have same kind of symptoms regardless of cause. Patients complain of burning, itching, foreign substance sensation, photophobia, reddened eyes, ocular fatigue and excessive lacrimation. (National Eye Institute 2009; MayoClinic 2009)

4.1. Aetiology of dry eye

There are numerous causes and risk factors for the tear film instability including age, hormonal deficiencies, use of the systemic anticholinergic medication, surgery that worsens the corneal sensory nerves and systemic autoimmune disease. The tear film, epithelia of the cornea and conjunctiva, the lacrimal glands and eyelids act as a functional unit, which means that they share feedback mechanisms which result in simultaneous, coordinated reactions to stimuli (Johnson & Murphy 2004). Pathogenic events which disturb homeostasis of the eye surface and are not promptly neutralized by the ocular system create a deleterious cycle that expose to dry eye disease.

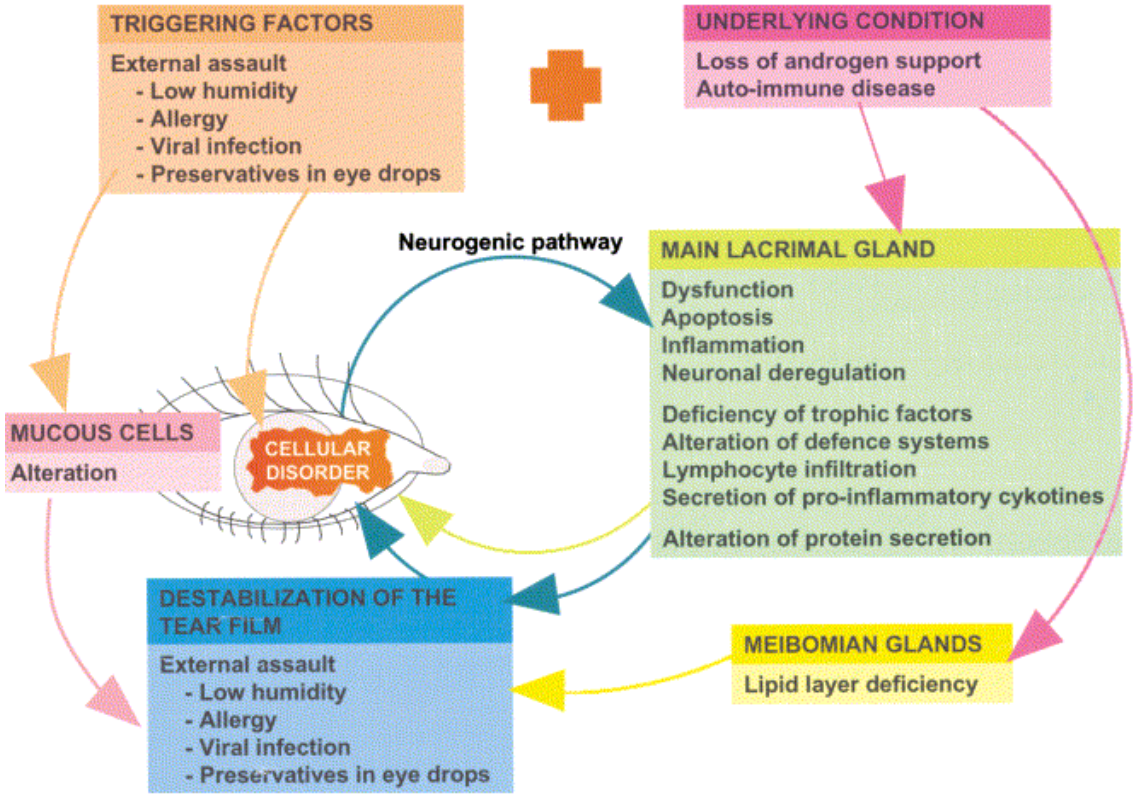


Figure 11. *The cycle of dry eye disease. Cellular disorder causes lacrimal gland disturbance, which further causes more cellular disorder. Underlying conditions such as Sjögren's syndrome disturbs lacrimal gland and meibomian glands function and DES is initiated. External assaults worsen symptoms, further alter epithelial cells and keeps the cycle going on. (Boudoin 2001)*

All types of dry eye are associated with ocular surface inflammation that may further increase tear secretion and cause ocular surface disease (Stern et al. 1998). In Figure 11 is presented the cycle of dry eye disease. In following chapters are discussed the causing factors of DES and these factors is summarized in Table 2.

4.1.1. Tear film instability

Tear stability is essential to minimize evaporation and keep proper tear coverage of the ocular surface between blinks. Tear film instability is a procedure which has been associated with tear deficiency, reduced quantity and quality of lipid layer, altered tear composition, ocular surface irregularities and ocular surface inflammation (Johnson & Murphy 2004). The traditional hypothesis of the tear film instability is that hydrophilic lipids diffuse and absorb to the mucus-aqueous interface (Holly & Lemp 1971). An alternative mechanism of the tear film rupture suggest that break up is started by long-

range apolar van der Waals forces that cause rupture of mucous layer at the thinnest spots, allowing the aqueous layer to come in direct contact with exposed patches of the epithelium (Sharma 1998b). The epithelium is normally wettable because of the glycocalyx, but small microscopic nonwettable areas are present due to cell desquamation. Nonwettable areas grow rapidly in size causing tear film rupture. Hydrophobic interactions are energetically favourable in damaged spots of the epithelium, because hydrated mucus is lost. Tear film rupture is also accelerated by meniscus induced thinning of the tear film, because of its concave curvature. (McDonald 1968)

Table 2. Main reasons for causing DES (Johnson & Murphy 2004).

Initiates DES	**Reason**	**Footnote**
insufficient tear flow	ocular surface hypoesthesia	stimulation of the nerves is not appropriate
	medication, which inhibits cholinergic signal	diuretics, antihistamines, betablockers, antidepressants and antianxiety agents
	lacrimal deficiency	Sjögren's syndrome, autoimmune disease, or non-Sjögren's tear deficiency
	obstructive lacrimal disease	scarring, narrowing secretary duct and orifices of the lacrimal glands
tear instability	instability in lipid layer, reduced level of the lipocalin, too high evaporation rate of the aqueous layer, nonwettable areas on the mucin, thin areas of the tear film	one mechanism does not act in isolation, but instability is often sum of the many reasons
tear hyperosmolality	osmolality increased by tear evaporation	direct and indirect (by triggering inflammation) ocular surface damages.
tear clearance	abnormal blinking	infrequent or too frequent blinking
	lacrimal dysfunction	composition of tear film is insufficient
inflammation	ocular surface inflammation	metaplasia (nonwettable keratinized epithelia)
	ocular allergy	inflammation products may be antigens
	epithelial surface damage	i.e. by surgery or accident
	Sjögren's syndrome	autoimmune disease

4.1.2. Insufficient tear film production

Insufficient supply of tears to ocular surface may occur due to abnormal stimulation of lacrimal glands, lacrimal glands disease and/or scarring of the lacrimal glands secretary ducts. Reduced water production and impaired lacrimal secretion decreases the supply of tear components, which are vital to the health, growth and repair of the ocular surface. Supply of the stabilization proteins of the tear film is disturbed and weakens characters of the overlying lipid layer. (Johnson & Murphy 2004)

4.1.3. Tear clearance

Tear clearance refers to the rate of the turnover and is a function of tear production and elimination (Xu & Tsubota 1995). Delayed tear clearance increases the residence time of the contents of the tear film, including toxic cell waste products, environmental antigens and proinflammatory cytokines and can activate surface inflammation (Barton et al. 1997). Insufficient tear flow can cause harmful concentration gradients of tear components and furthermore worsen dry eye symptoms.

4.1.4. Infrequent blinking

Reduced tear elimination and delayed tear clearance may arise from infrequent or ineffective blinking. Ineffective blinking may occur due to eyelid laxity, normally as a result of age-related degenerative changes, but can also be present following trauma or in floppy eyelid syndrome. Supplementary to their unfavourable effects on tear clearance, infrequent or ineffective blinking intensify evaporation tear loss by prolonging interblink periods and decreasing lipid layer thickness. Infrequent blinking can be involved in the development of DES, but reverse situation of increased blinking induces DES by reducing tear stability. (Johnson & Murphy 2004; Korp et al. 1994)

4.1.5. Tear hyperosmolarity

The number of osmotically active particles in a solution is a property of a solution called osmolarity or osmolality referring to numbers of osmoles of solute per mass of solvent and volume of solution, respectively (Johnson & Murphy 2004). Hyperosmolality of tear film is characteristic of DES and occurs through increased tear evaporation (Lucca et al. 1990). A hyperosmolar tear film results in ocular surface damage directly (Ciprandi et al. 1994) and indirectly (Baudouin 2001) by triggering inflammation.

4.1.6. Inflammation

In patients suffering from tear deficient DES, the disorder may be due to lacrimal gland inflammation, which is due to autoimmune disease such as Sjögren’s syndrome or ocular surface inflammation. Whatever the initial aetiology of DES, once it has developed, inflammation becomes the key mechanism of the ocular surface injury (Baudouin 2001). Ocular surface inflammation may be initiated by desiccation,

hyperosmolality, microtrauma from eyelids during blinking, proinflammatory substances released by the lacrimal glands or a reduced supply of supportive factors from tears and corneal nerves. When inflammation is on-going, the cytokines are secreted by damaged surface epithelial cells, and lymphocytes and leucocytes are infiltrated from expanded conjunctival blood vessels (Gamache et al. 1997). Patients with severe DES can become trapped in an increasing cycle of inflammation and ocular surface injury. Ocular surface inflammation leads to squamous metaplasia, loss of the glycocalyx and goblet cells dropout, which reduce ocular surface wettability and tear film stability (Rivas et al. 1992). The affecting mechanism of the inflammation is not quite clear, but cytokines are believed to have main role. Cytokines have been associated with epithelial keratinisation (Kawasaki et al. 2003) altered mucin expression (Hibino & Watunabe 2002) and corneal neovascularisation (Dana et al. 1998). Ocular surface inflammation in DES is typically limited to superficial layers of the ocular surface, because passages of cytokines and lymphocytes to the deep layers of the cornea are prevented by an intact epithelium and Browman's membrane (Johnson & Murphy 2004). If these layers become damaged through trauma, surgical operation or as a result of severe ocular surface inflammation by cytokine interaction, results are subepithelial scarring and stromal melt (Wilson et al. 2001).

Blepharitis and ocular allergy frequently coexist with DES in a mutual cause-effect relationship (Nelson 1994; Baudouin 2001). Blepharitis worsens DES by acting as a source of antigenic and proinflammatory substances and adversely influencing lipid production (Shine & McCulley 1998). DES may predispose to blepharitis, because of decreased tear antimicrobial activity and tear clearance. Also reduced dilution and washout of allergens increase likelihood of allergic conjunctivitis. Inflammatory products released by allergic response and associated eye abrasion can worsen ocular surface inflammation (Johnson & Murphy 2004).

4.2. Therapy for DES

The aetiology of DES is complex and it is a sum of many causing factors. Environmental conditions (e.g. humidity of the air) together with a human anatomic property can predispose to dry eye or finally to DES. Diagnosis of DES can be complex with several interacting factors implicated, and it is often difficult to determine the

relative significance of each. Artificial tears are palliative and serve only to wet the eye and are inadequate in all but the mildest case of DES. It is very important to find a suitable therapy for a patient suffering from DES and that makes knowledge of the causing mechanism momentous. (Johnson & Murphy 2004)

4.2.1. Condition improvements

Dry eye symptoms can be reduced by environmental conditions, which are clean and moist air. A humidifier provides moisture to the air and an air cleaner filters dust and other particles. A mini-atmospheric area can be prepared for the eyes by wearing glasses that fit closely to the face (Johnson & Murphy 2004). Some contact lenses made of silicone elastomers, which do not absorb water or permeate gas, protect the tear film. These contact lenses reduce evaporation and recurrent epithelial breakdown (Bacon et al. 1994). Contact lenses are discussed in the Chapter 4.3.3.

4.2.2. Dietary manipulation

Malnutrition is normally associated with lack of food in developing countries, but also occurring in developed nations as a result of inappropriate food selection, eating disorders and dictatorial fat diets. Inadequate nutrition can contribute to DES and other ocular surface diseases. It does not necessarily mean that DES can be successfully treated by dietary means with a good diet. Nutritional supplements are likely to be most beneficial in a patient with reduced ability to digest and absorb nutrients, due to age, chronic diseases, alcoholism or those with poor eating habits. Also pregnant and lactating women may benefit from a supplement, because of their increased nutritional requirements (Beers & Berkow 1999). Dietary supplement of essential fatty acids, such as linolenic and gamma-linolenic acids, omega-3 and omega-6-fatty acids have been recommended in the treatment of DES. Patient suffering DES should follow a well-balanced healthy diet, where vitamins such as A-, B_6, C-vitamins and mineral substances as potassium and zinc are recommended. (Caffery 1991)

4.2.3. Artificial tears

Artificial tears and lubricants are a palliative type of therapy and the most widely used therapy for dry eye today. The goal of using tear substitutes is to increase humidity at the ocular surface and improve lubrication. Artificial tears smooth the corneal surface of

the patients suffering of DES and some substances help restore the ocular surface after surgery. Natural tears have a complex composition of water, salts, hydrocarbons, proteins and lipids, which artificial tears cannot completely substitute. The integrity of the three-layer lipid, aqueous and mucin structure, vital to the effective functioning of the precorneal tear film, cannot be totally reproduced by artificial components. (Murube 1998a; Pflugfelder 1998)

Artificial tears can be delivered to the eye mainly by drops and ointments, which are dosed intermittingly rather than continuously as are natural tears. To overcome this problem the artificial tears are composed of ingredients that increase contact time with the ocular surface. These ingredients are designed to have mucoadhesive properties and formulate viscous gels. Viscosity can cause irritation, blur vision, make eyelids sticky and create a sensation of heavy eyelids (Ludwig 2005; Murube et al. 1998b). Highly viscous materials disadvantages can be overcome by different formulation strategies such as less viscous formulation which remains on the surface thanks to mucoadhesive properties. Artificial tears must also contain preservatives, stabilizers and other additives. These components supply stability and delay microbiological contamination and growth, but also ensure long shelf-life required for storage and commercialization. Most common preservatives are quartenary ammonium compounds such as benzalconium chloride, alcohols such as chlorobutanol and other compounds including chlorohexidin, sorbic acid, potassium sorbate, boric acid and biguanides. Preservatives are harmful when their presence is prolonged. Other common additives are buffers, whose purpose is to maintain pH, and electrolytes, which make artificial tears slightly alkaline (Murube 1998b). The buffering capacity of the natural tears depends mostly on bicarbonates and it is an essential component in the recovery of damaged corneal epithelial barrier and maintaining of normal structure (Ubels et al. 1995). Artificial tears are hypotonic electrolyte-based formulations, which are developed based on the recognition of the importance of tear osmolarity and electrolytes maintaining the ocular surface (Gilbard & Rossi 1992). Table 3 lists components that are used in artificial tear compositions to increase the retention time. Later on in Chapter 7 polymer properties and their trends are discussed more.

Table 3. Artificial tears and eye lubricates used in the therapy of the dry eye (Murube 1998a; Murube 1998b).

Dosage form/ brand name [1)]	Retentive polymer	Advantage	Notable
Drops/ Artelac ®, Celluvisc®, Ointment/Lacrilube® Lamella/Lacrisert® (Fimea 2010)	Cellulose ethers (CE) (hypromellose, methylcellulose, carmellose)	• Good retention time • Mix well with other ophthalmic products • Newtonian fluid	• Aqueous tear deficiency • Can cause crusting of eyelid, mimicking blepharitis
Drops/ Oftagel®, Viscotears®, (Fimea 2010)	Carbomer (CARP) (polyacrylic acid, PAA)	• Good retention time on ocular surface	• Tend to blur vision
Drops/ Oxyal®, (Santen 2008)	Hyaluronan (glycosaminoglycan, GAG)	• Support epithelial cells • Mimics mucin	• Low mechanical strength
Drops/ Oftan®, Liquifilms tears®, (Fimea 2010)	Polyvinyl alcohol (PVA)	• Beneficial in lipid, aqueous and mucin layer deficiencies	• Short retention time • Does not mix well with other ophthalmic products
Drops/ Oculac® (Fimea 2010)	Povidone (polyvinyl pyrrolidone, PVP)	Beneficial in mucin deficiency	-
Drops/ Lenzmoist® (Apteekkituotteet 2009)	Poloxamer (POL), carbomer (CARP)	• Surface activity • Emulsifier	• Insoluble recidues • Low mechanical strength • Low sol-gel-transition temperature
Drops and ointments/ SootheXP® emolient, (Bausch&Lomp 2009)	Lipids (vaselin, paraffin, lanolin, castrol oil)	• High viscocity and retention • Useful adjunct to consumption at night	• blur vision
Drops/ Restasis® (Allergan 2009)	Polysorbate with glyserin, castrol oil	• Long retention	• blur vision recommend night usage
Drops/ Bion® tears (USAEye 2009)	Dextran and hydroxyl propyl methylcellulose (HPMC)	-	-
Drops/Systane® (Tauber 2005)	polyglycol , polyethylene glycol (PEG)	• forms protective shield in the eye after administration induced by pH	-

[1)] Reference of the brand name is mentioned in parentheses in this column.

4.2.4. Punctal occlusion

Drainage holes, small circular opening at the inner corners of the eyelids where tears drain from the eye into the nose, can be plugged. Lacrimal plugs, also called tamponade methods or punctal plugs can be made of hydroxyl propyl methylcellulose (HPMC), gelatin, COL, silicone, teflon or polyethylene. These plugs are reversible, but in severe cases permanent plugs may be considered. Drainage holes can also be closed permanently in surgery called punctal cautery. Punctal occlusion retains both tears and supportive factors in the ocular surface, and has been shown to be more effective than frequent dosing with ocular lubricants (Murube & Murube 1996).

