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Modulation of Inflammation Driven Wound Healing after Glaucoma Surgery

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Abstract

Dysregulated wound healing contributes to most currently unanswered ophthalmological morbidity. Opacification and structure altering contractures compromise the delicate ocular anatomy upon which ocular function and healthy vision are reliant. Glaucoma filtration surgery, corneal stromal injury, proliferative vitreoretinopathy and age-related macular degeneration are major contributors to ocular morbidity – all with myofibroblast transdifferentiation and pathognomonic scarring activity at their core.

This thesis aims to revaluate the means by which dysregulated ocular wound healing is combated with evidence describing a novel strategy to mitigate its effects. A translational approach was used. An initial retrospective analysis of over ten thousand glaucoma surgeries found that perioperative NSAID exposure was significantly associated with surgical success. The current standard of care, corticosteroids, showed no such association. This was surprising and provided impetus to evaluate these clinical findings within the basic science lab.

The subsequent project examined the relative effects of NSAIDs to that of corticosteroids on the in vitro wound healing activity of ocular fibroblasts. Relative to steroids, NSAID exposure resulted in more ordered extracellular matrix remodelling, less cell-mediated collagen contraction and greater impairment of myofibroblast associated protein expression.

We hypothesized that these differences were due to NSAIDs more specific targeting of COX enzyme activity. By sparing lipoxygenase activity, competitive NSAIDs leave intact the biosynthetic machinery responsible for signaling the endogenous resolution of inflammation. This system involves the collective effects of the pro-resolving superfamily of lipid mediators and promotes the active resolution of inflammatory processes.

To assess the anti-fibrotic potential of inducing resolution within inflammation-induced ocular fibroblasts, two COX2 Ser516 acetylating molecules were utilized to modify the COX2 enzyme such that it: 1) ceases prostaglandin production, and 2) gains the capacity

to produce pro-resolving lipid mediators. When applied to inflammation-induced ocular fibroblasts, a reduction in in vitro wound healing phenomena was observed with a corresponding shift in pro-/anti-fibrogenic transcription factor expression and downregulation of myofibroblast associated proteins.

Together these findings suggest that the resolution of inflammation and the resolution of fibroproliferation may be controlled by a common signaling system, and that interventions promoting the production of resolving lipid mediators could have significant anti-cicatrizing properties.

Keywords: wound healing, inflammation, fibrosis, scarring, ophthalmology, ocular, myofibroblast, fibroblast, transdifferentiation, immuno-suppression, immuno-resolution

Co-Authorship Statement

Manuscript: Secondary surgical intervention after primary glaucoma filtration surgery: an Ontario population-based study

Canadian Journal of Ophthalmology. 2018; 54(2): 212-222

James J. Armstrong	Developed research question with assistance from CMLH, formulated database coding strategy, analyzed data, drafted manuscript
Blayne K. Welk	Collaborator; supervised access to ICES data, assisted with development of database coding strategy, assisted with data analysis strategy
Jennifer N.S. Reid	Executed the database coding strategy within ICES data holdings, returned data for analysis
Vinay Kansal	Determined the preservative concentration within ophthalmic eye drops for analysis
Cindy M.L. Hutnik	Supervisor; assisted in all aspects of experiments; all experiments conducted under her grant approval

Manuscript: Differential effects of dexamethasone and indomethacin on Tenon's capsule fibroblasts: Implications for glaucoma surgery

Experimental Eye Research. 2019; 182(Jan); 65-73

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Grant Application / Protocol: An investigator-initiated multi-center prospective clinical trial to examine the efficacy of peri-operative NSAID vs. corticosteroid treatment in trabeculectomy wound management

Glaucoma Research Society of Canada, Funded 2018, Patient Recruitment Ongoing

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Manuscript: Acetylsalicylic acid mitigates cytokine induced myofibroblast transdifferentiation and activity within human ocular fibroblasts

Experimental Eye Research, In preparation - planned submission: June 2019

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Manuscript: Acetylation of COX2: an immuno-resolving and anti-cicatrizing ocular intervention

