Prevention of posterior capsule opacification

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Table of contents

Summary

Interventions in IOL design and material have led to a reduced level of PCO over the past decade, but have not eradicated the problem. The desire to implant accommodating IOL's during cataract surgery in the future is held back by knowing that the success of this technology will be greatly affected by PCO formation. Resolving the problem of PCO is therefore very important.
Our understanding of the biological mechanisms contributing to PCO formation is growing, and therefore there is new development in interventions for prevention of PCO formation. New development pharmacological agents for prevention of PCO has recently caught attention. If pharmacological agents could suppresses or prevent signaling systems of PCO in a safe and effective manner. It would potentially improve the lives of millions who suffer of secondary cataract after implantation of an IOL.

1. Introduction

Cataract surgery is at present the only way to treat cataract. Cataract is a clouding of the lens that develops in the crystalline lens of the eye, varying in degree from slight to complete opacity and obstructing the passage of light. Most of the time, cataract is age-related. It is predicted that the number people with cataract will increase of the years, due to an increase in increased-aged population.
Cataract surgery involves removal of a segment of the anterior capsule and extraction of the opacificied lens. This produces a lens capsular bag that can house an intraocular lens implant (IOL).
However, posterior capsular opacification (PCO), which is also termed secondary cataract, is a common long-term complication of modern cataract surgery.
Following the mechanical insult of surgery, the remaining lens epithelial cells can rapidly grow and could ultimately encroach on the visual axis, which can give rise to secondary visual loss[1]. Lens epithelial cells (LECs) left behind in the capsular bag after cataract surgery are mainly responsible for PCO development. Proliferation, migration, epithelial-to-mesenchymal transition (EMT), collagen deposition, and lens fiber regeneration of LECs are the main causes of opacification[2].
There are 2 morphological types of PCO, the fibrosis type and the pearl type. Fibrosis-type PCO is caused by the proliferation and migration of LECs, which undergo transdiffentiation, leading to significant visual loss by producing folds and wrinkles in the posterior capsule[2]. The pearl-type PCO is caused by the LEC's located at the equatorial lens region causing regeneration of crystallin-expressing lenticular fibers and forming Elschnig pearls and Soemmering ring, responsible for most cases of PCO-related visual loss[3].
There are several reasons to eradicate PCO. One of the reasons is that PCO remains the most common complication of cataract surgery[4]. Another reason is that Nd:YAG laser capsulotomy, currently the only effective available treatment of PCO, has several significant complications. The technique clears the visual axis, but can bring damage to the intraocular lenses, increasing intraocular pressure, cystoid macular edema, retinal detachment and iris hemorrhage[5]. Above that, this procedure imposes a financial burden on the health care system.
Therefore it's necessary to find a safe and effective way to reduce or eradicate PCO. Nowadays, investigators are mainly busy with interventions to reduce PCO. Interventions for preventing PCO fall into three main categories.

Surgical modifications

Because PCO is predominantly caused by residual LECs in the capsular bag after cataract surgery, several surgical techniques have been attempted for the removal of these LECs at the time of lens extraction[6]. These techniques include polishing the capsule to reduce the number of lens epithelial cells remaining in the capsule bag after surgery with the aim of delaying the formation of PCO and reducing the amount of PCO[7].
Another surgical strategy is to control the size of the capsulorhexis (the opening in the anterior capsule). A smaller capsulorhexis usually results in a complete overlap of anterior capsule and IOL optic. This sealing is thought to enhance the PCO inhibiting effect of the IOL optic[8].
One of the new surgical techniques, which is in development, is the 'bag-in-the-lens' concept.
This involves the performance of anterior and posterior capsulorhexis of the same size. If the capsules are well stretched around the optic of a twin-capsulorhexis

intraocular lens design, it significantly reduces the interaction of the lens with the capsular bag. The LEC's will not be able to proliferate across the lens, so the visual axis will remain clear[9].

Therapeutic Agents

The development of an alternative medical treatment of PCO is of critical importance.
The use of therapeutic agents during or after surgery has recently received attention. The goal of pharmacological interventions during or after surgery is depleting or inhibiting regeneration of remaining lens epithelial cells. When selectively destroying residual LECs, it's important to avoid toxic effects on other intraocular tissues.
The commonly used methods for this application are direct injection into the anterior chamber, addition to the irrigating solution, or impregnation of the IOL[8].

Intraocular lens material and design

The interventions in IOL's are in geometric and material properties.
Several advancements have been made in IOL materials and designs to improve biocompatibility.
Biocompatibility in terms of the eye's foreign body reaction against the IOL and interaction of the IOL with residual LEC's within the capsular bag. Especially interaction with LEC's, because it has shown that this type of interaction influences LEC proliferation, migration, epithelial-to-mesenchymal transition (EMT), resulting in PCO.
Interventions in design are the introduction of sharp optic edges. This appears to reduce the incidence of PCO.
Interventions in design, in combination with intraocular lens material, which range from the high water content hydrophilic acrylic material, low water content hydrophobic acrylic material, to hydrophobic silicone material, gives differences in biocompatibility. Because these materials differ in their physical and chemical properties[8].

