CHAPTER 6

General discussion and conclusion

Part I: EMT in the eye lens

Under stimuli, for example associated with wounding, epithelial cells are able to modify their phenotype and genotype to mesenchymal-like cells in a process called Epithelial-Mesenchymal Transition (EMT). This is an intermediate process between the presence of epithelial cell functionality and end-stage fibrosis, which is characterized by the presence of myofibroblasts. During this transition the cells are constantly under modification and therefore it still is difficult to define a specific marker for EMT. Instead, EMT should be seen as a process of constant cell modification until the formation of the fibrotic tissue. In that sense, the loss of epithelial markers gradually transforms to the presence of more specific fibrotic markers. The detection of EMT should occur at both the protein and gene expression, i.e. phenotype and genotype level.

Fibrosis occurs in several organs such as kidney, lungs, liver, heart and eye lens. In the eye lens fibrosis appears after a cataract surgery in which the lens material is extracted and an intraocular lens (IOL) is inserted in the capsular bag. In the eye lens the lens fiber material is enclosed by the capsular bag that consists of collagens and glycosaminoglycan, separated by a monolayer of lens epithelial cells in the anterior part. The lens is crystalline and extremely elastic to allow its movement and consequently a focused vision (accommodation process) up till the age of 45. Cataract is the opacification of the eye lens that eventually leads to blindness in the patients concerned. The restoration of clear vision by insertion of an intraocular lens is accompanied by EMT of the lens epithelial cells, including fibrotic tissue formation on the posterior side and gradual, secondary loss of clear vision. The progression of the posterior capsule opacification (PCO) requires a YAG laser capsulotomy. The intense energy laser crosses the cornea, opens a hole in the fibrotic layer on the posterior lens capsule and may reach the retina. This procedure can bring some eye problems as macula oedema and retinal detachment and therefore it should be avoided if possible.

The research in this thesis is based on studies of new bioactive hydrogels that could prevent or avoid PCO by inhibition of EMT. To that goal suitable formulations of hydrogels were selected taking into consideration low markers of EMT in lens epithelial cells. Linkages of adhesion-mediating peptides were also investigated aimed to decrease EMT markers. Both *in vitro* and *ex vivo* models were developed to be used in these studies.

Biomaterials used in cataract surgery

The most common biomaterials used in IOLs are PMMA (poly-methyl methacrylate), hydrophobic acrylates, hydrophilic acrylates and less frequently silicone. Acrysof® lens are a well-known brand that produces the most frequently used IOL in the market. Brands have a narrow range of combination of materials to create IOLs that could suit the patient optical needs. Nevertheless they are based in a mix of acrylates and cross linkers that can have less or more hydrophobic properties.

As mentioned before, any of these biomaterials demonstrates PCO problems after insertion, with different percentages of the patient population involved. The presence of glistenings and also multiple microvacuoles in the IOL that affect vision are related with patients with Acrysof® lenses [1]. Hydrophobicity is a parameter that has a large influence in cell adhesion and spreading and it is also an important factor in the IOL materials. Several studies have reported that PCO could be delayed by using hydrophobic IOLs materials instead of hydrophilic materials [2, 3]. A recent in vitro study [4] with human lens epithelial cells-B3 (HLECB3), similar with cells used in **chapter 2** of this thesis, has shown that the largest cell adhesion and the lowest EMT could be seen on hydrophobic acrylic IOLs. In contrast, our studies reported in **chapter 2** comparing different levels of hydrophilicity and surface morphology led us to conclude that hydrophilic and compact materials are preferable above hydrophobic materials to promote cell adhesion and proliferation while at the same time normal epithelial cell behavior and lower EMT signals are preserved. The differences in these studies are the material surface morphology and also the chemistry of the materials. In Wang *et al*. IOLs were used and in our study hydrogels coated on a flat surface. The hydrogels differ from each other, not only with respect to the wettability, but also to fibers shape and compactness. As a conclusion, not only the wettability of the material should be taken into consideration but other parameters as chemistry and surface should be an important focus of attention in the development of a new biomaterial to be used to replace the lens material, although it is realized that these materials must be fully transparent and possess proper biomechanical properties to allow their lens function.