4.2.5. Stimulation of the tear production

Therapies that stimulate tear production have been limited by their side effects such as sweating. Muscarinic agonists have been used to increase lacrimal secretion by activating the cholinergic signal in transduction pathway in lacrimal acinar cells. For instance, orally administrated pilocarpin or cevimeline is used. (The dry eye digests 2009)

4.2.6. Treatment of inflammation

The only medication for chronic dry eyes approved by the FDA is cyclosporine A. It is a fungal-derived peptide and an immunosuppressive agent (Allergan 2009). Treatment effect is observed when systemically administered by mouth. Tetracyclines are compounds that have traditionally been recognized for their antibiotic properties. Nowadays they are also considered as antiinflammatory characters (Pflugfelder 2004) and used in the therapy of inflammation in dry eye patients.

4.2.7. Transplantations

In very severe cases of corneal damage, fetal amniotic membrane is used for ocular surface reconstruction (Sangwan et al. 2007) and limbal stem cells (Levin et al. 2004) have been transplanted by using autografts or allografts.

4.3. Potential therapeutic methods

Damage on the surface of the cornea is not improved rapidly without a therapeutic agent and without therapy the damage may lead to DES. Therapies mentioned above are mainly symptomatic and do not repair damaged areas. These methods keep the situation steady and more harm is not caused or damaging is significantly slowed down. (Murube 1998a) In following chapters, methods or molecules that have been shown potential therapeutic effects and are under development are discussed.

4.3.1. Trehalose

Trehalose is disaccaride composed of two glucose molecules, which are bound by α,α-1→1 lingage. The reducing end of trehalose is connected to other molecule, therefore trehalose is nonreducing sugar. Trehalose is a source of energy in most living organisms and can be found in bacteria, fungi, insects, plants and invertebrates. Organisms are

protected from stress, dryness, freezing and osmotic pressure by trehalose. Organisms which synthesize large amount of trehalose have the ability to tolerate lack of water. Trehalose plays an important role in stabilizing membranes and other macromolecular assemblies under extreme environmental conditions. Trehalose is one of the most stable saccharide with high thermostability and wide pH-stability range. Trehalose has suppressive properties for starch retrogradation, protein denaturation and lipid degradation. Trehalose is degradated by trehalase enzyme, which is produced in the small intestine, where it is digested to glucose. (Higashiyama 2002)

Trehalose was earlier produced by extraction from yeast, but low yield and high cost inhibited utilization. Later on a generated method provided a better yield at lower cost by enzymatic trehalose production from starch. After innovation of the effective production method the research of the trehalose applications has been increased (Maruta et al. 1995). In *in vitro* tests trehalose was found to protect corneal epithelial cells from death by drying. Lipids and proteins on the membrane of the cell are stabilized by trehalose under desiccation (Matsuo 2001). Protective property under desiccation is an ability which is helpful in DES. Therefore trehalose has been investigated *in vivo* as an eye drop agent (Matsuo et al. 2002; Matsuo 2004). In these studies trehalose-saline solution was dropped to the eye four times a day and was found effective and suitable for therapy of DES.

4.3.2. Autologous serum

Fox et al. described in 1984 that autologous serum had therapeutic characters for the ocular surface disorders by growth factors, electrolytes and vitamins, which exist in serum (Tsubota et al. 1999). They found that corneal erosion healed with autologous serum faster than with conventional treatment (Shimmura et al. 2003; Schrader et al. 2006). Autologous serum has been used as medical treatment of DES, but it is available only in clinical use. Autologous serum has low stability and it has to be produced just for individual needs. It is difficult to offer it to common markets due to producing difficulties and costs (Geering et al.2004). Shimmura et al. (2003) researched serum derived human albumin effects on the corneal epithelium. They found that albumin as a protein supplement in artificial tear solution had viable approach in the treatment of ocular surface disorder dealing with tear deficiency.

4.3.3. Contact lenses

Contact lenses are eye inserts whose first aim is to improve vision, but other advantages might be the protection of the epithelial cells and the prevention of the tear film evaporation. In a moderate case of DES, symptoms can be reduced by wearing contact lenses. The organ of seeing is sensitive and therefore the contact lenses should be biocompatible, appropriate wettable, mechanically adequate, having high gas permeability and resistance to degradation. (Bacon et al. 1994; FDA 2009; Johnson & Murphy 2004) In Table 4, contact lenses available commercially are collected.

Table 4. Contact lens classification (FDA 2009).

Contact lens type	Base matrix polymer	Advantage	Disadvantage
rigid lens	polymethyl-methacrylate	• easy to handle	• poor oxygen permeability
hydrogel soft lens	poly(hydroxyethyl-methacrylate)	• good swelling properties	• corneal abscess
rubbery soft lens	silicone rubber, polydimethylsiloxane	• flexible • high oxygen permeability	• hydrophobic
gas permeable rigid lens	cellulose acetate butyrate	• high oxygen permeability	• tends to wrap and scratch easily

Contact lenses have been found to be a potential drug or lubricate agent delivery platform. Therapeutic agent is loaded to the lens and released during wearing. One day lasting delivery is achieved by a cross-linked, modified, molecular imprinted or liposome laden contact lens. Three days' delivery is formed with nanoparticulate laden contact lenses and even five days is attained by biomimetic HA loaded lens *in vitro* (Venkatesh 2006). Scahrader et al. (2006) found that serum eye drops with hydrogel bandage contact lens gave beneficial effect to the corneal epithelium.

5. Requirements of the therapeutic system in the eye

The eye, especially a damaged one is a very sensitive organ. It is very essential that polymers used in the eye are non-toxic, biocompatible and cause no irritation, inflammation or any harm. Polymers should mimic mucus, swell and retain water, stay for a long period of time in the precorneal area and therefore provide a protecting effect by strengthening and substituting the mucus layer (Ludwig 2005). The polymer should not affect the clear vision. High turnover of the tear film and another flux of the fluids are challenging for a polymer, which is supposed to lubricate and sustain in the eye as long as possible, but still it must be biodegradable. Polymer should retain on the surface of the eye, in the other words should be muchoadhesive by physical, chemical and/or biochemical interactions. Dosing of the polymer into the eye is meant to be done seldom (once a day or less often) and it is suppose to be biodegradable almost in same time. If polymer is a carrier of the drug, bioavailability, loading capacity and long sustained release are characteristics that are also required. Concentration of the drug molecule in the eye should stay even between dosing times and side effects should be minor. (Johnson & Murphy 2004; Ludwig 2005; Saettone 2002)

6. Therapeutic delivery and delay methods

Tear film is the first layering that polymer in the corneal surface that therapeutic or lubricate eye drops makes contact with. Mucus layer on the eye is main component which interact with polymers and causes retention. Therapeutic impact is formed by delaying the polymer on mucus, sustaining release of the drug and finally biodegradation and disappearing of the polymer. Viscous eye drops sustain on the surface of the eye due to viscous character, but blur vision. Cationic and anionic polymers act by ionic interaction and/or weak hydrogen and van der Waals bonds. (Ludwig 2005) In Table 5, sustaining methods useful in ocular drug delivery are presented.

Table 5. Delay methods of the eye drops.

retentive property	**advantages**	**disadvantages**	**example of commercial applications in DES**	**references**
viscous delay	thicken tear film	mainly non soluble in water	oil, glycerol	Felt et al. 1999
hydrogel	good swelling behaviour	low mechanical strenght	HA	Nanjawade et al. 2007
in situ forming hydrogel	administration in liquid form and gelation in the eye	transition temperature optimization is troublesome	POL	Hoffman 2001
nanoparticle	large surface area, penetration to the mucus and drug loading cabacity	behaviour in the eye needs more research	not available	Sahoo 2008
thiolated polymer	mucoadhesion by covalent bonds	instability in aqueous solutions	not available	Bernskop-Schnűrch et al. 1999
other functionalized polymer	mucoadhesion and penetration	behavieour optimization can be troublesome	CE	Ludvig 2005

In situ forming hydrogels are formed by administration of the liquid dosage eye drops into the corneal epithelium and transforming is induced by temperature, pH and/or osmolality change (Felt et al. 1998). Many polymers have mucoadhesion properties but more effective adhesion properties are preferred. Modification of the polymers has

achieved better adhesion, which can be executed by adding functional chemical groups in to polymer (Bernskop-Schnűrch et al. 1999; Ludwig 2005). Functional group gives to the polymer partial hydrophilic, hydrophobic or charged characters, which are causing more hydrogen bonds, self linkages or even covalent bonds between the mucus layer and the polymer. Nanoparticles are submicron level molecules that penetrate effectively to the mucus layer and better bioavailability is achieved (Sahoo 2008).

6.1. Viscous delay

Viscosity induces and increases contact time in corneal epithelia by reducing the drainage rate and improves the overall bioavailability of an instilled solution. The viscosity of the polymer solution is a measure of resistance to flow, which is complex function of its MW, concentration, temperature and shear stress. Flow properties of the fluids can be divided into two categories: the Newtonian and non-Newtonian fluids. Newtonian fluids are independent of the shear rate and viscosity stays constant during blinking and fixation, while viscosity of the non-Newtonian fluids decreases during blinking and eye movements. (Nanjawade et al. 2007) Although viscous solutions lubricate well, blur of vision and sticky eyelids are their main disadvantages (Saettone 2002).

6.2. Hydrogel forming

As mentioned in the previous chapter, the most common way to improve polymer and drug retention on the corneal surface is to use polymer matrix that increases viscosity of the solution, which character occurs in hydrogels (Felt et al. 1999). Hydrogels form a cross-linked hydrophilic insoluble network and may absorb up to 1000-fold (Hoffman 2001) their dry weight in water, giving them physical characteristics similar to soft tissue. Hydrogels swell in aqueous environment and liquid-gel transition is induced. Hydrogels are highly permeable, which assist exchange of oxygen, nutrients and other water soluble metabolites. Preformed hydrogels are viscous solutions, which are not undergoing any modifications after administration (Felt et al. 1999). *In situ* forming hydrogels are meanwhile liquid, suspension or solution formulations that induce gel-form after installation due to changes in temperature, pH or electrical composition (Felt et al. 1998). *In situ* forming gels are easily administered in liquid form to the eye, and the effect is the same as the effect of eye drops made of preformed gel. Temperature

induced hydrogels are performed when polymer is contact with body fluids in temperature 35 – 37 ^{o}C (Hoffman et al. 1986). Intramolecular hydrogen bonds promote temperature induced gelation. A polymer used on the surface of the cornea should have a suitable phase transition temperature range and form gel just in ocular temperature. The phase transition temperature, in which a gel is formed, can be adjusted for example by adding or lowering amount of NaCl in liquid or cross-link polymers by substituent. Change of pH induces gel formation from the liquid state of the polymer. Interactions that form gelation are electrostatic interactions, hydrogen bonding, hydrophobic interactions and interdiffusion. Functional groups of the polymer interact with protons at low pH-values and keep the polymer in an inactive form. When the pH is increased the protonated functional groups of the polymer lose their protons and form interactions. Osmotically induced gelation is induced by ions that exist in physiological liquids in tear film. The divalent ions such as calcium and magnesium form better gel than monovalent ions due to ionic bridge. However concentration of the monovalent sodium (2.6 mg/l) is sufficient to induce gelation. (Felt et al. 1999)

6.3. Nanotechnology

Nanotechnology applies principles of engineering, electronics, physical and material science and manufacturing at a molecular or submicron level. Materials at nanoscale could be a device or a system or supramolecular structure, complexes or composites. In nanotechnology dimensions and tolerances are in the range of 1 nm up to 1 µm, while microspheres are in the range of 1µm to 1mm (Institute of nanotechnology 2009). Surface of the cornea is less irritated by small particles compared to the original suspension and nanotechnology provides this property. Particles in nanoscale have a large reactive area of the surface and penetration to the mucus is better compared to the original suspension. Better gel formation is also achieved by increasing formation of the interactions due to hydrogen bonds. Nanotechnology gives promising ways of delivering poorly soluble drugs to the eye (Sahoo 2008), but also delivery of the wetting polymer to the surface of the cornea is easier. Nanoscale suspensions and particles with inert polymeric matrix can be utilized for drug delivery systems which are capable of prolonging drug release and enhancing bioavailability. Nanotechnology-based drug delivery is also found very efficient in crossing membrane barriers, such as the blood retinal barrier in the eye (Sultana et al. 2006).

A microemulsion is a dispersion of water and oil with a surfactant and co-surfactant in order to stabilize the interfacial area. A microemulsion is easy to prepare and sterilize, is stable and has high capacity for drug dissolving (Vandamme 2002). A nanoparticle is a small polymeric colloidal particle with a therapeutic agent either dispersed in the polymer matrix as nanosphere or encapsuled in the polymer as nanocapsule. A nanosuspension consists of pure, poorly water-soluble drugs, suspension in an appropriate dispersion medium. A liposome is a bilayered artificial vesicle, which can be produced from natural phospholipids and cholesterol. A liposome is amphiphilic and well tolerated in the eye due to its lipid composition simulating cell membrane. A positively charged liposome is most affinitive to interact with the surface of the cornea, while a negatively charged one is less and a neutral liposome is least, which means that initial interaction between the surface of the cornea and liposome is electrostatic in nature. Liposomes have the ability to enhance corneal penetration of the drug by being absorbed onto the corneal epithelial cell membrane. Although liposomes seem to be potential for drug delivery device to the corneal surface, there is few targets for development. Liposomes have a short shelf life, limited drug loading capacity and also preparation and sterilization difficulties (Sahoo 2008). Niosomes are non-ionic surfactant vesicles and almost like liposomes in structure (Kaur et al. 2004), but they are chemically stable, can entrap both lipophilic and hydrophilic drugs (Carafa et al. 1998, Guinedi et al. 2005) and enhance bioavailability into lipophilic membranes (Carafa et al. 2002). A discome is a large structure (12 – 16 μm) derived from niosomes by addition of non-ionic surfactant. Discomes have better loading capacity of drugs and the disc shape provides a better fit in the cul-de-sac of the eye (Vyas et al. 1998). Dendrimers are macromolecule compounds made up of series of branches around an inside the core. Dendrimers are an attractive system for drug delivery due to its nanometric size, easy to prepare and functionalize, but also ability to carry multiple kind of functional groups. Drugs can be loaded on the inside of the dendrimer and outer branches are added by biological recognition processes (Ihre et al. 2002). Cyclodextrins are cyclic oligosaccharides composed of dextrose units joined through a 1→4 bond and capable of forming inclusion complexes with many drug molecules. Cyclodextrins have six to

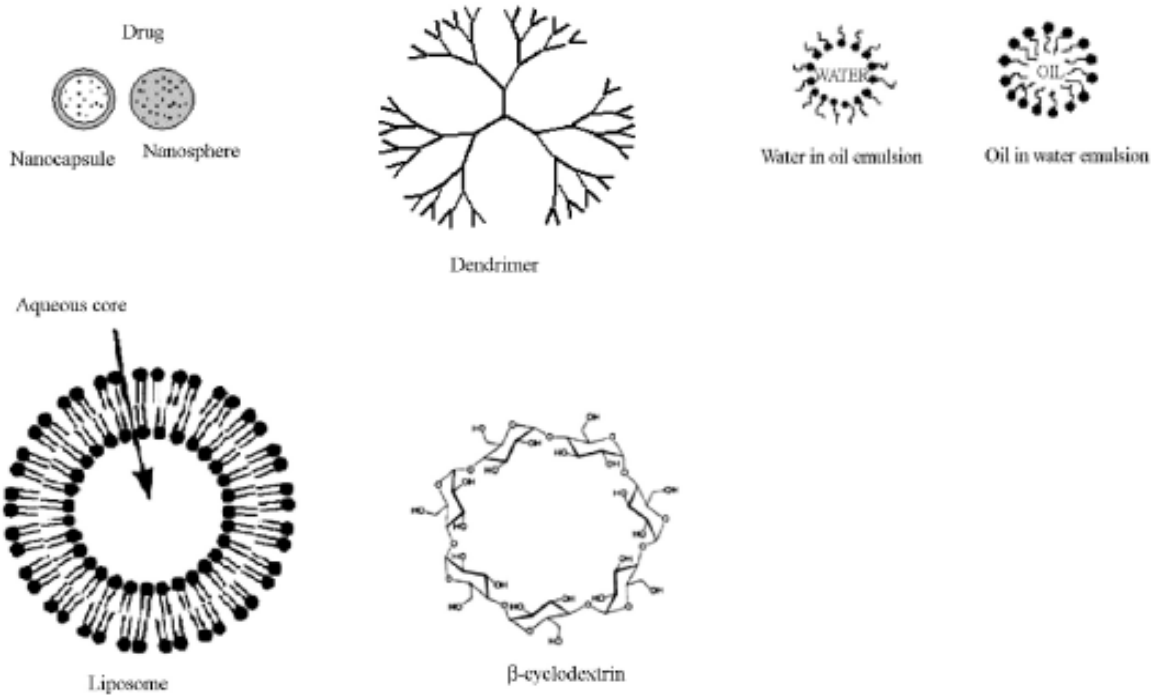


Figure 12 *There are different particular based drug delivery systems. Nanoparticles are small polymeric colloidal particles with therapeutic agent either dispersed in the polymer matrix or encapsulated in polymer. Dendrimers are mono dispersed symmetric macromolecule built around a small molecule with an internal cavity surrounded by a large number of reactive end groups. Microemulsions are dispersions of water and oil with surfactant and co-surfactant in order to stabilize the interfacial area. Liposomes are small artificial vesicles of spherical shape that can be produced from natural phospholipids and cholesterol. They can encapsulate drugs inside the cavity or between the bilayers depending on the hydrophilic or hydrophobic nature of the drug. Cyclodextrins are a group of cyclic oligosaccharides capable of forming inclusion with many drugs. (Sahoo 2008, Sahoo & Labhasetwas 2003)*

eight dextrose units in a cycle and named α-, β- , γ- cyclodextrin respectively (Rajewski & Stella 1996). Trough cyclodextrin some hydrophobic drugs can be enhanced without changing their molecular structure or their ability to permeate biological membranes. Cyclodextrin acts as true carrier by keeping hydrophobic drug molecule in solution and delivering them to the surface of the corneal epithelium (Sahoo et al. 2008). In Figure 12 some nanotechnology structures used in the drug delivery systems are represented.