In this study, I will lift out 2 interventions of PCO prevention.
Prevention by IOL design and material and prevention by pharmacological agents.
In my Bachelor thesis, I investigated 2 pharmacological agents. These results I will also review.

2. Prevention of PCO by IOL design and material

2.1. Influence of IOL optic material on the development of PCO

Materials used for the manufacture of IOL's can be divided into two groups: acrylic and silicone. Acrylic lenses can be further divided in:
- manufactured from polymethylmethacrylate (PMMA), these lenses are ridged.
- manufactured from hydrophobic acrylic materials, these lenses are foldable.
- manufactured from hydrophilic acrylics materials (hydrogels), these lenses are foldable.

Hydrophobic acrylic lenses and silicone lenses have a very low water content, while hydrophilic acrylic lenses have higher water contents.

2.1.1. Biocompatibility

When we look at the biocompatibility of the intraocular lenses after implantation, we can divide biocompatibility in 2 groups:

- Uveal biocompatibility: the eye's foreign body reaction against the IOL
- Capsular biocompatibility: interaction of the IOL with residual LECs within the capsular bag

A summary is shown in Fig.1.

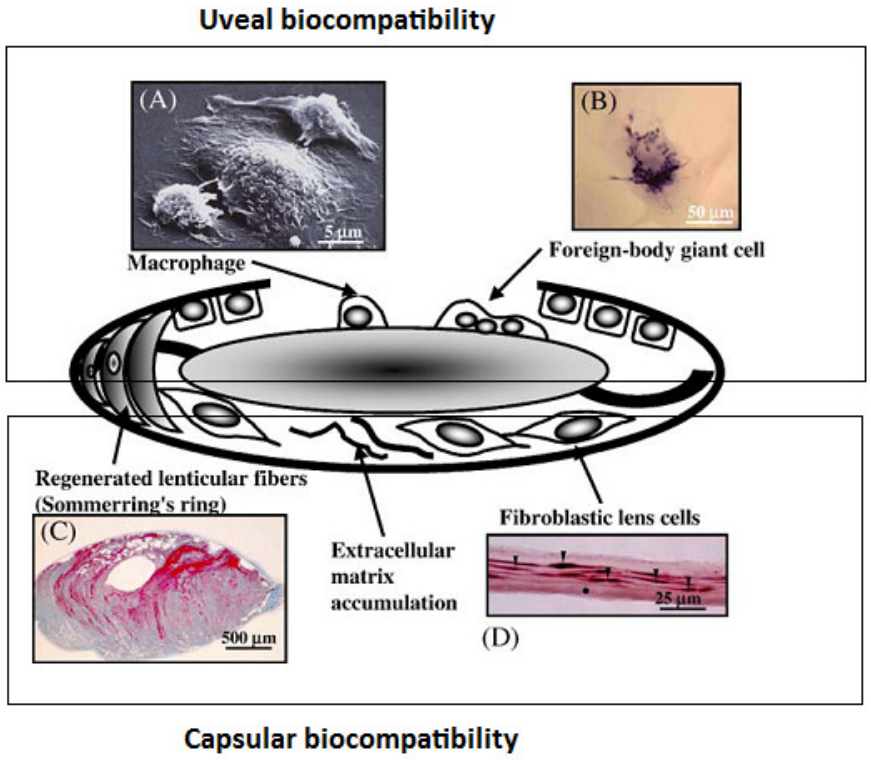

Fig. 1. Summary of biological reaction against an implanted IOL.
Foreign body reaction is mediated by macrophages (A) and foreign body giant cells generated through a fusion of many macrophages (B).
The equatorial region of the capsular bag is occupied by regenerated lenticular fibers of Sommerring's ring (C). Lens epithelial cells on the posterior capsule exhibit an elongated, fibroblast-like, shape (D) or Elschnig's pearl formation. (Reproduced from Saika[3], with a minor modification.)

2.1.2.Inflammatory response of the eye after intraocular lens implantation

Cataract surgery with IOL implantation produces a breakdown of the blood–aqueous barrier.
This will start a wound healing process, with release of proteins and cells into the anterior chamber.
Macrophages and foreign-body giant cells are covering the surface of the implanted IOL. This is shown in Fig.2[3], where IOL's of different materials where implanted in rabbit eyes and evaluated for macrophages and giant cells, 2 weeks after implantation.
Silicone lenses seems to be the most biocompatible to the eye, because on their surfaces no cellular deposits are seen. While on surfaces of PMMA IOL's many macrophages and giant cells are shown.