In order to avoid proliferation and migration of lens epithelial cells, coatings on the IOLs have also been studied. Bozukova *et al.* [5] and Okajima *et al.* [6] analyzed LEC proliferation and adhesion on IOLs coated with poly (ethylene glycol) (PEG) and poly(2-methacryloyloxyethyl phosphorylcholine) (MPC) respectively. The results have shown some decrease in cell adhesion but there was no evidence of a decrease in PCO.

In **chapter 3**, hydrogels based on nanofibers functionalized with peptides were chosen as a potentially feasible approach to prevent or reduce EMT and consequently PCO. The hydrogel used in **chapter 3** and also **4** was based on the 2D characteristics of the best coating for cell survival and proliferation from chapter 2. A low molecular weight gelator (LMWG) was developed that can be used as a stable hydrogelator, fulfilling a range of basic handling properties. This LMWG was the core for further functionalization with different peptides in different concentrations. The use of hydrogels can bring advantage over the acrylic compounds due to the intrinsically higher biocompatibility associated with the use of hydrogels. Water, hydrophilic and hydrophobic moieties can be entrapped or linked to the network of this hydrogel. The possibilities to serve as a drug carrier and cell storage depot are also beneficial for a future clinic application. Although no optical tests were performed during this thesis, this gel showed a high transparency for visible light. Due to its mechanical properties this hydrogel could be coated on commercial IOLs and can also easily be used as an injectable product in the capsular bag.

Functionalized hydrogels with cell instructor peptides

The functionalization with peptides can open new perspectives to avoid EMT. The integrin signal can be modified to avoid cellular transformation. The bioactive hydrogel when in contact with lens epithelial cells (LEC) can mediate cell alteration by allowing binding of the peptides to the integrins in the cell membrane. The peptide research was done considering one of the initial changes during EMT. Lens epithelial cells are located in a monolayer at the anterior part of the capsule bag. A defined polarity exists as they are attached to the capsular bag through the basement membrane. This condition is lost during surgery. The success of the surgery in terms of prevention of PCO relays on the removal of all the LEC from the capsule bag, which is practically very hard if not impossible to accomplish. Residual LEC respond to loss of adhesion and in general damage to the integrity of the epithelial layer by transforming into mesenchymal-like cells with capacity to migrate around the interior capsular bag.

In the eye lens the main constituents of the basement membrane are laminins and collagens. Both proteins are recognized by integrins and can be used as a trigger to maintain LEC in the epithelial phenotype and genotype. Also these proteins are frequently reported as a positive influence for cell attachment and survival. The composition of the basement membrane can diverge with the tissue of origin. In general, apart from collagen *type IV* and laminin, the basement membranes contain fibronectin and proteoglycans [7]. Fibronectin is a popular protein used to achieve cell adhesion and subsequent proliferation in many cell types but it is rarely detected in the mature eye lens. By our knowledge, cell instructing hydrogelators equipped with different peptide motifs have never been used to interfere with EMT in the eye lens. In **chapter 3** we studied the effect of the laminin α_1 and β_1 chain derivatives IKVAV (Ile-Lys-Val-Ala-Val) and YIGSR (Tyr-Ile-Gly-Ser-Arg), respectively. Their short sequence, low immunogenicity and their ability to promote cell survival made them a reliable choice to be coupled to the LMWG core. A mixture of adhesion peptides from the different basement membrane extracellular matrix proteins was also included in the study. This mixture consisted of peptides derived from fibronectin as RGD (L-arginine, glycine and L-aspartic acid) and PHSRN (Pro-His-Ser-Arg-Asn), peptides derived from collagen, DGEA (Asp-Gly-Glu-Ala) and the laminin sequences, IKVAV and YIGRS. It was concluded that a mix of laminin is not enough to improve the activity of the hydrogels to prevent EMT on LEC. Although both peptides are laminin derivatives, they show antagonistic roles in cancer cell invasion. IKVAV was reported as a promoter of angiogenesis in cancer cells [8]. However IKVAV was also mentioned as an in vivo reducer of inflammation on glial cells after a spinal cord injury [9]. On the other side, YIGSR was associated with a decrease of cancer cell invasion [10]. In our study, we concluded that this mixture enabled LEC survival but did not diminish EMT. However, a mix of different peptides derived from fibronectin, laminin and collagen enhanced LEC survival at the same time that did not promote EMT behavior. Several analyses performed in parallel with this study (not showed in this thesis) made us also concluded that the ratio of each peptide can have large influences in cell behavior.