6.4. Polymer modification

Polymers used in ocular therapeutic drug carrier, matrix or therapeutic agent have properties that suit somehow to the ocular environment, but may also have disadvantages. Mixing two or more polymers together result in blend that has the good characters of both components or resultant is something between that or even one that worsens the properties. Better combinations might be achieved by mixing, blending, cross-linking and functionalizing polymers to a formulation that penetrates more efficiently to the mucus and interacts with the mucus better than a polymer without modification. Swelling properties and drug carrying properties can be improved by modification with a functional group. A modified polymer can be turned to beads, particles, liquids, films or lamellas by which degradation speed, retention time or drug release profile can be adjusted. (Qi et al. 2007; Ma et al. 2007; Bonferoni et al. 2003; Ceulemans et al. 2002; Cao et al. 2007; Motowani et al. 2007)

6.4.1. Thiolation

Thiolation is one type of modification method of the polymer and has recently been succeeding protocol to achieve better muchoadhesive properties (Bernskop-Schnűrch et al. 1999).

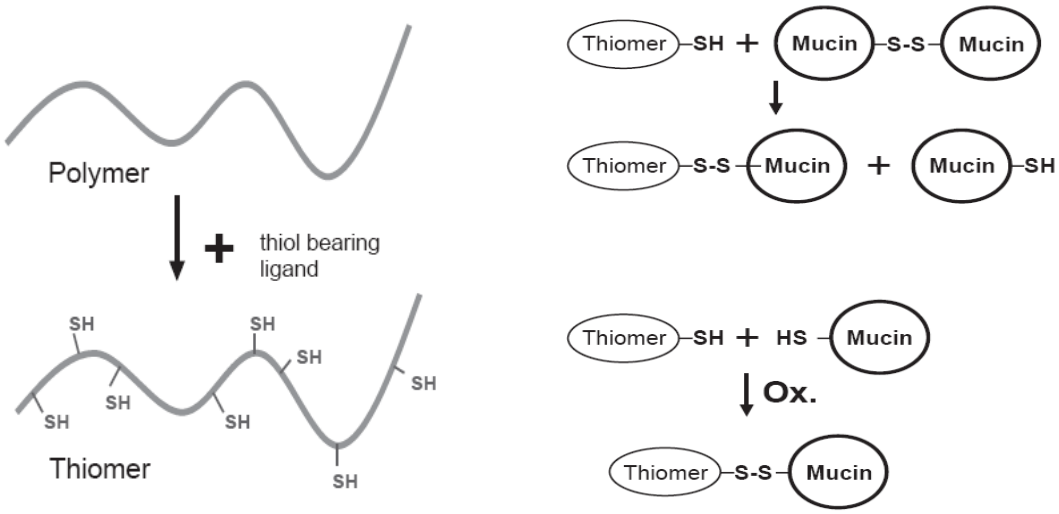


Figure 13. *Left: A polymer is coupled with a thiol group bearing ligand and the result is a polymer composing with reactive thiol groups. Right: Thiolated polymer couples with thiol groups of the mucin via thiol/disulphide exchange reactions or via oxidation reaction forming covalent sulphur bridges. (Leitner et al. 2003; Bernskop-Schnűrch 2005).*

The principle of the thiolation is that a sulphur containing molecule, peptide or amino acid residue is connected to the polymer resulting in a thiomer (Figure 13). Thiolated polymers are capable of forming covalent bonds, disulfide bridges to the Cys-rich subdomains of mucus glycoproteins (Leither et al. 2003). Thiomers mimic secreted mucus glycoproteins, which are also anchored covalently to the transmembrane mucin. Thiomers are capable of forming cross-links *in situ* itself, which can be another mechanism for improved mucoadhesion (Figure 14). Thiolated polymers perform *in situ* gelling properties due to oxidation of thiol groups at physiological pH-values, which result in the formation of inter- and intramolecular disulfide bonds. Disulfide bonds are not influenced by factors such as ionic strength, but velocity and extent of disulfide bond formation depends on the concentration and on thiolated anions representing in reactive form for thiol/disulfide exchange reaction and oxidation processes. The concentration of the thiolate anions depends on pKa of thiol group, pH of the thiomer and pH of the surrounding medium (Bernskop-Schnűrch 2005).

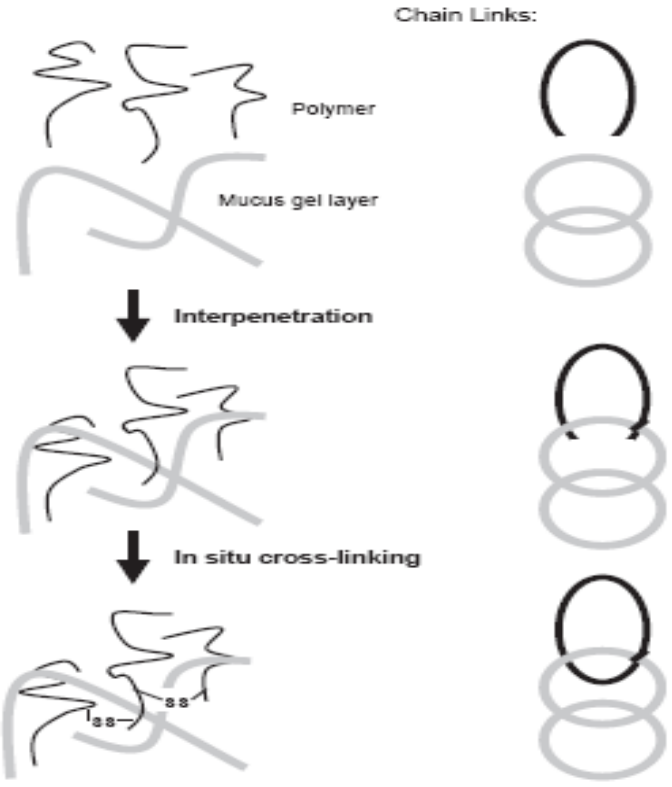


Figure 14*. Presentation of improved mucoadhesion by an* in situ *cross-linking comparison to the chain links according to Bernskop-Schnűrch (2005). The uppermost chains are free, the middle chains are penetrated to each other and lowest chains are additionally cross-linked by themselves.*

Thiomers are shown stable when stored in dry form. In aqueous solutions, thiomers form disulfide bonds in a pH dependent manner (Bernskop-Schnűrch 2005). At higher pH values the decreasing H^+-consentration leads to a higher amount of negative thiolate anions representing active form for oxidation resulting inter- and intramolecular disulfide bonds (Hornof 2003). Thiomers have not usually been used in liquid formulations due to this instability in aqueous solutions, but liquid formulations can be produced under inert conditions and vessels are packed in an aluminium foil containing an oxygen scavenger such as iron-oxides inside (Bernskop-Schnűrch & Hornof 2003a).

6.4.2. Other type of the polymer modifications

The main idea in the polymer modification or functionalization is to achieve better behaviour in a specific environment (Ludwig 2005). The Ocular environment is composed of hydrophilic areas: tear film outer layer and epithelial cell plasma membrane and hydrophilic areas: tear film aqueous layer and mucus layer. (Lang 2006) Good spread, delay and lubrication on the eye are achieved with polymers having both hydrophilic and hydrophobic character. Naturally lubrication is done by glands (Lang 2006), which administer tear film components straight to the right place, but drop wise dosed polymers are derived topically (Johnson & Murphy 2004). Therefore the composition of lubricating eye drops is preferred to be ambiphilic. The backbone of the hydrophilic polymer can be functionalize by hydrophobic substituent. Added substituents affect to polymer solubility, gelation and degradation rate, drug loading capacity and mucoadhesion ability (Wang 2007). Transmembrane mucin is positively charged and mucoadhesion occurs with negatively charged polymers. Polymers can be substituted by charged functional groups, which intensify interactions to the mucin or cross-links with itself forming a complicated net. (Hahn et al. 2006; Krauland et al. 2002; Liu et al. 2008; Nochos et al. 2008)

7. Polymers and their derivatives

Polymers used in ocular surface application can be classified in many ways. The pharmaceutical polymers are usually classified according to their origin, but can also be classified according to the state or administration dosage form. The production or modification method can be based on the classification, but also the therapeutic or eye surface sustaining method (Ludwig 2005). In Table 6 polymers are classified by origin, but in further chapters classification is done by charge.

Table 6. Classifying of polymers by its origin.

Type	**Polymer**	**origin or synthesis**	**sustaining method**	**charge**	**reference**
Natural origin polymers	Hyaluronan (HA)	connective tissue	swelling and adhesion	non-ionic	Chong et al. 2005
	Chitosan (CS)	cuticles of crustaceans	gelation and adhesion to the mucus	cationic	Kurita 2006; Di Mario 2008
	Alginate (ALG)	brown seaweed and bacteria	gelation	anionic	Nanjawade et al. 2007
	Dextran (DEX)	*Lactobacillaceae* fermentation	gelation	non-ionic	Pharmacosmos 2009; Cyper-Colloids library 2009
	Xanthan (XAN)	*Xanthomonas campestri* fermentation	gelation	anionic	McCormic et al. 2004
	Carrageenan (CARR)	red seaweed	sulphur bridges	anionic	FAO 1990
Semi-synthetic polymers	Cellulose ether (CE)	cellulose derived from plants and etherified	swelling	non-ionic	Feller & Wilt 1990
Synthetic polymers	Polyvinyl alcohol (PVA)	vinylalcohol polymerization	swelling	non-ionic	Marten 2002
	Carbomer (CARB)	crosslinked PAA	swelling and adhesion	anionic	Hosmani et al. 2006
	Poloxamer (POL)	PEO-PPO-PEO blocks	swelling and adhesion	non-ionic	Ruel-Gariépy & Leroux 2004
	Polyvinyl pyrrolidone (PVP)	polymerization of vinyl pyrrollidone	swelling and adhesion	non-ionic	Login 2004.
	polyglycol	polymerization of ethylene oxide	viscous	non-ionic	Tauber 2005

This chapter discusses polymers used in the disorders of the eye, which are dosed topically to the eye. There are covered physical and chemical properties, drug carrier

characters and sustaining protocol of the polymer, but also latest researched conquests. In appendices 1 - 4 is a listed summary of polymers discussed.

7.1. Anionic polymers

7.1.1. Hyaluronic acid

Properties of hyaluronic acid

Hyaluronic acid or hyaluronan (HA) is an anionic biodegradable and natural linear polysaccharide with broad MW range from 1 kDa to 10.000 kDa (Hahn et al. 2006). In a large molecule of HA, there can be over 30.000 repeating units (Price et al. 2007). HA is a main component of an extracellular matrix (ECM) and the only non-sulphated GAG, which control tissue hydration, contribute tensile strength and elasticity as a result of strong interactions with other components of the matrix (Kogan et al. 2007). HA regulates cell adhesion and motility, but also mediates cell proliferation and differentiation and for example takes part in wound healing. HA is naturally found in many tissues of the body, such as skin, cartilage and the vitreous humour. HA is soluble in water and forms a clear, highly viscous solution that serves lubricants in the synovial fluids of joints and jellylike gel into aqueous and vitreous humour of the eye (Kogan et al. 2007). HA may be produced from a number of sources but the two most common are extraction from rooster comps and recombinant production using *Streptococcus*-bacterium (Chong et al. 2005). Each source produces slightly different kinds of HA and have different kinds of rheological properties. In animals, HA is formed at cell membrane of fibroblast by extrusion into the ECM. Fibroblasts also elaborate hyaluronidase (HAse), the HA degradation enzyme. (Kogan et al. 2007)

HA is a disaccharide, which contains alternating residues of D-glucuronic acid and N-acetyl-D-glucoasmine with β-(1→4) interglucoside lingage (Figure 15). Hydroxyl and carboxyl groups are functional groups of the HA, whereupon mucoadhesion mainly occurs (Chong et al. 2005). HA is a water-soluble polymer and is degraded and eliminated by oxidation and enzymes. Native HA have poor biomechanical properties. Better biomechanical strength without loss of biocompatibility or biodegradation is achieved by chemical modification of the hydroxyl and carboxyl groups of the HA (Prestwich 2001).

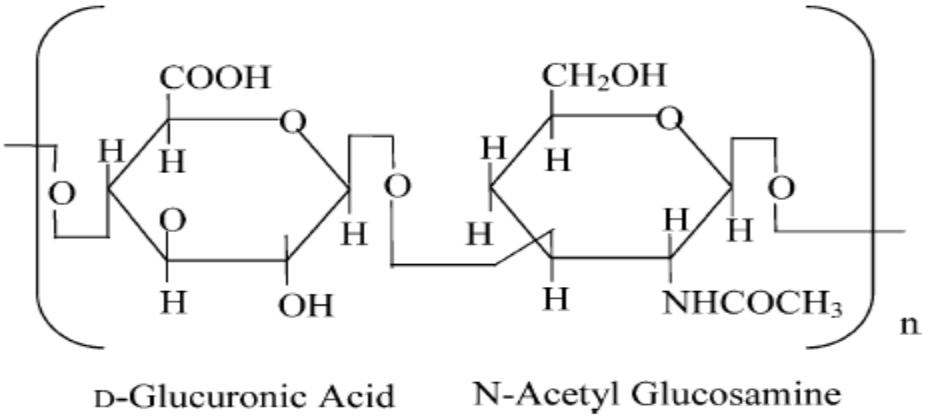


Figure 15*. Molecule structure of the HA. Repeating unit is D-glucuronic acid and N- asetylglucosamine linked together by alternating β-1→-3 and β-1→-4 glycoside bond (Chong et al. 2005).*

Trends of hyaluronic acid

HA is a polymer found naturally in many tissues of the body and therefore it is well suited for biomedical applications in the human body. At present there are available retentive, hydrative and epithelial cell conditioning artificial tears composed of HA from many producers (Apteekkituotteet 2009), but further development is needed to achieve improved mucoadhesive properties, mechanically robust and metabolically stable HA derivatives (Prestwich 2001).

HA has been the subject of studies where the aim has been to generate a polymer conjugate that degrades slower and has better biomechanical properties in physiological solutions than bulk HA. Although HA is naturally mucoadhesive (Chong et al. 2005), this character has been attempted to improve. Thiolation takes place usually by covalent coupling with L-Cys residue or conjugating it to HA molecule via formation of an amide bond with force of cross-linking mediator. HA-Cys conjugates can form cross-links between thiol groups by oxidation in air at room temperature resulting disulfide bonds and network trough out polymer. This network and *in situ* forming intermolecular disulfide bonds with tissue matrix lowered biodegradation rate and ability to release therapeutic molecule up to 12 h. *In vitro* studies with freshly excised porcine intestinal mucosa in the rotating cylinder method has been shown that adhesion time to the intestinal mucus were 6.5-fold greater than unmodified HA. Cross-linking of the HA-

Cys conjugate via disulphide bonds presence of HAse in aqueous solution reduces degradation rate comparing to non-cross-linked HA (Kafedjiiski et al. 2007).

In another application of the thiolated HA Shu et al. (2003) was coupled with electrophilic diacrylate (DiAc) conjugate of PEG (PEG-DiAc) to achieve injectable scaffold hydrogel, which form cross-links rapidly *in situ*. Cross-linking between PEG-DiAc and thiolated HA occurs in 9 minutes. The polymer complex was found cytocompatibile *in vivo* with mice and only little degradation was noted without HAse, but was increased with it. HA has also been cross-linked by adipic acid dihydrazine (ADH), metacrylic anhydride (MA) and imminothiolane (ISH) aim to form stronger hydrogels. *In vitro* and *in vivo* environments HA-ISH hydrogel degradated totally within 2 weeks whereas HA-ADH and HA- MA hydrogels degradated only partially in 29 days (Hahn et al. 2006). These above-mentioned cross-links and modifications provide slower degradable HA conjugate, which is also the aim of ocular surface applications. These HA studies showed longer degradation time (Prestwich2001; Kafedjiiski 2007; Hahn 2006) and faster gelation *in situ (*Shu et al. 2003), therefore operation in the eye environment may be possible, though more information is needed about appropriate composition, biocompatibility, mucoadhesive properties and behaviour in liquid state. These applications are mainly focused to soft tissue reconstructions and surrounding is different than in the eye, but may be potential as *in situ* -forming hydrogel in the surface of the eye.