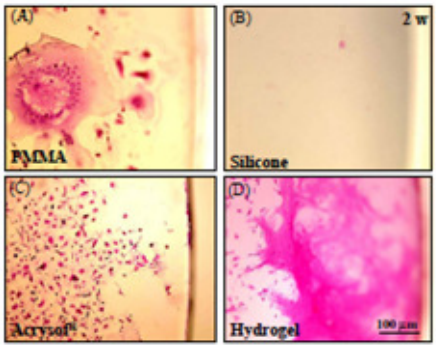

Fig. 2. Histological observation by hematoxylin and eosin staining of the edge area of the optic of an IOL of each material 2 weeks post surgery in rabbits. Many macrophages and foreign-body giant cells are observed on the optic surface of a PMMA IOL (A), while no cellular deposits are seen on a silicone IOL(B). On a hydrophobic acrylic IOL (C) many macrophages are detected, but foreign-body giant cells are less as compared with a PMMA IOL. The edge of the optic of a Hydrogel IOL is covered with a sheet of lens epithelial cell outgrowth(D)

2.1.3.Lens epithelial cell response after intraocular lens implantation

The epithelium of the natural crystalline lens consists of a sheet of anterior epithelial cells that are in continuity with the cells of the equatorial lens bow .
Following cataract surgery residual lens epithelial cells trans-differentiate to (myo)fibroblast-like cells on the IOL surface and on the inner surface of the residual lens capsule. They express a-smooth muscle actin that is not detected in normal lens cells[1].
The lens cells in the equatorial area differentiate into lenticular fibers of Soemmerring's ring and differentiate into Elsching's pearls on the posterior capsule, both characterized by the presence of crystallins-expresssing fiber cells that lack nuclei[3].
These cellular responses are believed to be induced by rises in growth factor concentration following cataract surgery[10]. However, cellular responses to cataract surgery may also be further influenced by the surface property of an IOL besides growth factor modulation[11].

As we showed before, the rank order of macrophage adhesion to an IOL is: hydrogel IOL>PMMA IOL>hydrophobic acrylic IOL>silicone IOL. This is part of the inflammatory response of the eye.
But when we look at the lens epithelial cell response, less deposition of cells on a

silicone IOL does not implicate less fibrotic reaction on the capsule. There is more fibrotic tissue formed around a silicone IOL[9].
Because of this effect, it can be said that a silicone IOL is less biocompatible to lens epithelial cells than PMMA. Silicone IOL's seems to stimulate the epithelial–mesenchymal transition of lens epithelial cells (Fig. 3).

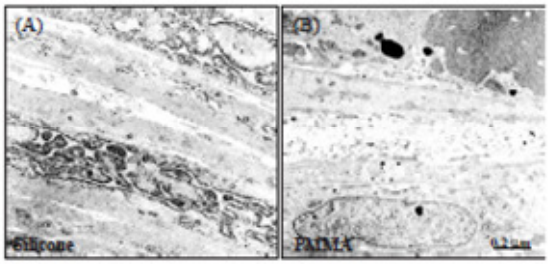

Fig. 3. Ultrastructure of rabbit lens epithelial cells 2 months following lens extraction and implantation of an IOL. The cells with a silicone IOL (A) are more markedly elongated as compared with those with a PMMA IOL (B). Endoplasmic reticulum is developed in cells with a silicone IOL, suggesting functional protein synthesis.
Regenerated lenticular structure is also seen in the specimens with a PMMA IOL. (Saika[3])

Hydrophilic acrylic material IOL's are more biocompatible to lens epithelial cells[12].
When we look at hydrophobic acrylic IOL's, there is no significant difference in adhesion of macrophages/foreign-body giant cells between a PMMA IOL and a hydrophobic acrylic IOL (Fig. 2).

2.1.4. PCO formation

When we look at the incidence of PCO formation there are also differences between the different IOL materials. In most studies, the incidence of PCO formation is quantified by PCO scoring systems. These scoring systems give a grade or a percentage of opafication behind the IOL . This grade or percentage multiplied by the fraction of capsular area involved, gives an individual PCO score of each IOL material. These scores can be generated from texture analysis (Aqua system), picture/pixel analysis (POCO system), morphological assessment (EPCO analysis) or subjective analysis[13].
Findl[8] compared the PCO scores of different studies of different IOL materials. I will summarize these results.

Silicone vs. PMMA IOL's
There was no significantly different PCO score between these IOL materials, because in some studies the PCO score were lower in the silicone group than in the PMMA group[14]. (Fig. 4)
But in one study, the PCO score was higher in the silicone group than in the PMMA group[15].

PMMA vs. hydrophilic acrylic IOL's
The PMMA IOL's were favorite over hydrophilic acrylic IOL's[14,16]. (Table 1)

Silicone vs. hydrophilic acrylic IOL's
In hydrophilic acrylic IOL's, the PCO score was higher than in silicone IOL's[14]. (Fig.4)

Kugelberg[17] compared hydrophobic acrylic IOL material with hydrophilic acrylic IOL material. Her study showed that 2 years after surgery the implantation of hydrophobic acrylic IOL's significantly less PCO formation caused compared to hydrophilic acrylic IOL's. (Table 2)

An meta-analysis of the included studies showed significantly higher PCO rates in hydrophilic acrylic IOL's than in other IOL optic materials (PMMA, silicone and hydrophobic acrylic). But there was no significant difference between other IOL optic materials (PMMA, hydrophobic acrylic, silicone).
Some of the studies compared round edge IOL's with sharp edge IOL's. In those cases, the optic edge design probably had more effect on the development of PCO than the IOL optic material.