Since the laminin-derived peptides could not avoid EMT in LEC, fibronectin and collagen peptides in different compositions were studied in **chapter 4** using an *ex vivo* capsule model. RGD peptide is one of the most well documented peptides in promoting cell adhesion, spreading and proliferation. PHSRN sequence also gained interested since it was found that it acts synergistically with RGD in promoting migration of corneal epithelial cells [11]. DGEA can be used as an antagonist of integrins for collagen *type I* receptors, a collagen related with tumor progression [12]. Avoidance of collagen *type I* deposition could reduce EMT in LEC, as this deposition is often correlated with a fibrotic matrix. In vitro studies on rabbit LEC have shown a benefit on cell adhesion and migration in contact with fibronectin and collagen *type IV* [13]. Similar results were found on porcine LEC in contact also with coatings of collagen *type I* and *IV* but not with laminin [14]. Moreover, α-SMA expression was increased on fibronectin and collagen *type I* but not on collagen *type IV* and laminin. These results were in line with our work in **chapter 4**, where we demonstrated a regeneration and large cell differentiation of LEC on the porcine capsular bag in contact with our hydrogel mixture containing RGD and DGEA peptides (fibronectin and collagen *type I* derivate, respectively). It was seen that this peptide mixture had a strong benefit in the regeneration of the cell cytoskeleton and the promotion of cell survival after 2 days of culture. However this regeneration came in parallel with a significant cell differentiation towards myofibroblasts-like cells. Independent of LEC being in the natural capsular bag (**chapter 4**) or in direct contact with the culture plate (**chapter 3**) the laminin mixture did not show any signals of improved EMT-related cell behavior. Laminins are major compounds in the basement lens capsule and are essential in lens development [15]. Several laminin-binding integrins were detected in lens development, yet in literature, laminin is not reported as a cell protector, but the laminin receptor, $\alpha_6\beta_1$ integrin, is expressed after a cataract surgery [16]. In this sense, the laminin hydrogel should be an advantage for cell survival. The opposite was detected in our study, probably due to an inadequate concentration of peptides in the hydrogel. By our knowledge both the concentration and the ratios of the used peptides can have an important role in cell behavior. Therefore, different concentrations and also, maybe more importantly, ratios should be investigated further to investigate this phenomenon.

In **chapter 4** it was also discovered that the hydrogel mixture that resembles the basement membrane most had improved cell survival and did not show significant signs of cell dedifferentiation towards EMT after 5 days in culture.

IOL design vs injectable gels

Several studies support the idea that IOL design has a primordial impact in avoiding PCO after a cataract surgery. Studies indicate that the design of the lens can even suppress the influence of the IOL material. Hara *et at*.[17] found the importance of an equatorial capsule ring in preventing LEC invasion to the posterior capsular side. Since then two IOL shapes are being intensely investigated and used, the square-edge and the round-edge shape. These “arms” of the IOL are intended to be a mechanical barrier of LEC migration to the posterior side of the capsule bag. In this thesis, we hypothesize that if

the capsular bag is refilled with an injectable hydrogel, the lens could keep its natural shape and the hydrogel, by its properties, could avoid LEC transformation into EMT and thus PCO. Since the capsular bag would be refilled, all the residual LEC would be in contact with the hydrogel that would provide instructions by targeting the integrins and these new integrin-ligand interactions in conjunction with the presence of nanofibers would be able to maintain the epithelial phenotype (Fig. 1). Obviously, without the myofibroblasts-like cells the fibrotic tissue will not be created. While developing the LMWG its potential use in the clinic was taken into consideration. Although the viscosity proprieties were not measured, its potential applicability in a cataract surgery setting has been reported by our colleagues [18].