7.1.2. Carbomers

Properties of carbomers

Carbomer (CARB) is a cross-linked polymer of high MW poly (acrylic acid) (PAA) (Figure 16). Acrylic acid (AA) monomer has bifunctional character by carboxylic acid and conjugated unsaturated double bond. In the polymerisation of the AA double bond breaks and carboxylic acid residue stays constant, but acts as a functional part in the cross-linking. Cross-linking and copolymerization protocol is done mainly by radical polymerisation with vinyl monomers, which have highly reactive double bonds (Felt et al. 1999). CARB is hydrophilic and usually insoluble in water depending on its structure and cross-links, but acidic carboxyl groups of the CARB dissociate partially with water molecules producing flexible coil. CARB adsorbs water, gets hydrated and swells up to 1000 times to its original volume and 10 times to its original diameter to

form a long lasting gel when exposed to pH below 6. Carboxyl moiety on the polymer backbone is ionized in pH from 6 to 0.5, resulting in repulsion between negative charges and hydrogen bonding is formed. (Hosmani et al. 2006; Ceulemans & Ludwig 2002) Ionized carboxyl groups of the CARB and sialic acid of the mucin interact via hydrogen bonds, but at pH 7.4 ionized groups rebel with each other and decrease mucoadhesion (Ludwig 2005). The glass transition temperature of the CARB is 105^{o}C (Hosmani et al. 2006), but decreases significantly as the polymer comes into contact with water, where chains start to gyrate and radius of the gyration becomes increasingly larger (Craig et al. 1994). CARB is a well tolerated polymer in the topical administration due to its non-penetrative character into epithelial cells, but acidic nature of the CARB may irritate the eye tissue at higher concentrations (Nanjawade et al. 2007).

Figure 16. *CARB alias cross-linked PAA. Left: repeating unit of the PAA and right: cross-linked PAA (Hosmani et al. 2006).*

CARB is commercially available in a wide range of MW and either as linear, branched or cross-linked, which affects to the polymer properties. CARBs, which have a higher cross-linking level, have lower equilibrium swelling ratio and lower adhesion ability due to the lower amount of free reactive carboxyl moieties, but also due to the lower elasticity. CARB works as emulsifier in an oil-in-water system at elevated temperatures, but has also antimicrobial character. (Hosmani et al. 2006; CarboMer 2009)

Trends of carbomers

CARB is commercially used in many pharmaceuticals applications and also in lubricative therapy for DES. Different kinds of CARBs are available (table 7) depending on their polymerization technique and crosslink levels (Hosmani et al. 2006). The pharmaceutical grade of CARB 974, cross-linked PAA is widely used in eye drops of DES therapy and the administration rate of these is one drop four times a day according to the symptoms (Fimea 2010). Other CARB modifications have also been used in eye therapy and these types differ from each others in cross-linking density. CARB has been successfully used as a drug carrier on the eye drops due to its retentive affect to the drug molecule and ability to adhere into the mucin (Ludwig 2005).

Table 7. Structure and polymerization techniques of CARBs mentioned in this thesis (Hosmani et al 2006).

Carbomer grade	**Structure**	**Solvent used in polymerization**
CARB 974	homopolymer	ethyl acetate
CARP 980	homopolymer	cyclohexane and ethyl acetate
CARB 1342	copolymer with long chain alkyl acrylate	benzene

The interactions between CARB 974 and mucin are limited and better results with sonication have been tried to approach. Sonication is a homogenizing method, where MW of the polymer decreases, some polymers splits into smaller parts and formation of the clumps can be avoided. Better CARB derivatives have been evolved by cross-links and copolymerization with other polymers. CARB 1342 cross-linked with lipophilic long chain alkyl acrylate shows better mucoadhesive properties comparing to the CARB 974, but must be sonicated. CARB 980 is the most efficient thickener of all CARBs and has better adhesion to the mucus than CARB 974. Interactions between CARB and mucin occur in corneo-conjunctival epithelium, where the amount of the mucin is high. There are fewer interactions in the tear film; where amount of mucin is lower. (Ceulemans & Ludwig 2002; Lubrizol 2010) A lipophilic water insoluble drug is difficult carry with hydrophilic CARB. Trough addition of tetraglycol, which is absorption enhancer and dissolver of lipophilic drug molecule, to the suspension bioavailability of the drug molecule can increase. Thermal treatment is needed for clarifying and thickening of the suspension (Bonacucina et al. 2005).

7.1.3. Carrageenan

Properties of carrageenan

Carrageenan (CARR) is a linear and highly sulphated disaccharide produced by red seaweed. It is composed of a 1→3-linked β-D-galactopyranose and 1→4-linked α-D-galactopyranose units (Figure 17). CARR is a group of structurally related polysaccharides differing primarily in the proportion and position of galactose, ester sulphates and 3→6-anhydro galactose, depending on the producing seaweed. CARRs are divided into the three families according to the position of the sulphur groups in the 1→3- and 1,4-linked galactose residues. In the beta (β) family the galactopyranosyl residue is not sulphated, in the kappa (κ) family the 1→3-linked units are sulphated at C-4 position and in the lambda (λ) family sulphated substituent occurs at C-2 on the 1→3-linked units. (FAO 1990; Chaplin 2008; Verschueren et al 1996)

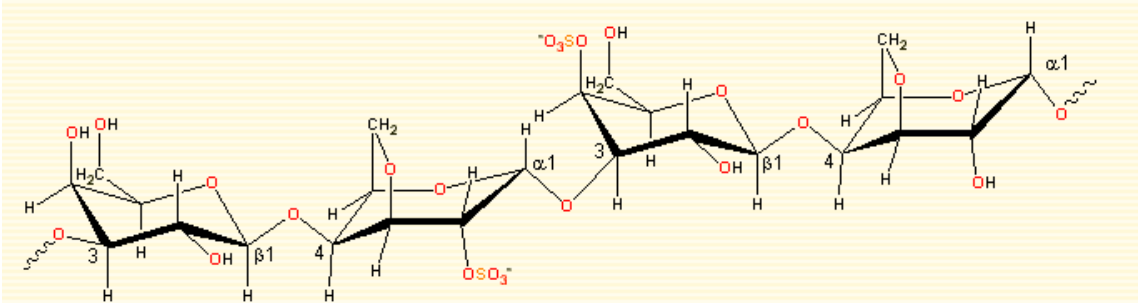


Figure 17*. Molecule structure of CARR (Chaplin 2008).*

Water solubility of the CARR depends on the molecule composition and it is more soluble in hot water than cold. Viscosity of the CARR, which increases almost exponentially with concentration, depends also on the temperature, the presence of other solutes, the type of CARR and its MW, which can be over 100 kDa. Viscosity of the CARR is lowered by salts, which reduces the electrostatic repulsion among the sulphate groups. CARR gel is thermally reversible, which means that they remelt upon heating and form a gel again upon cooling. Gelling temperature is depending on the type of the CARR and presence of the potassium and calcium ions may raise the gelling temperature with the concentration added. CARR is a strongly anionic polymer, which can interact with positive charges in the physiological fluids and with charged amino acid residues for example in mucin (FAO 1990; Verschueren et al 1996).

Trends of carrageenan

In pharmaceuticals, CARR is used in emulsions and suspensions (FAO 1990). Focus of the CARR in the eye drops is to be a viscosifier, but mucoadhesive properties and capability to interact with cationic drug molecules are also of interest. These properties have been a reason for further studies with a CARR and gelatin (GEL) polymer complex to prepare films and nanoparticles loaded with cationic drug. CARR-GEL polymer complex showed better mechanical properties and drug release profile in artificial lacrimal fluid than pure GEL or CARR. *In vivo* tested microspheres made of CARR-GEL showed better bioavailability than conventional eye drops. Detachment force increased with amount of the GEL in CARR-GEL complex, while pure CARR showed negative value comparing to the blank sample. GEL was in these circumstances responsible for mucoachesive properties. (Bonferoni et al. 2003)

7.1.4. Gellan gum

Properties of gellan gum

Gellan gum (GELG) polymer produced by spiesies of S*phingomonas* and is composed of glucose, glucuronic acid and rhamnose (Figure 18) in the molar ratio 2:1:1 (Johansson et al. 1983). These are linked together to form a linear tetrasaccharide repeating unit (BeMiller 2005) and partially esterified with L-glyserate (L-glyc) and acetate (Ac) (Kuo & Mort 1986). GELG has good thermal stability and its solutions make gel when any cations are present. Divalent cations are more effective than monovalents (Nanjawade et al. 2007).

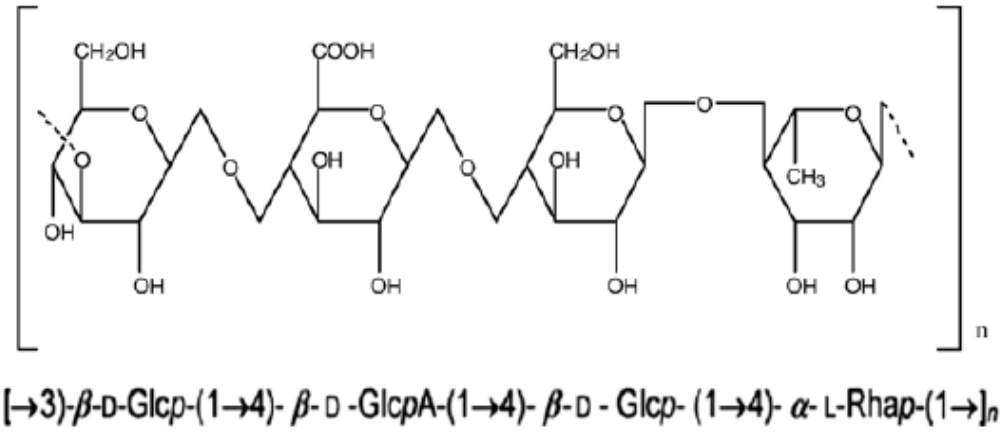


Figure 18. *Repeating unit of GELG, which is composed of glucose, glucuronic acid and rhamnose (Nanjawade et al. 2007)*

In an ion-free aqueous medium GELG forms double helix at room temperature. This solution has viscosity like water and helices have weak associations with each other. The gel is promoted by cations, which aggregate and cross-link the polymer. GELG properties can be adjusted by de-esterification at alkali treatment. When heating GELG solution in ion-free circumstances, the polysaccharide becomes a disordered coil, while heating in presence of cations, non-aggregated helices melt out first and the aggregated helices melt out at higher temperatures in a second transition. (Nanjawade et al. 2007)

Trends of gellan gum

Commercial product of GELG is Gerlite ®, which is totally deacetylated (DE). Gerlite® has been used as *in situ* gel-forming agent in the eye drops of the glaucoma therapy (Nanjawade et al. 2007). This composition has shown good bioavailability and administration is done once a day, while same eye drop without Gerlite® is dropped twice a day to achieve same drug response (Shedden et al. 2001). Krauland et al. (2002) developed deacetylated GELG occupied L-cys groups mediated by carbodiimide. This thiolated GELG was stored in a freeze dry form and hydration to aqueous solution at pH 4 to avoid disulfide bonds formation avoided during hydration. Thiolated GELG has been shown *in situ* gelation *in vitro* tests (Krauland et al. 2002).

7.1.5. Alginate

Properties of alginate

Alcinic acid as alginate (ALG) is a natural polysaccharide extracted from brown seaweed and bacterial source composed of linear (1→4) linked chains of the α-L-gulucuronic acid (G) and the β-D-mannuronic acid (M) blocks (Figure 19), which vary in size and in altering segments depending on the seaweed source and growing conditions (Nanjawade et al. 2007; CyperColloids library). The block structure dictates the gelling properties and more G blocks containing polymers tend to form more rigid gels in presence of cationic whereas higher M blocks form softer gels (Ludwig 2005). ALG is an anionic non-water soluble polymer and one of its most important features is capability to form clear hydrogels in the precence of divalent cations like calsium. The 3-dimensional structure of the hydrogel, mechanical strength and porosity are dependent on the G:M ratio, type of ionic cross-linker, concentration and viscosity of the initial ALG solution. This hydrogel forming character and suitable G:M ratio is found as a

capability to manipulate the swelling and gel formation. ALG hydrogels are considered biocompatible materials, which remain long in the mucus of the eye due to viscosity and interactions with mucus during fast *in situ* gelation. ALG has a low surface tension, which is lower than the surface tension of the mucin on the cornea, resulting in good spreading and adhesion to the corneal epithelium. (Cohen et al. 1997; CyperColloids library 2009).

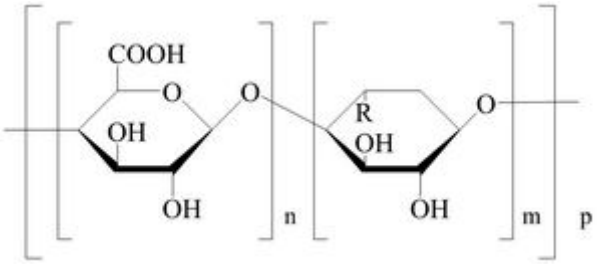


Figure 19. *Molecule structure of ALG, where block of n M-monomer is (1→4) linked to block of m G-monomes (CHEMnetBASE 2009).*

Trends of alginate

ALG is used as a gelling agent in pharmaceuticals. ALG based hydrogels are attractive scaffold materials because of their capacity to fill irregular tissue defects, suitable for minimal invasive procedures. ALG forms a strong gel, swells, is biocompatibile and has good endurance in the eye (Cohen et al. 1997), which has lead to commercial applications in the therapy of the glaucoma, but not DES (Ludwig 2005). In glaucoma therapy the drug molecule has been entrapped into the ALG solution, which forms gel in situ with cationic molecule and interact also with mucin resulting in delaying and sustaining release. Conventional eye drops without ALG support required administration twice a day, whereas the same response was achieved by dropping a drug with ALG once a day. In an *in vivo* test with rats and humans it has been observed that 1% ALG solution perform a viscosity low enough to allow topical administration without blurring effects. (Demailly et al. 2001)

In *in vitro* studies bovine serum albumin (BSA) has been used as a model protein in encapsulation into ALG/HPMC-polymer complex. Even small addition of HPMC to the ALG solution altered BSA release due to better swelling properties. ALG/HPMC matrix swelled rapidly when exposed to the dissolution medium resulting in enhanced BSA

diffusion outside the gel matrix. ALG, HPMC and BSA interactions were dominated by physical entrapments rather than electrostatic. At pH 7 BSA is negatively charged, ALG polyanionic and HPMC non-ionic, which suggests that electrostatic interactions were weak. According to the previous results drug releasing profile can be adjusted by optimizing ALG/HPMC/drug ratio. (Nochos et al. 2008)

BSA has also been used as a drug model in nanoparticle application of the ALG, where BSA was loaded into ALG microspheres embedded with COL hydrogel. The aim of this polymer combination was to deliver the drug or active agent during transplantation and matrix hydrogel was aimed to be used as corneal substitute. In *in vitro* studies COL and ALG interactions showed to become weaker in higher ALG concentrations due to discontinuous network and BSA loading capacity decreases. ALG/GOL complex was found non-toxic and it supported re-growth of the epithelial cells *in vitro.* (Liu et al. 2008)

7.1.6. Xanthan

Properties of xanthan

Xanthan (XAN) is extra cellular anionic heteropolysaccaride and is produced by the fermentation of the bacterium *Xanthomonas campestri.* XAN is composed primarily of 1→4-linked β-D-glucose backbone with side chains containing two mannoses and one glucuronic acid every other glucose at the C-3-position (Figure 20). Approximately half of the terminal mannose unit carries a pyruvic acid residue and the non-terminal residue usually carries an acetyl group at C-6 (McCormic et al. 2004). Side chains represent about 60% of the molecules and give XAN its anionic character and adhesive properties (Danisco 2009). MW of the XAN is estimated to be 2.000 kDa and its solutions are viscous at very low concentrations. XAN is pseudoplastic and is intensified by salt, temperature and pH over a wide range (McCormic et al. 2004). Single, double and triple helical structures have been proposed, but also quaternary double helix dimers exist, which are changed according to conditions of the solution (BeMiller et al. 2005).

Trends of xanthan

XAN polymer has been used in pharmaceuticals (Danisco 2009). XAN has moderate interactions with mucin, which was examined *in vitro* by BeMiller et al. (2005). Due to lacrimal salts, the XAN exist as an ordered double helix after installation in the

precorneal tear film. Interactions with XAN and mucin do not occur at low concentrations. Higher concentration solutions have higher viscosity and elasticity of the dispersion, but may cause uncomfortable feelings in the eye. Pre-treatment such as boiling or sonication promoted interactions with mucin, but optimal conformation of the helical structure is difficult to maintain due to pH and ions in mucin (Ceulemans et al. 2001).

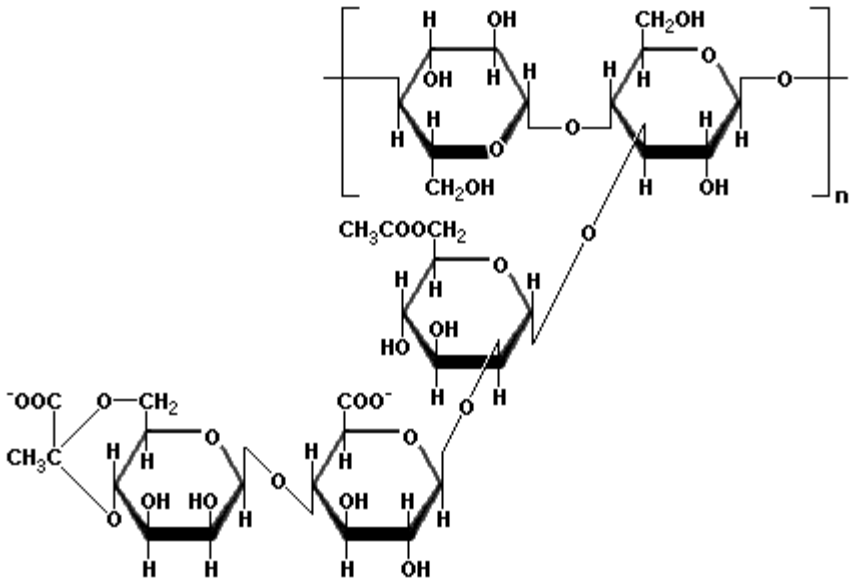


Figure 20. *Molecular structure of XAN, where backbone is composed of 1→4-linked β-D-glucose units with two mannoses and one glucuronic acid side chains in every other glucose in C-3. A pyruvic acid residue is linked to terminal mannose (Scientific psychic 2005).*

7.2. Cationic polymers

7.2.1. Chitosan

Properties of chitosan

Chitosan (CS) is a glycosaminoglycan (GAG) and is obtained by alkaline deacetylation of chitin, which is the second plentiful polysaccharide in the nature after cellulose. Chitin is the main component of protective cuticles of crustaceans such as crabs, shrimps, and cell walls of fungi. Economically thought, marine crustaceans are useful, because they are available as waste from seafood processing industry (Kurita 2006; Di Mario 2008). CS is biodegradable, biocompatible and non-toxic and also has

mucoadhesive properties. CS promotes wound healing character, is antimicrobial and non-allergenic (Beaulieu 2005).