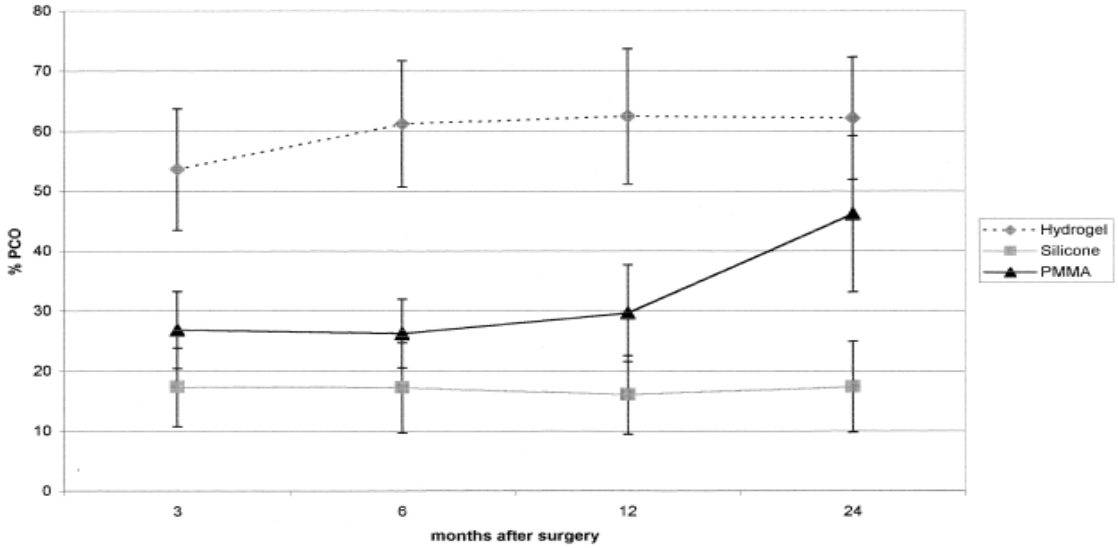

Fig. 4. Mean percentage area of posterior capsular opacification (PCO) plotted against time for the three intraocular lens materials (error bars represent 95% confidence intervals). PMMA = polymethylmethacrylate. (hollick[14])

Table 1. Mean percentage area of PCO postoperatively. (Meacock[16])

	Mean % PCO (SD)			
Group	**3 Months**	**6 Months**	**1 Year**	**2 Years**
Hydrogel	53.7 (28.3)	61.2 (28.9)	62.5 (31.0)	62.6 (23.7)
PMMA	26.9 (17.6)	26.3 (16.0)	29.7 (22.6)	45.6 (32.0)

PMMA = poly(methyl methacrylate)

Table 2. Percentage PCO of hydrophobic acrylic and hydrophilic acrylic IOL groups (copy from Kugelberg[17], with minor modification)

Parameter	hydrophobic acrylic IOL group (n = 58)	hydrophilic acrylic IOL group (n = 57)	p-value
PCO%	4.5 (0-71)	46 (0-100)	< 0.001
PCO severity	0.045 (0-0.83)	0.74 (0-2.2)	< 0.001

PCO = posterior capsule opacification

2.2 Influence of IOL optic design on the development of PCO

The design used for the IOL's is very divers. The influence of the IOL optic design on the development of PCO is different for different optic designs.
I will summarize the different IOL optic designs and their influence on the development of PCO.
Again, the comparison influence of different IOL designs on the development of PCO was made by the comparison of PCO scores.

Sharp versus round optic edges in PMMA, acrylic and silicone IOL's
There is a significantly higher PCO score in the round edge group than in the sharp edge group of every material used to manufacture the IOL's[8]. (Table 3)

Table 3: comparison sharp versus round optic edges (all materials) (copy from Findl[8], with minor modification)