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James J. Armstrong	Developed research question with assistance from CMLH, designed experiments, executed experimental work with the indicated assistance, analyzed data, and drafted manuscript
Erica A. Li	Assisted with western blot and immunohistochemistry
James T. Denstedt	Assisted with collagen contraction assays
Hong Liu	Assisted with MTT, western blot and immunohistochemistry assays
Cindy M.L. Hutnik	Supervisor; assisted in all aspects of experiments; all experiments conducted under her grant approval

Dedication

I would like to dedicate this thesis to my mother, Laurie Bruvall, as she dedicated her life to the formative years of my early education. It was through her guidance that a curiosity-driven love for discovery and inquiry, as opposed to dogmatic learning, was cultivated. She helped keep these ideals alive in me through high school and most importantly undergrad / medical school so that I could ultimately stumble into my true calling as a clinician scientist. There are no words enough to ever thank a mother, let alone one’s own.

Acknowledgments

I would like to acknowledge my advisory committee, Dr. David O'Gorman and Dr. Blayne Welk, who offered guidance and support throughout my studies. Dr. Welk developed my fluency in statistics, a skill that will never go without need. Dr. O'Gorman developed my command of myofibroblast biology and *in vitro* wound healing related assays - without him and his lab's guidance the results included within this thesis would be rather sparse.

Table of Contents

List of Tables

List of Figures

List of Appendices

List of Abbreviations

AA:	arachidonic acid
AC:	anterior chamber
AE:	adverse event
AHGF:	aqueous humor growth factors
AMD:	age-related macular degeneration
ANOVA:	analysis of variance
APHS:	N'-(3-aminopropyl)homospermidine 2-(20heptynylthio)-phenol acetate phenol 2-(2-heptynylthio)-acetate o-(Acetophenyl)hept-2-ynyl sulphide o-(Acetoxyphenyl)hept-2-ynyl sulphide
ASA:	acetylsalicylic acid
BAK:	benzalkonium chloride
BCA / BCVA:	best corrected visual acuity
BID:	twice daily
BSA:	bovine serum albumin
CAI:	carbonic anhydrase inhibitors
CIHI:	Canadian institute for health information
COX:	cyclooxygenase
CS:	chitosan sulfate

DAD:	discharge abstract database
DAMP:	danger / damage associated molecular pattern
DAPI:	4′,6-diamidino-2-phenylindole
DHA:	docosahexaenoic acid
DIN:	drug identification number
DMEM:	Dulbecco's modified eagle medium
DMSO:	dimethyl sulfoxide
DNA:	Deoxyribonucleic acid
ECCE:	endocapsular cataract extraction
ECM:	extracellular matrix
EMT:	epithelial to mesenchymal transition
EPA:	eicosapentaenoic acid
FBS:	fetal bovine serum
FDA:	fluorescein diacetate
GAPDH:	glyceraldehyde 3-phosphate dehydrogenase
GFS:	glaucoma filtration surgery
HEPE:	hydroxyeicosapentaenoic acid
HETE:	hydroxyeicosatetraenoic acid
HR:	hazard ratio
HTCF:	human Tenon's capsule fibroblasts

ICES:	institute for clinical and evaluative sciences
IDD:	indwelling drainage device
IFN:	interferon
IL:	interleukin
IOP:	intra-ocular pressure
IQR:	inter-quartile range
LC-MS/MS:	liquid chromatography - tandem mass spectrometry
LDH:	lactate dehydrogenase
LDL:	low density lipoprotein
LM:	lipid mediators
LOX:	lipoxygenase
LXA:	lipoxin A
MIGS:	micro-invasive glaucoma surgery
MMC:	mitomycin c
MMP:	matrix metalloproteinases
MTT:	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NO:	nitric oxide
NSAID:	non-steroidal anti-inflammatory drug
OAG:	open angle glaucoma
ODB:	Ontario drug database

OHIP:	Ontario health insurance plan
PAMP:	pathogen associated molecular pattern
PBS:	phosphate buffered saline
PCO:	posterior capsule opacification
PG:	prostaglandin
PGA:	prostaglandin analogue
PI:	peripheral iridotomy
PNM:	polymorphonuclear cells
POAG:	primary open angle glaucoma
PPAR:	peroxisome proliferator-activated receptor
PS:	penicillin and streptomycin
PUFA:	polyunsaturated fatty acids
QID:	four times daily
RCT:	randomized controlled trial
REB:	research ethics board
RM:	resolving mediators
RPDB:	registered persons database
SD:	standard deviation
SEM:	standard error of the mean
SMA:	smooth muscle actin