Chitin is a straight homopolymer of β-(1→4)-linked N-acetyl-glucosamine units while CS is made from chitin by deacetylation (Figure 21). Isolation of the chitin is challenging, because chitin is not dissolving to the ordinary solvents. Chitin is diluted by hydrochloric acid to remove salts, and then heated with sodium hydroxide to decompose proteins. White powdery α-chitin is obtained after washing and drying. Deacetylation of the chitin is carried out by treatment with sodium hydroxide, while the degree of the deacetylation increases with repeating cycles of the alkaline treatment. If the degree of deacetylation, which means amount of amino groups, is less than 40% the one is chitin and when deacetylation is higher than 40 % the one is CS. CS is a weak alkali and insoluble in water and organic solvents, but soluble in dilute aqueous acidic solution (pH < 6.5). An acidic environment converts the glucosamine units into ionic soluble form (Sinha et al. 2004). Low water solubility is a limiting factor of the CS usage in medical applications especial in the eye, but can be improved by adding hydrophilic functional groups to the backbone of the CS. (Chung et al. 2006). The mucoadhesion of CS is formed by hydrogen bonds or ionic interactions between the positively charged amino groups and the negatively charged sialic acid residues of mucins (Ludwig 2005). The quality of the interaction depends on environmental pH and mucoadhesive performance of CS is significantly better at slightly alkaline pH as in the tear film (Lehr et al. 1992). Hydrophilic CS swells and forms a gel like layer in aqueous environment, which is favourable for interpenetration of polymer and glycoprotein chains in to the mucus. *In vitro* environment degradation of the CS is carried out by acidic hydrolysis breaking glycoside linkages forming low molecular weight oligomers. The biodegradation of the CS is mediated by the hydrolytic actions of the lysozyme and other entzymes, which produce oligomers and monomers. Lysozyme interacts with the acetamide groups but it does not interact with the free amino groups. Therefore, the rate of the degradation depends on the degree of deacetylation, which way the releasing profile of the polymer and the drug can be controlled (Li & Xu 2002).

CS is suitable in pharmaceuticals applications because of its low toxicity, biodegradability, good biocompatibility and mucoadhesive capacity. Characters that limit usage in ocular applications are high molecular weight (MV) causing low

solubility in physiological solutions resultant of its crystalline structure. CS is only soluble in water containing acetic acid, which is causing problems with modification in neutral pH. It is possible that cytotoxicity increases after usage of acetic acid. CS is a polymorphous polymer and products such as films, beds, microspheres, nanoparticles, hydrogels and latest research water soluble nanoparticles has been made from CS. (Wang et al. 2007)

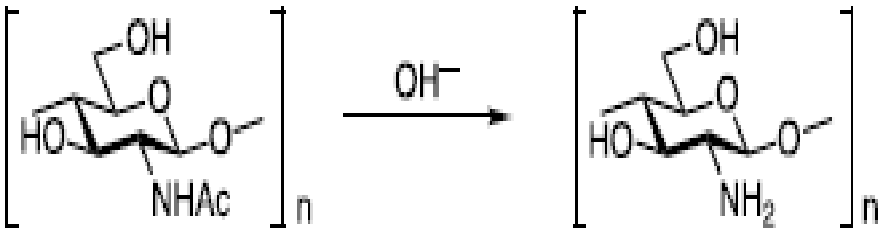


Figure 21. *Reaction that happens in modification of the chitin to CS, where the degree of the deacetylation rises up to 40%. Reaction occurs in an alkaline environment with sodium hydroxide. (Kurita 2006)*

Trends of chitosan

CS polymer has been widely used in commercial pharmaceutical applications in wound dressing of the skin and suture fibre, but also as gene carrier and in a dietary application as fat and cholesterol blocker. Drug delivery and sustained drug release applications are performed mainly in the intestinal mucus environment. Despite of CS's favourable characters on the mucoadhesion and non-immunogenicity the eye drop applications on the corneal surface have not yet become onto the market, though a lot of research has been done. CS has been subject for many researches of the drug delivery vehicles in surface of the cornea, because it has suitable properties and a good loading capacity of the therapeutic molecule (Beaulieu 2005). CS can be modified easily, because of reactive amino groups in backbone. CS has been made nanoparticles (Motowani et al. 2007), incorporated functional groups (Cao et al. 2007) and blended with other polymers (Di Colo et al. 2002) to improve mucoadhesive, degradation and water solubility character (Zhu et al. 2006).

CS is known to carry drug molecules and perform sustained release well (Felt et al. 2001). Hydrophilic drug has been delivered with CS gel and has achieved over 3-fold increase of residence time on the cornea of the rabbits comparing to the drug compound without CS. Reasons for longer retention time does not depend on MW or concentration of the CS, but depends on viscosity, degree of the deacetylation and ionic interactions between mucus and CS. Since increasing the MW does not produce a better contact time, it allows the use of low MW CS giving solution of low viscosity, enabling easy administration to the eye (Felt et al. 1998). Large surface area and effective penetration to the mucus of the cornea are advantages of the CS nanoparticles and microspheres compared to the CS suspensions. CS nanoparticles (deacetylation about 85 %) have been found stable *in vitro* in presence of lysozyme and did not affect to the viscosity of the mucin dispersion. Amount of nanoparticles were fairly constant *in vivo* at least 24 h on the cornea and conjunctiva of the rabbits when nanoparticles were administered in the cul-de-sac of the eye. In cell culture studies, the degree of survival was nearly 100%. (de Campos et al. 2004) Nanoparticles made from a combination of CS and sodium alginate (NaALG) have been reported by Motwani et al. (2007*)*. CS is cationic and ALG is anionic polysaccharide and they together form a polyionic complex forming hydrogel, which has demonstrated favourable characters for drug entrapment and delivery. CS –NaALG complex gave *in vitro* test results that sustained drug release lasted over 24 h.

Diebold et al. (2007) reported evaluation of the liposome-CS nanoparticle complex, where the aim was to get well tolerated, easily applied eye-drops and improved drug penetration to the epithelia. Hydrophilic CS nanoparticles combined with hydrophobic liposomes and their properties utilized to easy interaction with biological surfaces and cell membranes and potentially deliver drug to the cell. The first layer of the tear film is a lipid layer in which liposomes as lipophilic molecules interact. An aqueous layer and mucus layers are hydrophilic fluids which respectively interact with CS molecules. The CS-liposome system was found non-toxic and well tolerated in the rabbit eye.

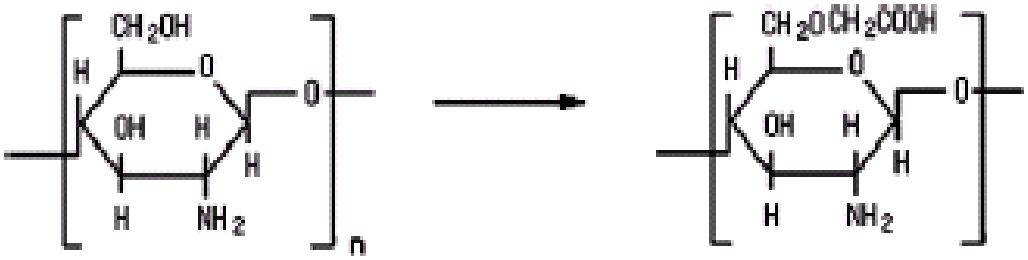
Muchoadhesion properties, drug loading capacity or penetration to the mucus can be improved by adding functional groups to the polymer. Added functional groups interact more effectively with functional groups of the transmembrane mucins and polymer stays longer on the surface. Cao et al. (2007) investigated properties of combination of

poly (N-isopropylacrylamide) (PNIPAAM) and CS aiming to get thermosensitive, gel-forming, drug releasing and mucoadhesive polymer. PNIPAAM-CS has thermosensitive phase transition temperature of 32^{o}C, which is close to human eye surface temperature. Thermal induced transition is the result of the change in its hydrophilic-hydrophobic balance. Hydrophilic interactions dominate in sol-gel transition, otherwise when temperature is below phase transition, hydrophobic interactions take place. PNIPAAM-CS copolymer was coupled by force of coupling agent and chain transfer agent, which can affect to the biocompatibility. Good tolerance was achieved in corneal epithelial cells in less than 72 hours cultivation. Twice higher time of release of the drug loaded to the PNIPAAM-CS copolymer was achieved compared to the conventional eye drops.

CS is a polymer studied widely these days and the last part of this chapter includes studies, where the target has been other tissues, which might be of interest in ocular surface applications. Yuan et al. (2006) reported that CS microspheres were cross-linked with albumin by help of genipin, which is a natural and non-toxic cross-linking reagent. *In vitro* cross-linking tests revealed that release of the albumin reduced about 30% up to 24 days and 60% up to 31 days, when the reaction time of the cross-linking was 4 hours. A higher amount of the genipin raised cross-linking efficiency and reduced the ratio of the release. The swelling ratio of the complex decreased with the cross-linking time and amount of the genipin reagent, because of a lower amount of the hydrating hydroxyl and amide groups. According to Yuan *et al.* (2006) the albumin release rates and swelling ratio of CS microsphere may be controlled by the degree of cross-links. Albumin has therapeutic effects to the ocular surface by grow factors like proteins and CS is mucoadhesive, therefore this combination is of interest in the treatment of DES.

As mentioned above CS has limited solubility in water and toxicity raises presence of lytic molecules. To solve this problem Zhu et al. (2006) have described the delivery system of antibiotics from O-carboxymethyl-CS (O-CM-CS), which is a water soluble CS derivative. O-CM-CS is formed by substituting hydroxyl group of each monomer with a carboxylethylic group trough the ether-bond formation (Figure 22). O-CM-CS is amphifilic polymer, which forms aggregates in an aqueous solution. Aggregation is mainly driven by the intermolecular hydrogen bonds and hydrophobic interactions in critical aggregation concentration of about 0.5 mg/ml. This CS derivative has a

hydrophilic character in hydroxyl and amino groups, hydrophobic character in acetyl groups but also in the dissociated carboxylic groups. These characters make O-CM-CS potential drug delivery polymer for hydrophilic molecules. Calvo et al. (1997) developed for BSA delivery water soluble CS-polyethylene oxide (PEO) and CS-PEO-polypropylene oxide (PPO) nanoparticles, which were formed by force of tripolyphosphate (TPP). These nanoparticles were found to have great protein loading capacity, transformable surface charge, pH-dependent dissolution behaviour. Wang et al. (2007) incorporated polyethylene glycol (PEG) to the water-soluble CS nanoparticles and achieved in *in vitro* study faster BSA release rate compared to the water-soluble CS without PEG.



Chitosan *O-carboxymethylchitosan (O-CM-CHS)*

Figure 22 *Water soluble CS conjugate O-CM-CS is formed by substituting hydroxyl group of each monomer with a carboxylethylic group trough the ether-bond formation (Zhu et al. 2006).*

CS interacts with mucin by weak bonds. Forceful interactions are achieved by thiolation, which means incorporation of free sulphur groups to the CS-polymer backbone. Sulphur groups are interacting with sulphur rich areas of mucin forming covalent disulphide bonds. Characters such as controlled release, permeation enhancing and enzyme inhibition are improved by thiolation. Kafedjiiski et al. (2005) reported *in vitro* test of CS conjugation with glutathione (GSH), which is a tripeptide consisting of glutaminic acid, Cys and glycine (Figure 23). GSH is found in every cell and acts as an antioxidant. CS-GSH conjugate is synthesized by the amide bond formation between amine group of CS and carboxylic acid groups of the GSH, which is placed in glycine. An *in vitro* test of CS-GSH tablets with freshly excised porcine intestinal mucosa in

rotating cylinder showed that total work of adhesion (TWA) was 9.9-fold higher than with unmodified CS. Cohesive properties were improved over 5-fold and CS-GSH conjugate showed 3-fold stronger permeation-enhancing effect compared to the unmodified CS. Bernkop-Schnűrch et al. (2003b) prepared and evaluated *in vitro* nanoparticles generated from CS modified with 4-thiobutylamidine (TBA). Comparing to the unmodified CS nanoparticles CS-TBA nanoparticles showed 2-fold higher zeta potential and improved stability due to intra molecular covalent sulphide bonds and over 2-fold better mucoadhesive properties were achieved due to disulphide bonds between mucus and CS-TBA nanoparticles. (Bernkop-Schnűrch et al. 2003b)

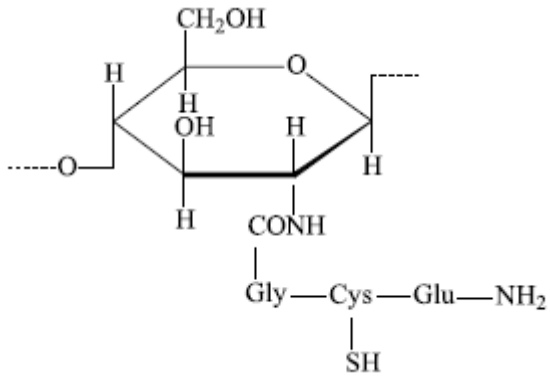


Figure 23 *Molecuar structure of CS conjugated with GSH (Kafedjiiski et al. 2005).*

7.3. Non-ionic polymers

7.3.1. Cellulose ethers

Properties of cellulose ethers

Cellulose is a polysaccharide consisting of a linear long chain of several hundred to over ten thousand β (1→4) linked D-glucose units. It is found from renewable resource as cell walls of the plants and it is most abundant polymer in the nature (Feller & Wilt 1990). In Figure 24 is illustrated structure of cellulose. Cellulose ether (CE) is moderate to high MW carbohydrate polymer derived from cellulose. Hydroxyl groups of the cellulose are reactive and easily substituted by an ether bond. CE exhibit inverse temperature-solubility behaviour in water, which mean that it is soluble in room temperature or below, but is insoluble at higher temperature. This behaviour derives

from association between water molecules and ether groups, which become weaker at higher temperatures and polymer separates from the solution (Feller & Wilt 1990; Nanjawade et al. 2007). The nature of the substituent placed in CE backbone influences the affinity to water. For example hydroxyl group makes polymer more hydrophilic and rises temperature of the clouding point, where solution blurs and gelation occurs by hydrophobic interactions. The gelation temperature and the solubility to the water decrease as salt concentration increases, because water molecules surround the salt molecules (Nanjawade et al. 2007). CE requires adding to hot water followed by cooling to enable proper dispersion, hydration and gelation. Nowadays there is a cellulose ether derivative available, which can be dispersed in a cold solution by pH adjustment (Amerchol 2005). CE is degradated by heat, sunlight and cellulolytic enzymes. The more complete is the substitution the more resistant CE will be against enzymatic attack. Cellulose is poorly biodegradable in the body and is not digestible, but it can be made hydrolysable by structural modification. (Amercol 2005: Feller & Wilt 1990)

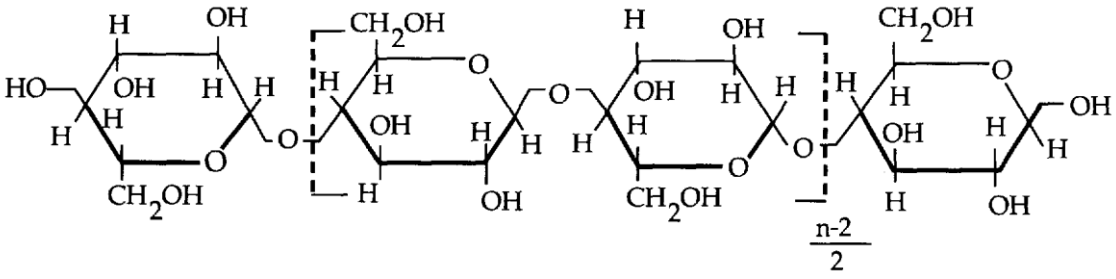


Figure 24. *Molecule structure of cellulose, which is composed of β(1→4) linked D-glucose units (Feller & Wilt 1990).*

Trends of cellulose ethers

CE polymers have been used commercially in pharmaceutics (The dry eye digest 2009), where it has shown good biocompatibility and non-toxicity. CE molecules swell and form gel in the presence of water and in pharmaceutical interests the CE retains well on the mucus (Nanjawade et al. 2007). CE is suited well in the body and used in commercial applications of the artificial tears. Commercial CE derivatives substituted with methyl (M), carboxymethyl (CM), hydroxymethyl (HM) hydroxyethyl (HE), or hydroxypropylmethyl (HPM) groups are used in eye drops due to their high swelling

and viscosifying properties (The dry eye digest 2009). Figure 25 displays the chemical structure of the MC, where the hydroxyl group is occupied by a methyl group. The hydroxyl group is an active part of the cellulose where functional groups are incorporated. Ether bonded substituent forms hydrophobic areas to the cellulose molecule, which generates non-water soluble domains. The degradation time can be modified by amount, but also size of the substituent. Blending two or more different kind of CE substituted polymer together is also achieved demanded characters and they are commercially available (Amercol 2005).