Study or subgroup	Sharp N	Mean(SD)	Round N	Mean(SD)	Mean Difference IV,Random,95% CI	Mean Difference IV,Random,95% CI
Buehl 2002	45	11 (7.5)	45	21.9 (12.7)		-10.90 [-15.21, -6.59]
Buehl 2004	40	7.1 (6.5)	40	14 (11.3)		-6.90 [-10.94, -2.86]
Findl 2005a	32	22.9 (32)	32	51.2 (17.6)		-28.30 [-40.95, -15.65]
Hayashi 1998	73	16 (10.3)	69	26.3 (12.2)		-10.30 [-14.02, -6.58]
Hayashi 2004	95	9 (6)	95	20 (12)		-11.00 [-13.70, -8.30]
Hayashi 2005	69	10.2 (3)	69	15.3 (4)		-5.10 [-6.28, -3.92]
Hollick 1998	19	10.2 (0)	23	56.1 (0)		0.0 [0.0, 0.0]
Kcksmer 2000	21	4 (10.3)	21	44 (30)		-40.00 [-53.57, -26.43]
Mester 2004	73	0.2 (1.2)	73	1.1 (3.1)		-0.90 [-1.66, -0.14]
Pohjalainen 2002	16	15.6 (0)	22	11.4 (0)		0.0 [0.0, 0.0]
Sacu 2004d	53	6.5 (10.5)	53	23.3 (18.8)		-16.80 [-22.60, -11.00]
Sacu 2005a	24	12 (6)	24	24 (14)		-12.00 [-18.09, -5.91]
Sundelin 2005	57	0.75 (5.54)	59	9.44 (17.98)		-8.69 [-13.50, -3.88]
Wejde 2003	40	5.8 (0)	43	15.5 (0)		0.0 [0.0, 0.0]
Zemaitiene 2004	84	0.4 (0.2)	42	3.1 (0.5)		-2.70 [-2.86, -2.54]

Square-edge optic design has been recognized to be one of the most important factors in the reduction of PCO in adults[18].
The reduction of PCO has been observed with all IOL material types. However, hydrophobic acrylic material with a square edge has been observed to further decrease the incidence of PCO in comparison with other materials. Especially when compared with hydrophilic optic IOL's[19].

3. Prevention of PCO by pharmacological agents

Pharmacological prevention of PCO is still in the experimental stage but is next to are lens design and materials and surgical technique, the third prevention method of PCO after cataract surgery.
Experiments to find a substance, which would reduce PCO, go back to the early 1970's, when many substances were rejected because they were too toxic to the corneal endothelium.
The goal of pharmacological interventions during or after surgery is depleting or inhibiting regeneration of remaining lens epithelial cells. When selectively destroying residual LECs, it's important to avoid toxic effects on other intraocular tissues.
There are several methods for depleting or inhibiting regeneration of remaining lens epithelial cells, but I will make a distinction between depleting by targeting signaling pathways of cell-apoptosis and inhibition regeneration of cells by targeting signaling pathways of PCO.

3.1 Targeting signaling pathways of cell-apoptosis

Theoretically, the most efficient way to prevent PCO is to eliminate all the cells within the capsular bag at surgery. Elimination of all cells can be reached by using an apoptosis inducer gene.
Apoptosis, or programmed cell death, is a physiological process essential for normal development and maintenance of homeostasis in many organisms[20].
This cellular suicide can be triggered by the expression of different genes known to act through different caspase-dependent or -independent pathways to induce apoptosis[21].
The main reason for using an apoptosis inducer gene to improve the efficiency is that all residual lens epithelial cells must be removed to avoid PCO.
Several experimental approaches have been reported in attempts to inhibit secondary cataract formation. Toxic agents, including mitomycin and 5-fluorouracil[22], in solution or attached to IOL's, have shown variable effectiveness in inhibiting LEC growth in vitro or in vivo.
Nontoxic compounds, such as diclofenac and cyclosporin A, have also shown variable ability to inhibit LEC proliferation[23].
To date, however, none of these treatments have yet to reach the clinic.
Therefore there is scope to develop further agents to prevent PCO and arsenic trioxide is a promising candidate.
Arsenic trioxide (As_2O_3) is a potent anti-tumor agent to treat acute pro-myelocytic leukemia[24].
In that study was shown that depletion of calcium store by As_2O_3 is effective and that ATP signaling through calcium is inhibited. This ultimately resulted in apoptosis.
Therefore, potential survival factors mediating their actions through calcium

mobilization will no longer be able to elicit a response. If a sufficient stimulus is not detected then apoptosis typically results[25].
Such observations support the notion that manipulating the apoptosis pathway(s) for clinical benefit may be possible since it theoretically would assure that all residual LEC would be unable to proliferate since they are dead by apoptosis.
But since there are a few studies that investigated the effects of those therapeutic agents on surrounding tissues, there has to be more investigation of those therapeutic agents.

3.2 Targeting signaling pathways of PCO

Another therapeutic way to prevent the formation of PCO, is by targeting biological signaling pathways of PCO. These signaling pathways are imported for normal cell survival an behavior and can be divided in different groups. A few of them I will discuss.