SPM:	specialized pro-resolving mediators
TBST:	tris-buffered saline and polysorbate 20
TGF:	transforming growth factor
TIMP:	tissue inhibitor of matrix metalloproteinase
TVT:	tube vs. trabeculectomy study
VA:	visual acuity
VC:	vehicle control
WGA:	world glaucoma association
α:	alpha
β:	beta
ɣ:	gamma
µ:	micro

Chapter 1

1 Introduction

1.1 Ocular Wound Healing

The mechanisms of wound healing within the anterior segment of the eye, specifically the subconjunctival tissues, are similar to most non-nervous tissues within the body.[1–4] After hemostasis, tissues undergo repair in three stages: 1) inflammation / resolution, 2) proliferation and 3) remodeling. These stages are partially overlapping sequential events, controlled through the integration of information from several dynamic signaling networks. These signaling networks incorporate several diverse classes of signaling molecules, several cell types and physical cues from the extracellular matrix (ECM) and external environment. For healthy wound healing to occur, as opposed to an anatomically impairing fibroproliferative response, orderly and efficient transitions between each phase are required. A non-anatomically disruptive wound healing response after ocular insult is critical to maintaining vision, as the slightest contracture, thickening or opacification of delicate ocular anatomical structures can have dire consequences to a patient's refractive status and/or ocular physiology.

1.1.1 Inflammation and Resolution in Wound Healing

Inflammation and its resolution are an endogenously controlled biological algorithm coding the intentional deviation and subsequent return to homeostasis that is required to overcome infection or trauma.[5,6] The magnitude of tissue damage (fibrosis/scarring) that results from an inflammatory insult is directly proportional to the intensity and duration of the inflammatory reaction the body levies in response to that insult (Figure 1-1). In the eye, it is especially critical to ensure inflammatory responses are kept to a minimum – as the anatomy upon which healthy vision is dependent can easily be disrupted by inflammation and fibrosis induced changes to tissue architecture, often with permanent consequences.

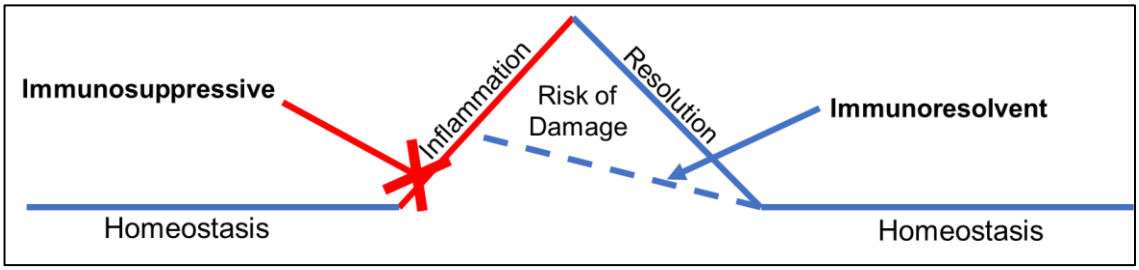


Figure 1-1 Diagram illustrating the risk of local tissue damage during inflammation-induced deviations from homeostasis. Immunosuppressive interventions aim to block the molecular signals contributing to the original, inflammation-induced deviation from homeostasis. Immuno-resolvent interventions aim to reduce the risk of tissue damage by both dampening the inflammatory deviation from homeostasis and hastening the subsequent endogenously controlled return.

Inflammation is involved in both normal wound healing and the development of fibrosis. Tissue damage caused by infectious, ischemic, autoimmune, traumatic or surgical insults causes localized cell death. The intracellular components of

these necrotic cells are released into the interstitum. Surviving cells within the area express danger associated molecular pattern (DAMP) receptors, which can sense intracellular components such as DNA, RNA, histones, mitochondrial DNA and ATP among others within the interstitum.[7–9] Once activated, DAMP receptors bind their ligands and initiate an intracellular signaling cascade culminating in secretion of IL-1β and the canonical commencement of inflammatory signaling.[7,8]