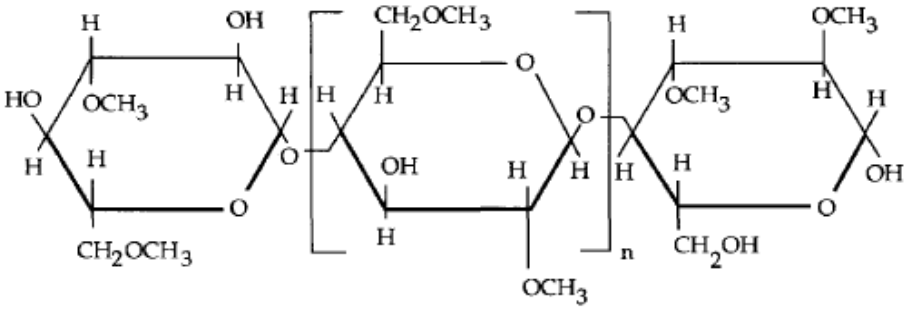


Figure 25. *Chemical structure of MC. Methyl groups is linked to the hydroxyl group by ether bond (Feller & Wilt 1990)*

The rate of gelation is essential in many applications of the hydrogel. Rapid gelation gives quickly strength and form to the polymer after administration. Under rapid gelation uniform structure is difficult to form and structure lacks strength properties, which are needed in artificial tear applications. Gelation of the CE derivatives occurs quite slowly when added to the eye and therefore Liu et al. (2006) has studied gelation properties of the blend of ALG and HPMC. In the same study drug loading capacity and releasing properties were measured. In this blend ALG formed a strong gel with cationic salts of the tear film and HPMC enhanced viscosity. Lower ALG concentrations improved patient compliance. It was found that ALG/HPMC-blend retained drug molecule up to 8 hours *in vitro* and were longer than ALG or HPMC solution alone. In *in vivo* the rabbit eyes had achieved better bioavailability and no irritation was found. Gupta et al. (2006) studied fast-gelation properties of the blend of HA and methylcellulose (HA-MC), which was developed for soft tissue scaffolds. A gel of the HA-MC is formed at room temperature, but further gelation is formed *in situ* due to increased temperature after injection (Gupta et al. 2006). *In vivo* studies with rats in intrathecal space HA-MC was found biocompatible and biodegradation rate was almost

one month. HA and MC are polymers suitable for ocular therapies, therefore faster gelation with blend may stay longer on the surface of the eye compared to the single polymer suspension and is also a potential combination in ocular therapies.

7.3.2. Poloxamer

Properties of poloxamer

Poloxamer (POL) is a non-ionic surface active agent, which is composed of triblock copolymers: PEO-PPO-PEO. Figure 26 demonstrates the POL molecule structure, where blocks vary by length. MW of the POL can vary from 1.1 to 14 kDa and PEO: PPO ratios from 1:9 to 8:2, respectively (Ruel-Gariépy & Leroux 2004). POL is composed of central hydrophobic PPO part and surrounded by hydrophilic PEO parts. The mucomimetic property of POL is due to its hydrophobic and hydrophilic sequences simulating mucin action by absorption of the aqueous layer of tears on the hydrophobic epithelium. Depending on the ratio and distribution along the chain of the hydrophobic and hydrophilic subunits, several MW variants are available leading to different solubility and gelation characters. Thermal gel formation by intramolecular hydrogen bonds is reversible and at room temperature the solution behaves like mobile viscous liquid and transforms rapidly into semisolid transparent gel at body temperature (Felt et al. 1999).

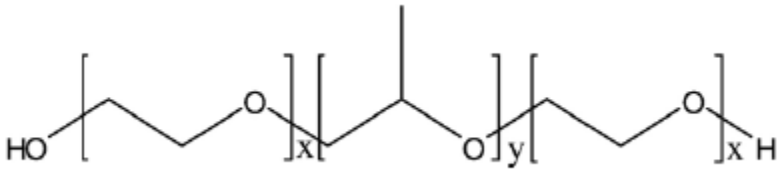


Figure 26 *Molecule structure of POL, which is composed of triblock copolymers PEO-PPO-PEO (Nanjawade et al. 2007).*

POL has a weak mechanical strength, erodes rapidly and is not biodegradable. The non-biodegradability character causes that high MW polymers are not eliminated by renal excretion and formation of the solid residues on the eyelid. These characters are the main drawbacks of POL use in the DES therapy, but by modification of the polymer by biodegradable substituent some drawbacks can be avoided. (Ruel-Gariépy & Leroux 2004)

Trends of the poloxamer

POL works as an emulsifier, solubilizer and dilute in both hydrophilic and hydrophopic domains. Due to its surface activity properties, POL has been used in pharmaceutical ointments, inserts, tablets and gels. In topical drug administration POL has been used widely and also in topical ophthalmic applications. A commercial product called Pluronic® is trademark of POL (Felt et al. 1999) and is used in a matrix of eye drops for DES therapy. Addition of CMC to the POL matrix improves administration and adjusting the ratio of POL:CMC to the proper level can gelation phenomena regulated *in situ*. POL and CMC containing eye drops are found well tolerated (El-Kamel 2002; Yliopiston apteekki 2009).

Properties such as low mechanical strength, low sol-gel-transition temperature and non-biodegradability, are characters of POL, but unwanted in a drug delivery system. Despite these characters, POL has potential in ophthalmic applications, because of biocompatibility, solubility, gel formation and emulsifying characters. Hydrophopic drug molecule as puerarin has been delivered to the rabbit's ocular surface with *in situ* gel forming blend of POL and CARB. In this study, it was found that an addition of a small amount of CARB into POL delivery system did not affect the rheological properties, but did enhance the mucoadhesion significantly compared to individual solutions. At room temperature, the combination solution was liquid and transformed to a firm gel after administration. Gel formation prevented rapid corneal elimination and attraction to the ocular mucosal surface improved the bioavailability of the drug (Qi et al. 2007).

POL grafting with PAA results in a copolymer, which has an optimal gelation temperature *in situ*. Achieved copolymer formed temperature-responsive gelation at a lower concentration (4% w/v) than pure POL solutions (18% w/v). A drug release profile with a water soluble gatifloxacin (GFX) was zero-ordered when simulated in artificial tear flow *in vitro*. POL-PAA copolymer loaded with drug molecules, which were administrated into lower conjunctival sac of the rabbit's eye, improved bioavailability and precorneal residence time (Ma et al. 2007).

7.3.3. Polyvinyl alcohol

Properties of polyvinyl alcohol

Polyvinyl alcohol (PVA) (Figure 27) is a synthetic water soluble and non-ionic polymer, which is biologically inert and has the ability to adhere to surfaces (Tang et al. 2007). PVA is manufactured by hydrolysis of polyvinyl acetate and the end product of PVA is a copolymer of the vinyl acetate and vinyl alcohol. The final properties of the PVA are affected by a polymerization method and conditions of the parent polyvinyl acetate, hydrolysis, drying and grinding. PVA has the ability to crystallize, which is its most important physical property as it controls water solubility, tensile strength, oxygen barrier properties and thermoplastic properties. (Hassan & Peppas 2000)

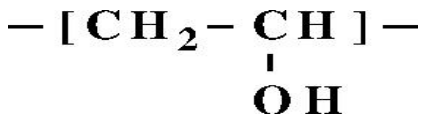


Figure 27. *Molecule structure of PVA. (PolymerProsessing 2001).*

The degree of polymerization and hydrolysis of the PVA affects the solubility to the water and other polar solvents. Fully hydrolyzed PVA is only soluble in hot water, partially hydrolyzed grades are soluble at room temperature, but grades with a hydrolysis of 70 - 80% are only soluble to water at temperatures 10 - 40^{o}C. Above 40^{o}C the solution becomes cloudy and then followed by the precipitation. Water solubility is reduced by the hydroxyl groups in PVA, which contribute strong hydrogen bonding both intra- and intermolecularly. The presence of hydrophobic acetate groups in partially hydrolyzed PVA weakens the hydrogen bonding and allows solubility at lower temperatures (Hassan & Peppas 2000). Viscosities of PVA solutions mainly depend on the MW and solution concentration, but also on the temperature and the degree of hydrolysis (DH). The viscosity increases with increasing DH. PVA forms gelation on standing, but viscosity is decreased at higher temperatures. PVA with a high DH adheres to the hydrophilic and one with a lower DH adheres to hydrophobic surfaces. PVA properties can be adjusted also by MW, crystallinity and modifying by cross-linking with multifunctional compound that react with hydroxyl group of the backbone. These modifications improve the water resistance of the polymer or increase formation of viscosity (Tubbs 1966). PVA is completely biodegraded producing water and carbon

dioxide. Degradation occurs by random oxidation of the hydroxyl groups with an aid of hydrolase leading to a reduction of the MW and formation of the carboxylic and the methyl ketone end groups. Continued degradation leads to formation of the acetic acid and finally to carbon dioxide and water (Marten 2002).

Trend of polyvinyl alcohol

PVA is used in pharmaceutical applications due to its biocompatibility, ability to form a viscous solution or protective membrane (Peppas & Berner 1980), but also due to its ability to adhere to the surfaces (Marten 2002). Above mentioned properties are also useful in therapy of DES and PVA is therefore used as a matrix in artificial tears (Fimea 2010).

7.3.4. Dextran

Properties of dextran

Dextran (DEX) is an α-1→6-glucose-linked glucan with side-chain 1-3 linked to backbone units of the DEX biopolymer (Figure 28). Branches are 1-2 glucose units long and degree of the branching is about 5%. The MW of the DEX can be varying up to 2.000 kDa and its distribution affects the physical properties such as viscosity. DEX is soluble in water and electrolyte solutions to form a stable solution, which is not significantly affected by pH. Although DEX forms a clear solution, the low MW

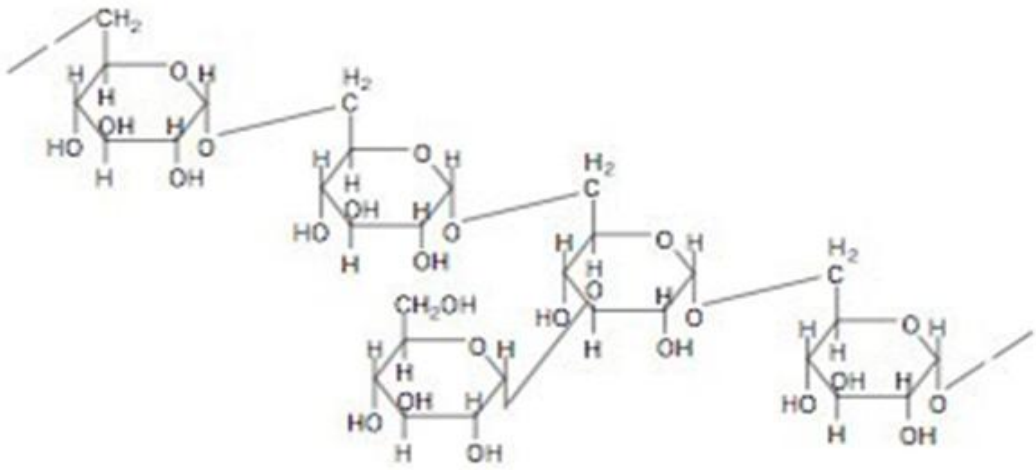


Figure 28 *Molecular structure of DEX (Pharmacosmos 2009).*

fractions may form turbid solutions on standing. From a pharmaceutical point of view, DEX is found biodegradable, biocompatible and it does not easily permeate to the human tissue due to its large dimension. Dextran can be obtained from fermentation of the sucrose-containing media by family of the Lactobacillaceae (Pharmacosmos 2009).

Trends of dextran

DEX is used as a component of the artificial tear with glycerine and HPMC. This composition provides palliative therapy for DES patients by lubricating and moistening eye surface (Drug digest 2008).

7.3.5. Xyloglucan

Properties of xyloglucan

Xyloglucan (XYL) is a hemicellulose of cell walls of the plants named dicotyledonous and monocotyledon. It is structurally related to cellulose with β-1→4-linked glucose backbone, but in XYL every glucose residue is substituted by α-1→6-xylose residues except fourth glucose in non-substituted (Figure 29). Some xylose units can further be substituted by α-1→2-galactose residue and can be further substituted by α-1→2-fructose residue (Fry 1989). Composition of the XYL depends on source of the isolation. XYL forms thermosensitive gels in water and gelation is reversible if XYL is

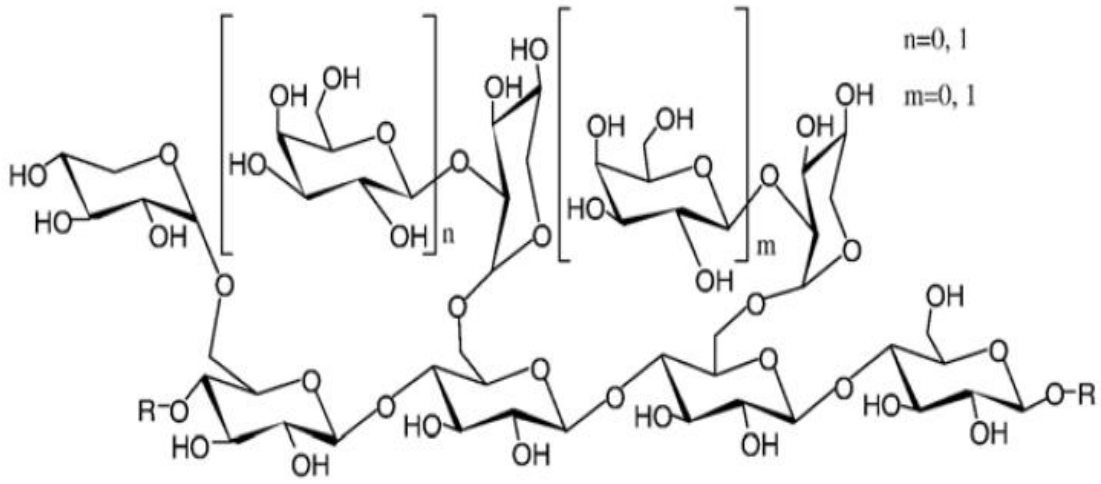


Figure 29 *Molecule structure of XYL, where backbone is composed of β-1, 4-linked glucose units. Every glucose is substituted with xylose residue, but the fourth glucose is not. Xylose recidues can be further substituted with functional groups (Nanjawade 2007).*

partially degradated by β-galactosidase. A native XYL solution does not exhibit reversible gelation. The amount of galactose plays an important role in the gelation process because the transition temperature decreases with galactose removal ratio (Shirakawa et al. 1998).

Trends of xyloglucan

Thermally reversible XYL has been studied as a gelling agent in sustained release of the drug. Bioavailability and sustaining of the miotic response time in the rabbit's eye were increased with the amount of XYL in eye drops. In low concentrations compared to the Pluronic F127, XYL was more viscous and had almost equal mechanical strength. XYL released drug faster and still lasted as long as Pulronic F127 matrix. At higher concentrations release rate was decreased. Although XYL has shown good biocompatibility in *in vivo* tests (Miyazaki et al. 2001) and good properties for eye drop gelling agent, no commercial applications have been developed due to the transition temperature (22 – 27 ^{o}C), which makes handling at room temperature difficult (Ruel-Gariépy & Leroux 2004).

7.3.6. Poly-N-vinyl-2-pyrrolidinone

Properties of poly-N-vinyl-2-pyrrolidinone

Poly-N-vinyl-2-pyrrolidinone (PVP) is a water soluble or dispersible polymer, which exhibit ability to interact with variety of surfaces by hydrogen or electrostatic bonding, resulting in protective and adhesive coatings. Vinyl pyrrolidinone (VP) is synthesized by vinylation of the 2-pyrrolidinone in alkali conditions and PVP is produced by polymerization with initiator. Cross-linked PVP can be produced by chemical modification or with radiation (Login 2004). The molecule structure is presented in Figure 30.

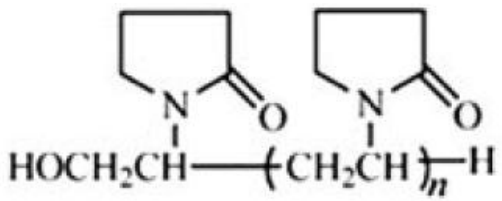


Figure 30 *Molecule structure of the PVP (Login 2004).*

PVP is mainly soluble in water, forms hydrogel and swells in aqueous environment. The PVP swelling ratio decreases with temperature, because swelling is an exothermal phenomenon. Cooling back to a lower temperature gives a higher swell ratio. Alcohols and other hydrogen bonding solvents cause the same effects but at a lesser extent. The solubility of PVP in water is high and only limited by the viscosity of the resulting solution. High dipole moment and polarity of the PVP affects the water molecule resulting hydration (Figure 31). Hydrated PVP forms a non-freezing suspension, which is used even in cryobiology. PVP has been found to have several protein-like characters due to its repeating amide linkage. Although proteins are extremely complicated molecules with sequence distribution, tertiary bonding and structural complexity, PVP has been considered as a uniform synthetic protein-like analogue. Unlike the proteins PVP is soluble in polar solvents and does not form intermolecular hydrogen bonding. PVP has the ability to form complexes with large anions such as dyes and surfactants, but also has potential to carry drug molecules (Login 2004; Mitra et al. 2009).