3.2.1. Growth factors

Transforming growth factor beta (TGF-β) is the major area of focus within the prevention of PCO.
TGF-β is a key regulator of many processes in both normal and pathological development[26].
Amongst the major effects of TGF-β signaling is induction of EMT and associated fibrosis and to suppress LEC proliferation[27].
However, immediately after surgery, when LEC's are exposed to increased levels of TGF-β□, FGF (fibroblast growth factor), and HGF (Hepatocyte growth factor) simultaneously, LEC's eventually proliferate and undergo changes that result in the formation of PCO. This suggests that FGF and HGF, which have been shown to induce proliferation, may eventually counteract the proliferation suppression of LEC's by endogenous TGF-β[28].
The recent studies, the suppression of LEC proliferation by proteasome inhibition in the presence of TGF-β_2, FGF-2, and HGF, served as a potential strategy to prevent PCO[28].
Therefore, MG132 was used as a proteasome inhibitor. MG132 is a reversible and cell permeable peptide aldehyde inhibitor of the proteasome,. It was used as an antiproliferative agent for LEC's, to prevent PCO-like changes[29].
MG132 is has the ability to inhibit the proteasome reversibly, so that normal cells can recover from its effect, while the challenged LECs that have survived surgery are more likely to be affected. This is shown earlier in studies that used proteasome inhibitor treatment to selectively killing tumor cells[30].
These findings indicate that MG132 strongly decreases the proliferation of LEC's, either alone or in the presence of TGF-β_2, FGF-2, and HGF.
TGF-β_2 also induces EMT, resulting in fibroblast/ myofibroblast cells. of PCO[31].
Previous studies[32] indicate that MG132 blocks TGF-β_2–induced EMT markers in HLE B3 cells, that suggest that proteasome inhibition can block TGF-β_2-induced EMT.

3.2.2. Matrix metalloproteinase's

Matrix metalloproteinase's (MMPs) are key modulators of important biological processes during normal and pathophysiological events, including cellular migration[29].

MMP's have been found in normal ocular tissues and their over expression is associated with excessive scarring[33]. And since MMP's are well know to cleave protein components of the extracellular matrix, they are involved in tissue remodeling[34].
One of the chances is lens capsule structure during PCO development include remodeling of the ECM by MMP's and the migration of residual LEC's to the posterior capsule plays a crucial role in PCO development[29].
But less is known about the cell signaling mechanism of migration. A study[35] showed an up regulation of MMP-2 and -9 after cataract surgery, that suggest the involvement of MMP-2 and -9 in PCO development (Fig. 5).
And in addition, a MMP inhibitor has been shown to prevent human LEC migration and contraction of the lens capsule, suggesting that MMP inhibition may have a role in the therapeutic treatment of PCO[36] (Fig.6).

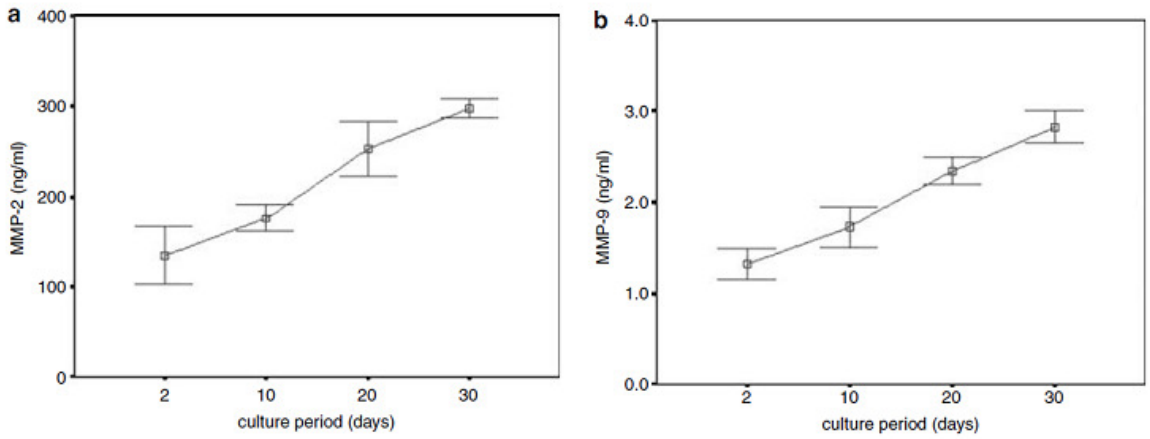

Fig.5. Total MMP-2 (a) and MMP-9 (b) protein production collected in the culture media of lens capsular bags quantified by ELISA. Both levers were increased with time with maximum levels reached on day 30. Error bars represent a 95% confidence interval. (copied from Li[35])