Replacing the interior of the lens with a viscous gel is a well investigated practice. A study to replace the lens material with silicone has been done by Koopmans *et al*.[19]. Here the success of the technique relied on a chemical treatment to kill all the residual LEC before the injection of the gel. The use of drugs such as anti-inflammatory drugs, immunotoxins, cytokines and TGF-β inhibitors as a suppressor of LEC is well documented in the literature. Despite the in vitro and *in vivo* benefits of these drugs, their toxicity in the eye often is a limiting factor for their use. The employment of cell instructing hydrogelators with linked peptides, for example the described LMWG BM mixture (**chapters 3** and **4**), can thus be seen as a new, valuable strategy to control LEC phenotype and genotype.

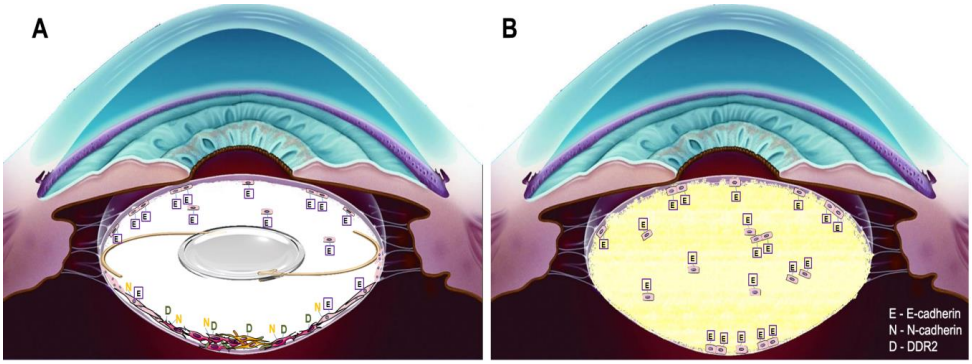

Figure 1: Schematic representation of eye lens after a cataract surgery. A) represents the current situation after an insertion of an IOL. The residual LEC express E-cadherins and along of the migration to the posterior side of the capsule bag, their phenotype change to mesenchymal–like cells. The expression of cell surface markers change to N-cadherins and DDR2 (discoidin domain receptor tyrosine kinase 2). The deposition of these cells together with fibronectin, collagens, α-SMA, among others create the fibrotic tissue, known as PCO. B) represents a future approach to avoid the formation of fibrotic tissue. The injection of a bioactive hydrogel enforces residual LEC to behave as epithelial cells, maintaining their phenotype and E-cadherin expression.

Lens model to study EMT

The eye lens has some peculiar characteristics that can bring advantages to study *in vitro* (**chapter 3**) and *ex vivo* (**chapter 4**) EMT. LEC display a well characterized EMT and a pronounced PCO limited by the capsular bag. This controlled environment inside the capsular bag together with its permeability to small molecules and fluids can be used to control specific parameters in an *ex vivo* model.

More specifically in EMT studies, the cellular (de)differentiation and migration are important parameters. The use of LEC in contact with the anterior capsule bag for *ex vivo* analysis can resemble more the natural environment and the reliability of the studies can be enhanced without exceptional surgical skills from the operator.

In the lens of the eye there is no innervation or vascular system, which allows the mimicking of the natural environment with high efficiency and reproducibility with less complexity than in other organs. In addition, the lens is composed by a LEC monolayer allocated on the anterior side of the capsular bag, which can be used in a more located *in vivo* situation than in other organ models.

Using the anterior capsular bag with the LEC monolayer in direct contact with gels in in vitro and *ex vivo* models, a post-operative scenario can be studied, because the interior of the capsular bag after surgery suffers a large stress and consequently an increase in EMT signals.