The cardinal signs of inflammation have long been tied to the increased production of the prostaglandin (PG) and leukotriene (LT) classes of lipid derived signaling molecules. The secretion of these lipid mediators requires the actions of intracellular phospholipases (PL), such as PLA2, acting on membrane phospholipids to generate arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).[10] These precursors are then oxygenated by cyclooxygenase 2 (COX2) to produce the prostaglandins and leukotrienes.[11] The expression of both COX2 and PLA2 is induced by IL-1β.[12] Due to their extremely potent chemokine and vasoactive properties, PGs and LTs function in wound healing mainly to perpetuate the inflammatory state, increase vascular permeability and increase platelet aggregation. Vascular permeability and platelet aggregation increase the relative numbers of local inflammatory cells, fibroblasts, pro-inflammatory growth factors and wound healing associated cytokines.

Due to the difficulty of measuring lipid derived signaling molecules (LMs), relatively little is known about their role in wound healing. Recent developments in liquid chromatography tandem mass spectrometry (LC-MS/MS) have enabled more in depth study. Mice with impaired AA lipid metabolism exhibit delayed

wound closure,[13] the PGF2α receptor has been shown to facilitate the development of pulmonary fibrosis,[14] and the long term use of PG analogs was shown to induce ECM contraction, upregulate pro-fibrotic cytokine release and collagen expression in human eyes[15] – thus it can be inferred that the AA derived LMs facilitate certain aspects of the wound healing process.