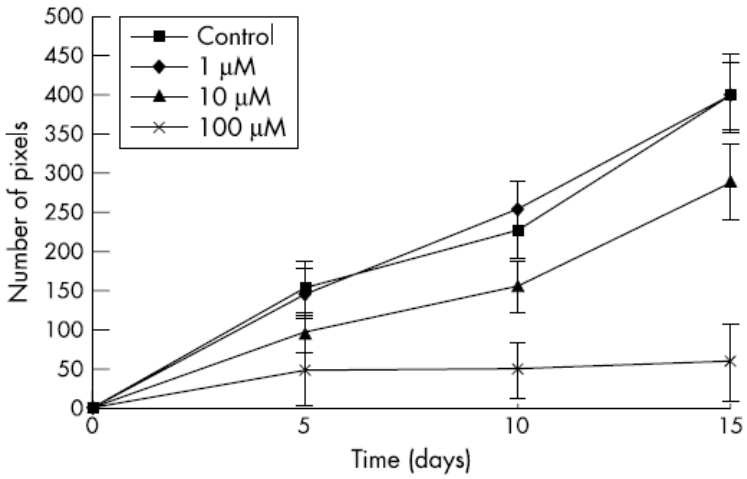

Fig.6. Serial digital photographs were taken of the lens capsules. The capsule diameters between the pins were measured using UTHSCSA (University of Texas Health Science Center in San Antonio) Imagetool Software, and the average diameter for each lens capsule was calculated from six repeated measurements from vertical and horizontal axes. All images were taken at the same marked area at 106 magnification. Three capsules were measured at each treatment group. The graph illustrates the mean distance travelled by the cells and the error bars represent a 95% confidence interval. (Copied from Wong[36])

Matrix contraction plays a key role in PCO development and cell migration is intrinsically involved in matrix contraction. And as shown above, reduction in cell migration can be induced by the inhibition of MMP activities. A recent study[29], has shown that TGF-β_2 treatment causes an increase in MMP-2 and -9 activities, suggesting the involvement of TGF-β_2 in MMP-2 and -9–induced cell migration and PCO development.
MG132 can also be used for decreasing MMP-2 and -9 activities in explanted IOL's and HLE B-3 cells, either alone or in the presence of TGF-β_2. This suggest that proteasome inhibition can prevent residual LEC migration and tissue contraction[28]. Therefore, it can be said that a proteasome inhibitor strongly suppresses LEC proliferation in the presence of TGF-β_2, FGF-2, and HGF, together with its ability to block TGF-β2 induced EMT markers and MMP activities, suggesting that MG132 should be considered as a target for prevention of PCO.

3.2.3. Integrins

As a receptor family, the integrins are linked to critical events in cell differentiation and tissue morphogenesis including cell attachment, proliferation, migration, polarity, and survival.
Altered integrin function is closely linked to the progression of fibrotic diseases of the lens. Changes in both integrin expression and in the composition of the matrix are reported in PCO and are linked to activation of TGF-β signaling pathways[37]. Thus stabilizing of integrin function plays a key role on inhibiting activation of TGF-β signaling pathways.

3.3 Developing therapeutic agents

Several attempts have been made to find an appropriate therapeutic target to prevent PCO, but none has proven effective either because of its toxic effect on other ocular tissues or because of only partial or differential effect on the causes of PCO, such as LEC proliferation, migration, and transdifferentiation.
Therefore, a good strategy to prevent PCO is blockage of most of the PCO-causing pathways, with less toxic effect on other ocular tissues.
The safest intraocular substances, which may be effective, are at present immunotoxins, 5-fluorouracil (5-FU) and lidocaine in the form of Xylocaine[37].

Lidocaine is the most used intraocular drug in cataract surgery and is considered safe when used as 1 mL unpreserved Xylocaine 1%. This preparation has the pH that is acceptable to the corneal endothelium[38].
5-FU is an antimetabolite and antimitotic agent used in glaucoma surgery to prevent closure of the filter created. In the cell it is converted to active metabolites that interfere with the metabolism of DNA and RNA[37].
Another drug that interfere with the metabolism of DNA and RNA is Actinomycin D. Actinomycin D is a cytotoxic drug that intercalates with DNA, resulting in a decreased RNA polymerase (DNA reading) activity inhibiting RNA synthesis.

In my study, I treated Human lens epithelial cells with different concentrations of Actinomycin D.
My goal was to inhibit proliferation of lens epithelial cells.
The in vitro study showed that the proliferation of LEC's was inhibited by all concentrations of Actinomycin D in medium. Proliferation was most inhibited by concentrations of 20 µg/mL with a treatment of 72 hours. But there was also significantly less proliferation with the same concentration of Actinomycin D with a treatment of 24 hours. (Fig.7)
This drug seems to be a potential drug for prevention of PCO, but this Is only tested in vitro. In vivo studies is necessary just as a study of the effect of Actinomycin D on surrounding tissue.

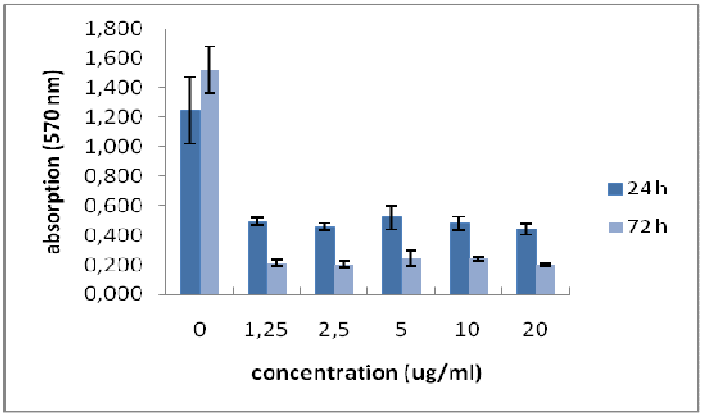

Fig.7. MTT conversion by HLE- B3 (expressed as absorption) exposed to Actinomycin D dissolved in Medium during 24hrs and 72hrs.