Part II: Detection of cornea epithelial cells damage

The cornea of the eye is the most external part of the eye and consequently more prone to mechanical damage. An external layer of lipids, aqueous and mucins is crucial to reduce the impact of possible damage and also to maintain the eye moisture. It has been shown that the eye drops available in the market have a low effect in restoring the cornea properties in dry eyes, mainly due to the fragile linkage with the cornea which causes a quickly disappearance of the ingredients. Samson *et al*. [20] studied the effect of a mucin-like glycoprotein, proteoglycan 4, in a human cornea. However, in this study the cornea was previously cut off. The removal of the cornea from the eye ball requires an accurate technique to avoid extra cornea damage and the elastic properties of the cornea change when taken from the eye ball. Therefore, new techniques to explore a more realistic effect of lubricants in the cornea are required. In **chapter 5** we describe the development of a new tribometer to evaluate damage of the cornea in the intact eyeball. Combined with an elegant and accessible fluorescence technique, damage can be visualized. This is based on the fact that intact cell membranes of the cornea epithelial cells do not allow penetration of the actin-cytoskeleton staining agent. In this sense, the damage was measured by the quantity of corneal epithelial cells with cytoskeleton staining.

The developed new tribometer associated with confocal staining of fluorescent agents penetrating the cornea epithelial cell layers allows a more rigorous and real evaluation of the effects of lubrication in the cornea epithelial cells.

Part III: Future perspectives

The work in this thesis had the goal to explore new insights in reducing EMT with focus on the eye lens, realizing that EMT is a widespread phenomenon that can be identified in many other organs. The use of native LEC on the capsular bag in direct contact with the biomaterials is a more realistic model, since the cells are still sensing their natural basement membrane. This model can also be used for other studies related to the control of cell behavior including EMT.

We showed that the idea of using hydrogelators equipped with peptides to activate integrins during the EMT process in lens epithelial cells has its merits for a sustainable approach to avoid or reduce EMT in these cells. Going along with the arguments provided by Eldred *et al*. [21] as the use of a lens model to study fibrosis, it makes sense to adopt a similar strategy for other organs. However, the relative and absolute quantity and also ratios of peptides coupled to the hydrogelators needs much more investigation as there seems to be an optimal composition for avoiding EMT. Compositions should be analyzed taking into consideration the requirements for obtaining an ultimate hydrogelator with appropriate viscosity that can be used as an injectable biomaterial.

Cells experience large changes during EMT with a total transformation of the integrin signals and a switch of cadherins. The loss of connection of epithelial cells with the basement membrane is only one of the important facts that occur during this process. These cells may respond to the presence of adhesive peptide motifs surrounding them. New peptides motifs could be investigated, as for example HAV (His-Ala-Val). This peptide has been found in the extracellular domains of classical cadherins and is reported as an inhibitor of N-cadherins [22]. Therefore, the typical switch from E-cadherins to N-cadherins during EMT could be avoided.

Since the adhesive peptides often are interacting with integrins, further research using anti-integrin antibodies should be performed. In our case, the detection of integrins participating in binding to collagens, laminin and fibronectin can be important to further identify which adhesive peptides are optimal to control the cell response.

Although the eye lens is not directly surrounded by blood (immune privilege) the ocular fluids are full of immunoinhibitors [23]. Studies on biocompatibility using T cells or macrophages could be a benefit for further implementation *in vivo*, especially when working with biologically active components.

The biomechanical properties of the hydrogels as viscosity and refractive index should also be analyzed since these hydrogels must resemble the natural lens for optimal visual acuity. Moreover, for a clinical application the hydrogels must be injected in the interior of

the lens and therefore should have a convenient viscosity that allows it to be used in a surgical needle and that for a sufficient amount of time.

Once some successful studies have been done in the attempt to kill or suppress the residual LEC with drugs before the insertion of the IOL [24, 25], this concept could also be used as a combined treatment with the hydrogelator. Actinomycin D is a powerful and aggressive drug amply used in *in vitro* studies. Its toxicity raises many questions about the benefit of its use. Also, actinomycin D, as most of the drugs, has a high molecular weight making it impossible to link it to our hydrogelator. In this sense, new approaches for the use of drugs in the eye lens can be studied. The importance of EMT in fibrosis and cancer is well described; therefore the use of anti-cancer drugs could bring new opportunities for treatments. For instance, drugs with tyrosinekinases as main targets could decrease the effects of TGF-β in the early beginning of the inflammation.