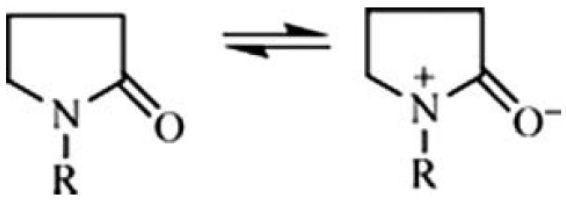


Figure 31. *In an aqueous solution PVP forms polarity to the five-membered planar lactam ring, where oxygen gets a negative charge and nitrogen a positive one (Login 2004).*

Trends of poly-N-vinyl-2-pyrrolidinone

Good adhering capability to surfaces and water solubility are characteristics of the PVP that is utilized in therapy of DES and development of the new copolymer for the use of ocular disorders. PVP has poor properties for carrying hydrophobic drug molecules, which is the main target of the development nowadays. To improve the carrying properties of the PVP, methacrylic acid (MAA) or AA has been cross-linked with PVP aiming to achieved hydrophobic areas to drug molecule interact. An *in vitro* test showed faster drug release with PVA-AA copolymer comparing to PVA-MAA, because MAA is more hydrophobic than AA, but AA swelled better. Wafers loaded with pilocarpine

drug and made from a PVP-MAA cross-linked polymer performed sustained release. The miotic effect held up to 300 min in *in vivo* test with rabbits. Swelling and hydrophobicity of the matrix were key factors in affecting controlled drug release with PVP-MAA copolymer. (Barbu et al. 2005)

7.3.7. Polyethylene glycol

Properties of polyethylene glycol

Polyethylene glycol (PEG) (Figure 32), also known as PEO, is a thermoplastic polymer manufactured by the heterogeneous polymerization of ethylene oxide. PEG with MW of less than 200 Da is clear viscous liquid and wax-like, while one with a higher MW is an opaque white and crystalline solid substance. At room temperature low MW PEG is perfectly water soluble, but higher MW or temperature decrease solubility (Marten 2002). The viscosity of aqueous solutions of PEG depends on the concentration, MW, solution temperature, the concentration of dissolved inorganic salts and the shear rate. Viscosity increases with concentration especially at a higher MW. PEG is a pseusoplastic polymer and the degree of the pseudoplasticity increases with the MW. A free electron pair of the ether oxygen in the backbone give strong hydrogen bonding affinity and can also take part in associating reactions with electron acceptors. In the backbone of PEG, there is a weak ether bond, which is easily degraded by oxidation in solutions and is accelerated with presence of oxidative salts or avoided by stabilizers (Back & Schmitt 2004). PEG polymers are used safely in many pharmaceutical applications and have shown low toxicity in animal studies. High MW polymers are poorly adsorbed to the tissue and are well tolerated in the eye. PEO has good adhesive properties to the mucosal surfaces, because of linear structure and fast hydration properties. In the ocular surface application PEO is used as contact lens fluids forming cushioning layer between lens and mucus where shear rate is low. Above of the contact lens the shear rate is high and viscosity of the PEG decreases during blinking and increases drainage loss. (Dimitrova et al. 1993; Ludwig 2005)

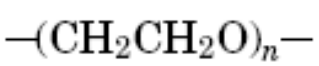


Figure 32. *Molecular structure on PEG (Back & Schmitt 2004).*

Trends of polyethylene glycol

PEG polymers with low MW form a clear lubricating wax-like gel with water. PEG is biocompatible and erodible in body fluids (Marten 2002). Therefore PEG is found in pharmaceutical applications and also used as components in eye drops for treating DES (Tauber 2005).

At present there are no liquid dosage forms of PEG applications under development, but few higher MW PEG inserts applications are worth mentioning. PEG has good compressibility and therefore it has been studied to form an erodible gel-forming ocular insert, which is loaded with drug molecules. Solid material surface starts to erode and forms a superficial gel in the presence of water. The drug molecule is released from the PEG matrix by polymer erosion and swelling or by drug diffusion trough the gel (Ludwig 2005). The MW of the PEG is an important character, which affects the swelling, erosion and release rate of the drug molecule loaded in the matrix. The MW 400 kDa of the PEG seems to have the best mucoadhesion ability and gives the best bioavailability of ofloxacin (OFL) drug molecule loaded in a disk-shaped insert comparing to the other MW inserts of the PEG. The PEG 900 kDa achieved the longest effective therapeutic time, which was 380 minutes. The effective concentration of PEG 400 kDa lasted 290 minutes (Di Colo et al. 2001). In another insert composition, an Eudragit L100 (EUD), which is partially hydrophilic copolymer of the MAA and methyl metacrylate (MMA), has been blended with PEG. Interactions in the blend were performed by hydrogen bonding via unionized carboxyl of the EUD and ether oxygen of the PEG. Protonation state of the existing carboxyl groups were adjusted by neutralization, which defined swelling and erosion characters. Neutralized PEG-EUD insert were loaded with OFL drug molecule and set to the rabbit's cul-de-sac of the eye, where muchoadhesive gel was formed and spread over the ocular surface. Effective time of the drug lasted up to 194 minutes (Di Colo et al. 2000). Di Colo et al. (2002) studied method where the microspheres of the CS diluted with HCl were added to PEG 900 kDa matrix and loaded with OFL drug. CS has ability to interact with negatively charged conjunctiva and enhance the permeability of epithelia. Matrix was pressed to flat-faced tablets and added to the cul-de-sac of the rabbit's eye. Presences of the CS in the matrix the erosion and the drug release rate of the insert were accelerated. The amount of the OFL in aqueous humour was raised and the effect lasted 300 minutes (Di Colo et al. 2002). Although these studies are of solid dosage forms, there may be

feasibility to turn these polymer applications to a liquid or semi-liquid form for administration as drops.

8. Simulating protocols

Polymers and their derivatives, which have retaining character on the cornea, are nowadays in the focus of the research. Lower administration rate, better bioavailability and less side effects of the drug molecule are main advantages of the sustaining polymers comparing to the conventional eye drops (Saettone 2002). The behaviour of the potential eye drops composition in the surface of the eye is found exactly only in real mammalian eye, but *in vivo* tests and animal suffering should be minimized. Therefore some simulation protocols *in vitro* have been developed. All characters such as tear flow, optimal mucus production and blinking are almost impossible to have in a same model, but different mechanisms are simulated partially (Crabovac 2005; Choy et al. 2007; Choy et al. 2008; Takeuchi et al. 2005). Simulation methods used in eye surface studies are listed in Table 8 and discussed further chapters.

Table 8. Method for different kind of simulation protocols.

Simulation protocol	**method**	**measured unit**	**author**
mucoadhesion	porcine mucin in rotating sylinder	TWA or time of detachment	Grabovac et al. 2005
	commercial Biocore® chip	Refractive index	Takeuchi et al. 2005
penetration	double chamber apparatus	drug concentration	PermeGear 2005
tear fluid flow	constant flow forming apparatus equipped with peristaltic pump	molecule concentration from effluent	Choy et al. 2007
	microfluid device	molecule concentration from effluent	Ali et al. 2007
	flow-through-cell	molecule concentration from effluent tear flow	Ma et al. 2008
	Automated cultivation well-plate system	Sensors for O_2, temperature and pH → viability of the cells	Kuncová-Kallio 2007
desiccation	96-wells plate with conjunctival cells	vitality of the cells	Paulsen et al. 2008
blinking	Excised eye with eyelids from porcine equipped with movable arm	viability of the cells	Choy et al. 2008

8.1. Mucoadhesion simulation

In vitro tests used to compare and describe mucoadhesive properties involve measurements of peel, tensile force and stress, which are necessary to detach a polymer formulation from mucosa. pH of the polymer has been recognized as characters that informs about mucoadhesion ability of anionic and cationic polymers. Water uptake ability of the polymer informs also about adhesive properties to the mucus, but results differ widely. Therefore better methods have been developed and adhesion to the mucus has been measured in rotating cylinder made of stainless steel. Freshly excised small intestinal mucosa of the porcine has been spanned onto the cylinder with compressed polymer. Detachment was performed by replacing mucosa to phosphate puffer until disintegration happened. TWA measurements were in correlation to disintegration times achieved with the cylinder. The porcine intestinal mucosa is better available and cheaper compared to the corneal mucosa (Grabovac et al. 2005). Porcine intestinal mucin particles have also been used in measurements of zeta potential (ZP), which describes stability of suspension. Change in ZP between beginning of the test and steady state indicates mucoadhesion. Biocore® system is a mucoadhesion test method, which is commercial available. Biocore® instrument is based on optical phenomena called Surface Plasmon Resonance (SPR). The response is a measurement of refractive index, which varies with solute concentration in a solution that contacts a sensor chip. When a detected molecule is attacked to the surface of sensor chip or when the analyte binds to detected molecule, the solute concentration on the sensor chip surface increases, leading to the SPR response. In detection of the mucoadhesion character of polymers using Biocore®, each polymer is immobilized on the surface of the sensor chip and the mucin suspension is passed through the sensor chip. Biocore® system is found applicable also in simulation of sustaining drug delivery system (Takeuchi et al. 2005). Although above mentioned mucoadhesion simulation and research methods are done with intestinal mucosa, they may be possible to test with ocular mucosa.

8.2. Penetration simulation

Before the drop wise administrated drug molecule is able to penetrate trough cornea, it has to adhere to the corneal epithelia. Adhering, penetration and enhancing characters of polymer or drug molecule can be studied with horizontal side by side cell apparatus (Majumdar et al. 2007). Double chamber consisting apparatus is loaded with excised

cornea between champers (Figure 33). Different appropriate fluids can be loaded in to chambers (PermeGear 2005). In this method corneal membrane from mammalian eye is needed, but can be achieved from slaughter and unnecessary sacrifices can be avoided.

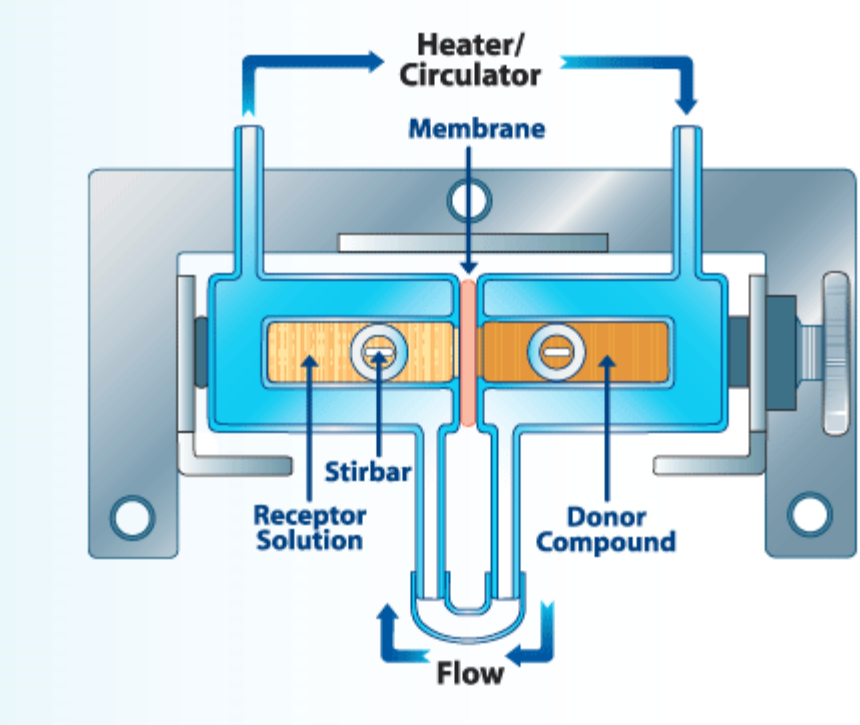


Figure 33. *Double chamber containing apparatus, where membrane (in this case exercised cornea) is located between. One chamber is filled with studied compound and the other chamber is filled with receptor solution. System can be cooled or heated (PermeGear 2005).*

8.3. Tear flow simulation

Artificial lacrimal fluid solution is used in *in vitro* simulations replacing native tear flow. Artificial tear solution is used in degradation and release studies, but also in mucoadhesion investigations. Choy *et al. (*2007) has developed an apparatus (Figure 34) where a peristaltic pump formed a constant flow over membranes which were soaked with porcine stomach mucin solution. The investigated polymer suspension was applied by single drop to the middle of the membrane. The membrane was immediately washed with continuous Hank's solution flow, which simulated artificial tears. A tear sample can be collected after membrane and also taken from the membrane itself.

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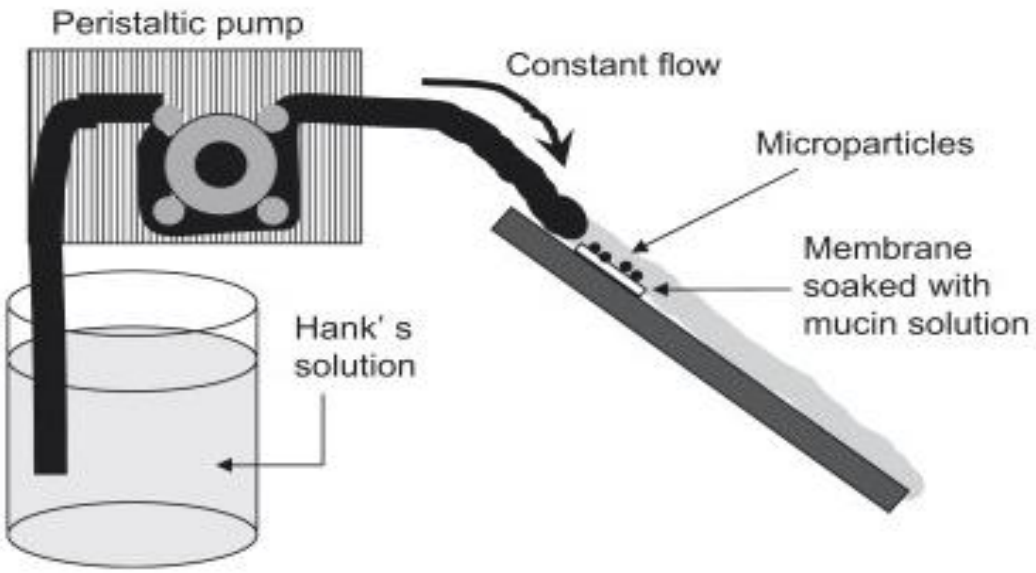

Figure 34. *Apparatus for simulation of the eye tear flow. Hank's solution, representing a artificial tears, is flowed over the membrane soaked with mucin, where the researched polymer is placed (Choy et al. 2007).*

Another tear flow simulation apparatus has been introduced by Ali et al. (2007), where a hydrogel and therapeutic drug containing contact lens was inspected in a microfluidic device that simulated the tear flow rate and composition. The composition of artificial tears is added to the device by syringe, which is introduced to the entrance channel of the microchips, which led tear flow to the chamber. Samples are collected from the effluent of the artificial tears (Figure 35).

Drug release from a matrix polymer is based on molecule diffusion from matrix and matrix erosion or degradation. An *in vitro* release test is carried out with artificial tear fluid. Figure 36 shows flow-through-cell -apparatus, which Ma et al. (2008) used for a drug release study. A matrix polymer loaded with drug molecules is placed to the lower rectangular part of the apparatus and artificial tear solution is led through pipeline to the upper semicircular section. Tear flow samples are collected from the other pipeline channel. This kind of apparatus can be use for tear flow simulation with a matrix polymer without a drug molecule and there might be a possibility to culture cells in the chamber.

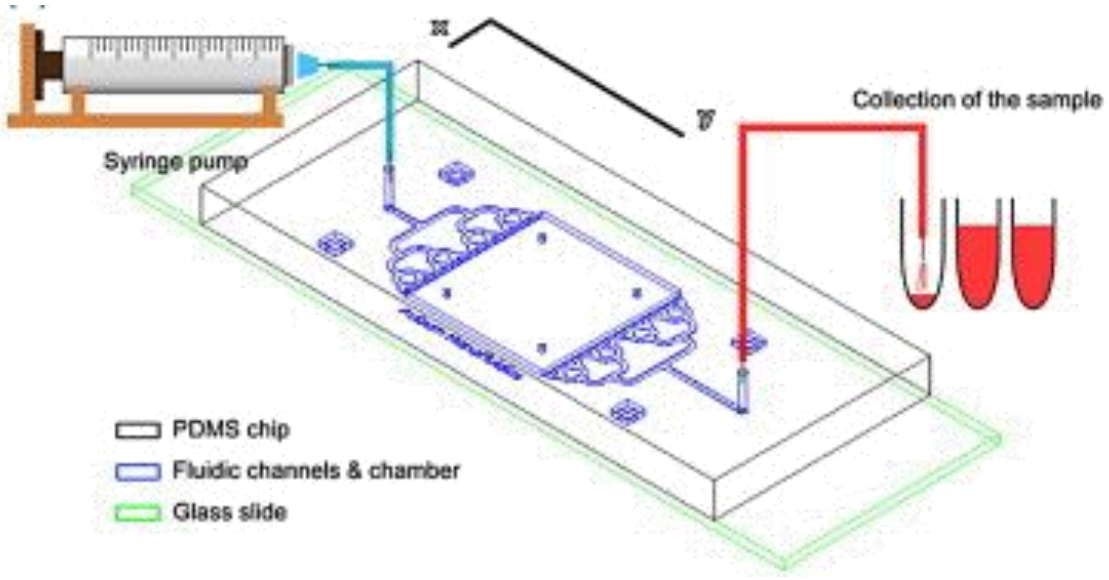

Figure 35. *Microfluidic polydimethylsiloxane (PDMS) chip for simulating tear flow. Hydrogel is placed in the microfluidic chamber. Artificial tear flow is input trough four ports and samples are collected from the output canal (Ali et al. 2007).*

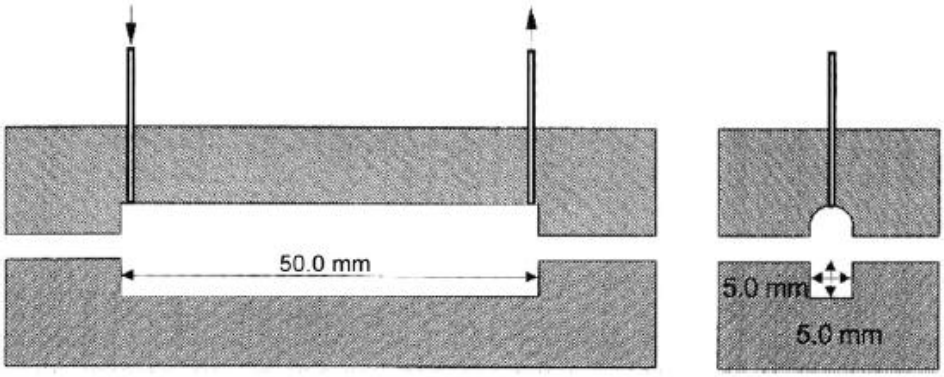

Figure 36. *The flow-through-cell for performance of drug release from matrix by artificial tear flow. Drug loaded polymer is placed in the lower section of the apparatus and artificial tear flow is led through pipelines as the arrows show (Ma et al. 2008).*

Kuncová-Kallio (2007) has presented an automated cell cultivation well-plate system for adherent cell, which was equipped with pH, temperature and O_2 sensors. Perfusion of the nutrients was supplied continuously by pulsating pumping. Every well had individual environmental control and remained fully untouched after closing. This system is feasible in a fluid flow simulation with or without cells.