In my study, I also investigated another pharmacological agent.
Alkylphosphocholines (APC's) are effective inhibitors of human ocular cell proliferation[39-42]. Beside being an inhibitor, at certain concentrations, they're also nontoxic for surrounding ocular tissue. Alkylphosphocholines are synthetic phospholipid derivatives and represent a class of pharmacologically active agents applied clinically for their anti-tumor properties[43].
This is an advantage on Actinomycin D, because Actinomycin D is not yet tested on his effect on surrounding tissue. Proliferation of HLE-B3 cells were significantly reduced after exposure of the cells to APC's added to the culture medium and after exposure of the cells to APC's added to PBS. (Fig. 8)

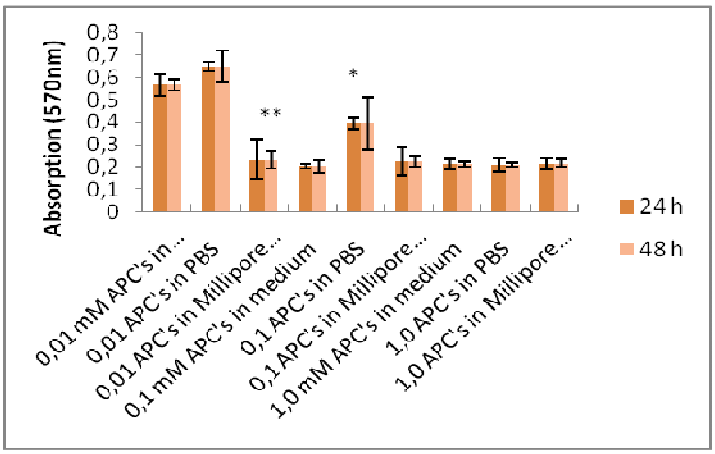

Fig. 8. MTT conversion by HLE- B3 (expressed as absorption) exposed to Alkylphosphocholines in different dissolvents during 24hrs and 48hrs.

Migration of LEC's was also investigated.
Concentrations of 1.0mM APC's in PBS lead to massive disruption of the actin filaments, while concentrations if 0.01mM APC's in medium and in PBS lead to less disruption.
This tells us that APC's are inhibiting cell migration, which is one of the properties of PCO formation. This was shown earlier in the study of K. Eibl[39], in which 0.1mM APC's gave rice to reduction of cell migration by 43.1% of the cells.
Pharmacologic prevention of PCO represents a valuable option for a new treatment of PCO, because the effectiveness of pharmacologic agents can be reached during short exposure time of the agents to the remaining epithelial cells. But the effect of the pharmacological agents on surrounding tissue remains the restrained on clinical use.

4. Discussion

In recent years, our understanding of mechanisms of PCO development has increased significantly. Therefore, several advances have been made to improve cataract surgery techniques, IOL materials and designs, and the use of therapeutic agents. Because of these improvements, PCO occurrence has decreased. But PCO remains the most common complication of cataract surgery. When we look at the two prevention methods, IOL design and material and pharmacological agent, both seem to be important to reduce PCO.
A combination of both may be necessary to achieve a result in reducing the occurrence of PCO.
When we look at IOL design and material, the development of new materials is hold back by the limited types of biomaterials that are biocompatible for the eye and for the human lens epithelial cells. When we look at the design of IOL's, the types of designs are also limited. This is because the optimal design must replace the lens, and the shape of the lens is determined by the function of the lens in the eye.
Pharmacological agents are relatively new in the development of prevention techniques of PCO.
Besides being effective as inhibitor of PCO formation they have a second criterion, nontoxic for surrounding tissue. During the development, investigators looked at different agents that are already
being used for other treatments. But still, there is no pharmacological agent found that is effective as inhibitor and nontoxic for surrounding tissue.
Therefore, research aimed at improving surgical techniques to eliminate almost all lens epithelial cells from the capsular bag at the time of surgery. Research aimed at optimizing IOL biocompatibility and minimizing postoperative inflammation reaction. And research aimed at targeting residual lens epithelial cells by pharmacological agents that have minimal or no effect on other ocular tissues is highly desirable.
I think the answer is a golden combination of all three. Since cataract surgery could not remove all lens epithelial cells, IOL's and surgery trigger lens epithelial cells to proliferate, transdifferentiate and migrate, and therefore a pharmacological drug is necessary to eliminate lens epithelial cells.
But as long as the development of the three types of prevention will be investigated separately, the problem of PCO will not be resolved at short time.

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