As a final step, *in vivo* studies using our best combination of peptides and hydrogelator (for example the BM mixture, but after further exploration most likely a different composition) should be performed. The main question here is which animal model should be used. Experiments performed in rabbits and monkeys have demonstrated that the local lens environment in both animals led to very different outcomes in terms of capsular opacification. In this sense the use of pig eye lens material as proposed in **chapter 4** also can be a matter of debate.

Developing new methods to study the damage in cornea epithelial cells has also been described in this thesis. A new tribometer, to measured coefficient of friction, was designed to use a full eyeball instead of a cornea fixed in a platform. This is a more realistic method which associated with immunohistochemistry is a reliable and simple method to detect cornea damage. This technology allows more sensible tests to analyze the effects of different lubricants in the eye cornea and further lubricants can be studied using this method.

CONCLUSION

EMT is considered to be the initial step for the deposition of a fibrotic tissue in the (posterior) lens capsule. Posterior capsule opacification is a phenomenon that originates from the replacement of the nucleus of the lens with an IOL, usually in relation to cataract surgery.

In this thesis new approaches to reduce and ideally avoid EMT in lens epithelial cells were studied. Lens epithelial cells behavior was modulated by the use of cell instructing hydrogelators designed with different peptide motifs in different concentrations towards a reduction in EMT.

The hydrogelator with peptides that resemble the basement membrane most showed a substantial reduction of EMT. This model of hydrogelator could be used for further design in order to totally avoid EMT of the residual lens epithelial cells on the capsular bag. Additional studies for its suitability in replacing the lens nucleus should be performed, although the data in this thesis support this idea.

A new method to detect mechanical damage in cornea epithelial cells was also described in this thesis. We were able to associate a new tribometer with fluorescence staining to perform reliable tests of cornea lubrication and related cornea epithelial damage.

REFERENCES:

[1] van der Mooren M, Franssen L, Piers P. Effects of glistenings in intraocular lenses. Biomedical Optics Express. 2013;4:1294-304.

[2] Heatley CJ, Spalton DJ, Kumar A, Jose R, Boyce J, Bender LE. Comparison of posterior capsule opacification rates between hydrophilic and hydrophobic single-piece acrylic intraocular lenses. Journal of Cataract & Refractive Surgery. 2005;31:718-24.

[3] Wejde G, Kugelberg M, Zetterström C. Posterior capsule opacification: comparison of 3 intraocular lenses of different materials and design. Journal of Cataract & Refractive Surgery. 2003;29:1556-9.

[4] Wang G-Q, Dang Y-L, Huang Q, Woo VC-P, So K-F, Lai JS-M, et al. In vitro evaluation of the effects of intraocular lens material on lens epithelial cell proliferation, migration, and transformation. Current Eye Research. 2017;42:72-8.

[5] Bozukova D, Pagnoulle C, De Pauw-Gillet M-C, Desbief S, Lazzaroni R, Ruth N, et al. Improved performances of intraocular lenses by poly(ethylene glycol) chemical coatings. Biomacromolecules. 2007;8:2379-87.

[6] Okajima Y, Saika S, Sawa M. Effect of surface coating an acrylic intraocular lens with poly(2-methacryloyloxyethyl phosphorylcholine) polymer on lens epithelial cell line behavior. Journal of Cataract & Refractive Surgery.2006;32:666-71.

[7] Yurchenco PD, Schittny JC. Molecular architecture of basement membranes. The FASEB Journal. 1990;4:1577-90.

[8] Grant DS, Kinsella JL, Fridman R, Auerbach R, Piasecki BA, Yamada Y, et al. Interaction of endothelial cells with a laminin A chain peptide (SIKVAV) in vitro and induction of angiogenic behavior in vivo. Journal of Cellular Physiology. 1992;153:614-25.