8.4. Tear film desiccation

The desiccation of the eye is phenomena happening in DES. Paulsen et al. (2008) simulated eye drying in 96-well plates with conjunctival epithelial and corneal cells. On confluent growth the cultures are wetted with artificial tear composition and exposed to the constant air flow. Vitality of the cells is determined. (Paulsen et al. 2008)

8.5. Blink simulation

Eye blinks are difficult to simulate, because its lacrimation, lubrication, muscles and nerves. Only living mammalian eye works perfectly and modelling gives only indicative behaviours. Choy et al. (2008) used porcine eye simulating dry eye conditions. Eyes with eyelids and conjunctiva were enucleated from porcine within minutes after being killed, kept in moist and cool environment. Experiments were done within one day after enucleation. Surrounding tissues of eyeball were removed except the nictitating membrane, lacrimal gland and the conjunctival tissue. The eyeball and lacrimal glands were fixed in a movable arm so that nictitating membrane was placed just above the cornea. The whole cornea was exposed to air, but a nictitating membrane and lacrimal glands were covered by cotton wool moistened with phosphate buffer saline. Above the cornea, a lacrimation set for the application of artificial tears onto the superior limbal region of the eye was placed. After lacrimation, the nictitating membrane swept and spread the artificial tear over the corneal surface. (Choy et al. 2008)

9. Discussion

For the therapy of DES, there are nowadays available eye drops of natural and synthetic origin, which lubricate and moisture the cornea and conjunctiva (see appendices 1-2). These so called artificial tear drops keep the situation steady, but do not treat the disease. Today there are commercially available eye drops for DES, which are non-toxic, biocompatible and have few side effects. Properties such as swelling and adhering are also found in the artificial tear drops repertoire. Today eye drops have quite a high administration rate due to low delay and low bioavailability. The research of the DES therapy is focused to develop new strategies to reduce the administration rate, improve delay and bioavailability, but not increase dosing volume or concentration. This combination is possible by mucoadhesion, which means that drops remain longer on the cornea by physical, chemical and biochemical interactions. Another target in the research of the DES therapy is to carry a therapeutic molecule or polymer on to the cornea with the help of eye drops and treat the damaged epithelia of the cornea. Through these new strategies drug molecules should achieve better bioavailability.

Traditional commercially available eye drops for DES are mainly composed of hydrogel forming polymers. Hydrogel is usually formed before administration and has viscous character. Eye drop composition of POL blended with CMC is only an *in situ* gel forming therapy for the DES and is induced by temperature. Osmotic *in situ* induced gelation is performed by ALG and GELG in treatment of glaucoma, but pH induced gelation has no eye applications. *In situ* gel formation is an advantage, because eye drops can be dosed in a liquid state and gel is formed afterwards in the eye. The composition of the polymer is needed to be optimized in order to achieve a specific transition point and good spread over the cornea. Researchers have found potential in this line of study and a couple of temperature sensitive combinations of polymers of synthetic origin are suitable to eye: blend of POL-CARB and craft of POL-PAA. In soft tissue demonstrations, blend of HA-MC has shown interesting behaviour in temperature sensitive gelation *in situ*. A thiolated HA-PEG-DiAc combination formed rapid *in situ* gelation after injection to the soft tissue, which character is also needed in

the eye surface applications. For the treatment of the DES more development is needed to optimize composition and gel forming when administrated to the eye.

Every polymer has some kind of interactions with surfaces; attractive or repulsive forces. Aim is to explore most adhesive ones and best for ocular surface. Scientists have been investigated polymers, that are suitable for eye drops and which are more mucoadhesive than commercial drops. CARB occupied by long chain acrylate, CARR - GEL and ALG - HPMC are combinations studied today and have shown feasible characters with mucoadhesion. Mucoadhesion is notably increased by covalent disulphide bridge formation between mucin and polymer containing thiol groups. CARR is the only polymer that is naturally composed of thiol groups and is capable of forming a strong gel trough intramolecular disulphide bridges. Thiol has been introduced to the polymer backbone and conjugates such as CS-GSH, CS-TBA, HA-Cys, HA-ISH and GELG-L-Cys have been developed. These conjugates showed improved mucoadhesion, but disulfide bonds were formed also intramolecularly resulting in polymer cross links. A dry form of thiolated polymers has a long shelf life, but in a liquid form as tear drops they are unstable. Although thiolated polymers are a very promising technology there is still need for research in storage techniques and dosage forms.

Better bioavailability of the drug molecule is achieved on the cornea by sustained release, which is depending on mucoadhesion and biodegradation, but also loading capacity of the drug molecule. A hydrophilic matrix cannot carry hydrophobic molecules and therefore amphiphilic polymers have been developed. Polymers are occupied hydrophobic conjugates for binding drug molecules which are then released in the eye surface. In addition, a nanoparticle or microsphere formulation made of hydrophobic conjugates relieves more active surface area and helps penetration to the epithelia. Hydrophobic drug carrier combinations are developed such as: CARB-tetraglycol, CS-liposome, PNIPAAM-CS, CS-PEG and CS-NaALG. Improving the drug loading and releasing character of CS has been attempted by making CS more water soluble. CS has been therefore occupied with substituent of PEO, PEO-PPO or O-CM. A combination of ALG-HPMC has been shown to have feasible characters in hydrophilic drug molecule delivery. Drug molecule release can be also regulated by degradation rate, which has been experimented with cross-links of HA-ADH and HA-

MA. It is not only the drug molecule carrying character or degradation rate that is important in eye drug delivery, but also the character to sustain on the ocular surface. These polymer combinations are potential for topical and local DES therapy with or without a drug molecule, but much more research is needed.

Benefits of eye therapies in a liquid state are easiness to manufacture and administer. However, the dosing frequency is too frequent and therefore other applications such as inserts, wafers, mini tablets or contact lenses are of interest in corneal applications. Inserts can carry more drug molecules inside, sustain longer on the cornea and release drug in appropriate rate comparing to traditional eye drops. There are no inserts on the market for DES therapy, but under investigation are a few: PVA-AA, PVA-MAA, high MW PEG and CS-PEG.

There are no eye drops available for DES to treat the disease. Researchers have found supportive and protective response with trehalose and albumin, which are molecules that can be loaded into appropriate eye drops or inserts. These molecules have interesting effects on the corneal epithelia and would be potential components of the new eye drop.

There are nowadays designed bioreactors, which help to simulate eye surface behaviour. Simulation of the whole eye is impossible, but mucoadhesion, tear fluid flow, desiccation, blinking and drug penetration trough exercised porcine cornea are performed in a laboratory environment. A very interesting method is an automated cultivation well-plate system with continuous nutrition. This method allows simulation of tear flow and mucoadhesion and long-term follow up studies.

10. Conclusions

Within last years have been done many studies for new corneal mucoadhesive eye drop to treat DES, but any big breakthrough has not been done. In topical administration the main point has been to improve polymer retention time on the corneal epithelia and lower dosing rate. The most interesting method in this mean have been thiolation of polymers, which has been studied actively, In this literary survey, natural origin polymers CS and HA substituted by a thiol group was found as a potential matrix polymer in DES therapy. In *in vitro* studies, CS was occupied with GSH or TBA, and HA with Cys. Mucoadhesion feature of these polymers have been studied with intestinal mucus *in vitro.* Therefore the suitability to the eye environment will need to be studied in the future. The automated cell cultivation well-plate system provide long-term follow-up for these studies. In addition, trehalose or albumin molecule suitability as a therapeutic agent in a thiolated polymer matrix, is very interesting and worth further investigation.

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APPENDIX 1
Polymers, which form hydrogel before administration

polymer	**appr.**	**structure**	**functional groups**	**charge**	**attraction to water**	**gelation**	**degradation**	**other characters**	**commercial eye application**	**reference**
Hyaluronic acid	HA	GAG	hydroxyl and carboxyl	anionic	hydrophilic	gelation in water	hyaluronase, oxidation	+ mucoadhesive + mimic mucin - low mechanical strength	yes (DES)	Apteekkituotteet 2009; Chong et al. 2005; Kogan et al. 2007; Price et al 2007
Dextran	DEX	polysaccharide	hydroxyl	nonionic	hydrophilic	gelation in water	biodegradable	-	yes (DES)	Drug Digest 2008; Pharmacosmos 2009
Cellulose ethers	CE	modified cellulose	hydroxyl, ether lingage	nonionic	hydrophilic	thermal gelation	poor in the body	+ mucoadhesive - poor biodegradation	yes (DES)	Amercol 2009; Feller et al. 1990
Carbomer	CARB	cross-linked PAA	carboxyl group	anionic	hydrophilic	gelation in water	enzymatic and osmotic	+ mucoadhesive	yes (DES)	CarboMer 2009; Fimea 2010; Hosmani et al. 2006; Ludwig 2005
Polyvinyl alcohol	PVA	Vinyl acetate	hydroxyl	nonionic	hydrophilic	gelation in water	-	+ mucoadhesive	yes (DES)	Marten 2002; PolymerProsessing 2001; Fimea 2010
Poly N-vinylpyrrolidone	PVP	N-vinyllactam	oxygen	nonionic	hydrophilic	gelation in water	-	+ mucoadhesive + protein analogue	yes (DES)	Login 2004
Polyethylene glycol	PEG	PEO	ether bond	nonionic	hydrophilic	gelation in water	-	+ mucoadhesive + strong hydrogen bonding affinity	yes (DES)	Back & Schmitt 2004; Chemindrustry 2009; Tauber 2005
Chitosan	CS	GAG	amino	cationic	ambiphilic	gelation in water	lysozyme, acidic hydrolysis	+ mucoadhesive - non-soluble to water	yes (contact lens)	Beaulieu 2005; Chung et al. 2006; Di Mario et al. 2008; Kurita 2006; Li & Xu 2002;Ludwig 2005; Wang et al. 2007; Sinha et al. 2004

APPENDIX 2
Polymers, which form hydrogels *in situ*

polymer	**appr.**	**structure**	**functional groups**	**charge**	**attraction to water**	**gelation**	**degradation**	**other characters**	**commercial eye application**	**reference**
Poloxamer	POL	PEO and PPO bloks	hydroxyl group	nonionic	ambiphilic	thermal gelation	erodes rabidly, non-biodegradable	+ mucoadhesive + *in situ* gelation with precence of CMC - low transition temperature	yes (DES)	El-Kamel 2002; Felt et al. 1999; Nanjawade et al. 2007; Ruel-Gariépy & Leroux 2004; Yliopiston apteekki 2009
Alginate	ALG	polysaccharide	hydroxyl and carboxyl	anionic	hydrophilic	osmotic induced	biodegradable	+ makes strong clear gel *in situ* + well spreading	yes (glaucoma)	CyperColloids library 2009; Demailly et al. 2001; Nanjawade et al. 2007; Ludwig 2005
Gellan gum	GELG	polysaccharide esterified with L-glyc and Ac	hydroxyl	anionic	hydrophilic	osmotic induced	biodegradable	+ helical structure + mucoadhesive	yes (glaucoma)	BeMiller 2005; Johansson & Lindberg 1983; Kuo & Mort 1986; Nanjawade et al. 2007
Xanthan	XAN	polysaccharide with pyryvate residue	hydroxyl	anionic	hydrophilic	thermal and osmotic induced	biodegradable	- helical structure is difficult maintain - pseudoplastic	no	BeMiller 2005; Danisco 2009; McCormic et al. 2004
Xyloglucan	XYL	hemicellulose	hydroxyl	nonionic	hydrophilic	thermal gelation	biodegradable	+ low transition temperature - handling at room temperature is difficult	no	Fry 1989; Miyazaki et al. 2001; Ruel-Gariépy et al. 2004
Carrageenan	CARR	sulphated polysaccharide	sulphur and hydroxyl	anionic	hydrophilic	gelation in water	biodegradable	+ mucoadhesive + interact with cations + sulphur bridge formation *in situ*	no	FAO 1990; Verschueren et al 1996

APPENDIX 3
Modified polymers under development

Polymer	**conjucation**	**state/ dosage**	**change of the property versus unmodified polymer**	**study**	**target in body**	**Reference**
POL-CARB	blend	solution	+ mucoadhesion + *in situ* gel formation	*in vivo*	eye surface	Qi et al. 2007
POL-PAA	grafted	solution/ eye drops	+ temperature sensitive gelation *in situ* + precorneal recidence time	*in vitro* and *in vivo*	eye surface	Ma et al. 2007
CARR – GEL	cross-linked	microspheres	+ mechanical strength + mucoadhesion	*in vivo*	eye surface	Bonferoni et al. 2003
CARB - long chain acrylate	cross-linked and sonicated	hydrogel	+ mucoadhesion	*in vivo*	eye surface	Ceulemans & Ludwig 2002
ALG-HPMC	blend	solution/eye drops	+ drug molecule retaining	*in vivo*	eye surface	Liu et al. 2006
CS-liposome	complex	nanoparticle	+ drug molecule delivery to the biological surfaces and cell	*in vivo*	eye surface	Diebold et al. 2007
PNIPAAM-CS	coupled with agents	solution/eye drops	+ optimal transition temperature + sustained drug release	*in vitro*	eye surface	Cao et al. 2007
CS-PEG	blend	microspheres/ flat tablets	+ sustained drug release	*in vivo*	eye surface	Di Colo et al. 2002
CS - NaALG	cross-linked	nanoparticles/ eye drops	+ sustained drug release + penetration to the corneal epithelia	*in vitro*	-	Motowani et al. 2007
CS-albumin	cross-linked with genipin	microspheres	+ swelling ratio + able to adjust of the drug release rate	*in vitro*	-	Yuan et al. 2006
O-CM-CS	coupled	nanoparticles	+ water solubility + drug delivery character	*in vitro*	-	Zhu et al. 2006
CS-PEO and CS-PEO-PPO	coupled wit TPP	nanoparticles	+ water solubility + sustaining drug release	*in vitro*	-	Calvo et al. 1997
CS-PEO/CS-PEO-PPO + PEG	coupled with TPP	nanoparticles	+ water solubility + higher drug release rate	*in vitro*		Wang 2007
CS - GSH	coupled	nanoparticles	+ mucoadhesion by thiol groups + permeation	*in vitro*	-	Kafedjiiski et al. 2005
CS - TBA	coupled	nanoparticles	+ stability + mucoadhesion by thiol groups	*in vitro*	-	Bernkop-Schnűrch et al. 2003b
high MW PEG	-	insert	+ drug release rate	*in vivo*	eye surface	Di Colo et al 2001

APPENDIX 4
Modified polymers in other tissues

Polymer	conjucation	state/ dosage	change of the property versus unmodified polymer	study	target in body	Reference
Other soft tissues						
HA - MC	blend	hydrogel	+ temperature sensitive gelation *in situ*	*in vivo*	soft tissue	Gupta et al. 2006
HA – Cys	cross-linked by mediator	hydrogel	+ mucoadhesion 6.5 fold + intermolecular disulfide bonds lower degradation rate	intestinal mucus in bioreactor (in vitro)	-	Kafedjiiski 2007
CARB - tetraglycol	blended	suspension	+ lipophilic drug dissolving	*in vivo*	skin	Bonacucina et al. 2005
thiolated HA-PEG-DiAc	coubled	hydrogel	+ rapid gelation *in situ*	*in vivo*	soft tissue	Hahn et al. 2006
HA-ADH	cross-linked	hydrogel	+ lower degradation rate	*in vitro* and *in vivo*	soft tissue	
HA-MA	cross-linked	hydrogel	+ lower degradation rate	*in vitro* and *in vivo*	soft tissue	
HA-ISH	cross-linked	hydrogel	+ lower degradation rate	*in vitro* and *in vivo*	soft tissue	
Other potential *in vitro* tests						
ALG – HPMC	cross-linked	solution	+ swelling properties + faster drug release	*in vitro*	-	Nochos et al. 2008
ALG- COL	embedded	micro-spheres	+ drug loading capacity	*in vitro*	-	Liu et al. 2008
GELG-L-Cys	coupled by mediator	suspension	+ gelation	*in vitro*	-	Krauland et al. 2002
PVP-AA	cross-linked	wafer	+ drug release rate	*in vitro*	-	Barbu et al. 2005
PVP-MAA	cross-linked	wafer	+ drug release rate	*in vitro*	-	Barbu et al. 2005