[9] Tysseling-Mattiace VM, Sahni V, Niece KL, Birch D, Czeisler C, Fehlings MG, et al. Self-assembling nanofibers inhibit glial scar formation and promote axon elongation after spinal cord injury. The Journal of Neuroscience. 2008;28:3814-23.

[10] Sakamoto N, Iwahana M, Tanaka NG, Osada Y. Inhibition of angiogenesis and tumor growth by a synthetic laminin peptide, CDPGYIGSR-NH_2. Cancer Research. 1991;51:903-6.

[11] Hattori A, Hozumi K, Ko J-A, Chikama T-i, Oomikawa K, Kato J, et al. Sequence specificity of the PHSRN peptide from fibronectin on corneal epithelial migration. Biochemical and Biophysical Research Communications. 2009;379:346-50.

[12] Salaam AD, Hwang P, McIntosh R, Green HN, Jun H-W, Dean D. Nanodiamond-DGEA peptide conjugates for enhanced delivery of doxorubicin to prostate cancer. Beilstein Journal of Nanotechnology. 2014;5:937-45.

[13] Olivero DK, Furcht LT. Type IV collagen, laminin, and fibronectin promote the adhesion and migration of rabbit lens epithelial cells in vitro. Investigative Ophthalmology & Visual Science. 1993;34:2825-34.

[14] de Jong-Hesse Y, Kampmeier J, Lang GK, Lang GE. Effect of extracellular matrix on proliferation and differentiation of porcine lens epithelial cells. Graefe's Archive for Clinical and Experimental Ophthalmology. 2005;243:695-700.

[15] Walker J, Menko AS. Integrins in lens development and disease. Experimental Eye Research. 2009;88:216-25.

[16] Linnola RJ, Werner L, Pandey SK, Escobar-Gomez M, Znoiko SL, Apple DJ. Adhesion of fibronectin, vitronectin, laminin, and collagen type IV to intraocular lens materials in pseudophakic human autopsy eyes. Journal of Cataract & Refractive Surgery. 2000;26:1792-806.

[17] Hara T, Hara T, Yamada Y. "Equator ring" for maintenance of the completely circular contour of the capsular bag equator after cataract removal. Ophthalmic Surgery, Lasers and Imaging Retina. 1991;22:358-9.

[18] Nibourg LM, Gelens E, de Jong MR, Kuijer R, van Kooten TG, Koopmans SA. Nanofiber-based hydrogels with extracellular matrix-based synthetic peptides for the prevention of capsular opacification. Experimental Eye Research. 2016;143:60-7.

[19] Koopmans SA, Terwee T, van Kooten TG. Prevention of capsular opacification after accommodative lens refilling surgery in rabbits. Biomaterials. 2011;32:5743-55.

[20] Samsom ML, Morrison S, Masala N, Sullivan BD, Sullivan DA, Sheardown H, et al. Characterization of full-length recombinant human Proteoglycan 4 as an ocular surface boundary lubricant. Experimental Eye Research. 2014;127:14-9.

[21] Eldred JA, Dawes LJ, Wormstone IM. The lens as a model for fibrotic disease. Philosophical Transactions of the Royal Society B: Biological Sciences. 2011;366:1301-19.

[22] Blaschuk OW, Sullivan R, David S, Pouliot Y. Identification of a cadherin cell adhesion recognition sequence. Developmental Biology. 1990;139:227-9.

[23] Caspi RR. A look at autoimmunity and inflammation in the eye. The Journal of Clinical Investigation. 2010;120:3073-83.

[24] Fernandez V, Fragoso MA, Billotte C, Lamar P, Orozco MA, Dubovy S, et al. Efficacy of various drugs in the prevention of posterior capsule opacification: Experimental study of rabbit eyes. Journal of Cataract & Refractive Surgery. 2004;30:2598-605.

[25] Legler UFC, Apple DJ, Assia EI, Bluestein EC, Castaneda VE, Mowbray SL. Inhibition of posterior capsule opacification: The effect of colchicine in a sustained drug delivery system. Journal of Cataract & Refractive Surgery. 1993;19:462-70.