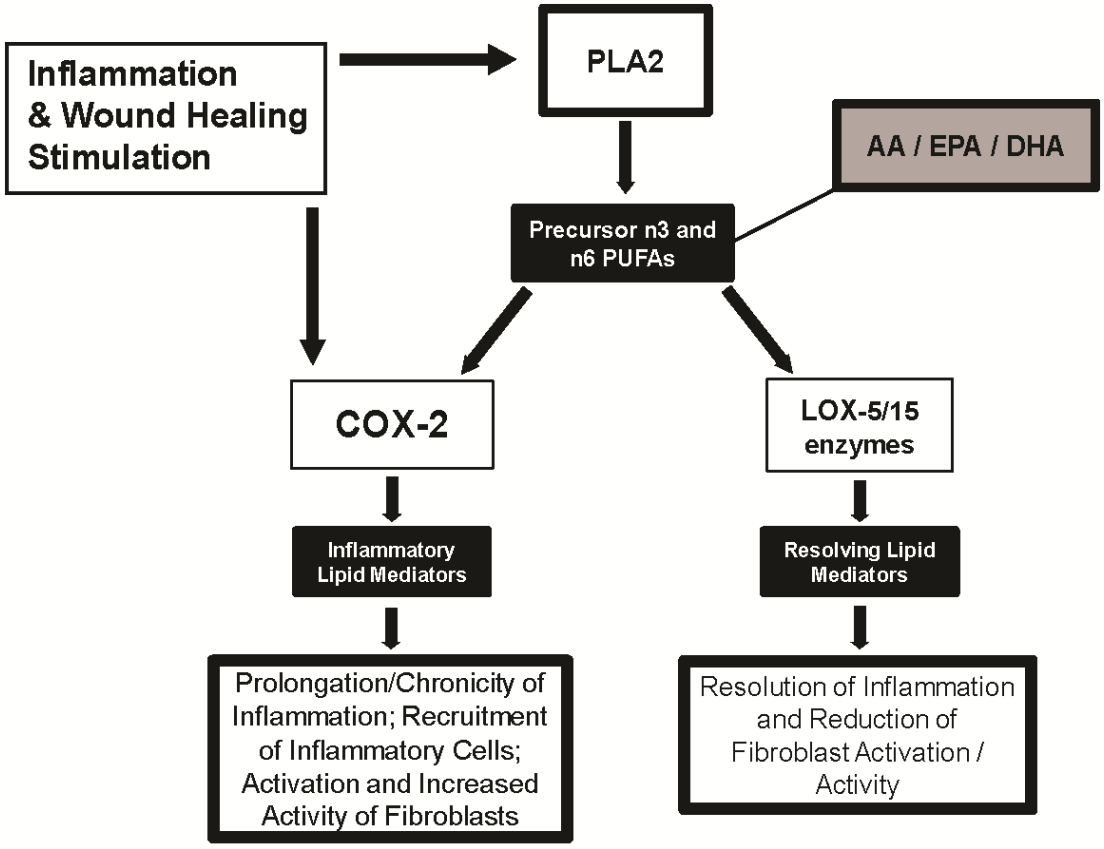


Figure 1-2: Diagram of pro-inflammatory and pro-resolving lipid mediator synthesis pathways. COX2 is responsible for utilizing n3 and n6 polyunsaturated fatty acid precursors to create PGs. The LOX enzymes cooperate to give rise to many different AA, EPA and DHA derived RMs. PLA2: phospholipase A2; AA: arachidonic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; PUFAs: polyunsaturated fatty acids; COX2: cyclooxygenase 2; LOX: lipoxygenase.

Over the past twenty years, our understanding of how inflammation undergoes spontaneous resolution has evolved. Previously, resolution was thought to occur through the passive diffusion and dilution of inflammatory mediators. However, it is now understood to be an active process; tightly controlled by a novel superfamily of lipid derived signaling molecules – the specialized pro-resolving mediators (RMs).[5,16–18] RM synthesis is triggered (after a slight delay) by pro-inflammatory mediators. They are generated in relatively large quantities and are extremely bioactive (nanomolar level). Grossly, they are derived from the actions of the lipoxygenase enzymes (as opposed to COX2) on AA, EPA and DHA (Figure 1-2).[6,19,20]

In the eye, SPMs signal for the active termination of inflammatory processes such as inflammatory cell trafficking, activation and vascular permeability.[21] At a cellular level, there are cell surface and nuclear receptors for SPMs,[22–25] the activation of which downregulates PG production,[21,26] vascular cell adhesion molecules, monocyte chemotactic protein 1, IL-8, IL-1β and TNFα signaling.[21] Their effects also stimulate resolution-associated processes such as macrophage-mediated phagocytosis of wound debris, inflammatory cell clearance and efferocytosis.[6,27] Overall, SPMs function to protect tissues from an over exuberant inflammatory response via several cellular and organ-level mechanisms, facilitating an ordered return to homeostasis after inflammatory insult.[28,29]

1.1.1.1 Fibroblasts as inflammatory cells

Fibroblasts can be conceptualized as sentinels of the immune system.[30] They express functional toll-like receptors,[31] DAMP receptors, and normally exhibit little cellular activity. However, fibroblasts are often the first cell type to encounter an inflammatory stimulus. Indeed, when activated by DAMPs[9] and pathogen associated molecular patterns (PAMPs),[30] fibroblasts begin to secrete their own inflammatory cytokines and chemokines such as TGFβ1, IL-1β, IL-6, IL-13 and IL-33 as well as exhibit strong induction of COX2.[26,32] These protein based mediators act in a paracrine manner to activate nearby fibroblasts, vascular endothelial cells and recruit circulating inflammatory cells. They can also act in an autocrine manner to illicit transdifferentiation of fibroblasts into myofibroblasts – the main cellular drivers of fibrosis and scarring.[1,33,34]

The effects of RMs on fibroblasts within an inflammatory and wound healing microenvironment are starting to be elucidated. The experimental overexpression of LOX enzymes was found to impair the scarring observed in a mouse model of pulmonary fibrosis and was attributed to increased LOX-mediated RM generation.[35] Exogenous application of individual RMs has impaired the development of experimental renal fibrosis in an obstructed kidney model through the inhibition of fibroblast proliferation and a mitigation of the inflammatory response.[36] Further studies have demonstrated that RMs have the capacity to interfere with TGFβ1-induced collagen production and fibroproliferation.[37] These data would seem to suggest that RMs may function to attenuate fibroblast-mediated tissue fibrosis and wound healing phenomena.

1.1.2 Proliferative Stage of Wound Healing

The proliferative stage spans the end of the inflammatory and beginning of the resolution stages. Inflammation induces the secretion of growth factors which stimulate the proliferation of local fibroblasts and inflammatory cells. These cells deposit ECM molecules and generate new granulation tissue to fill lesions and restore barriers to the external environment.[38] Contraction of the ECM is elicited by inflammation-induced myofibroblast transdifferentiation,[39] with the novel expression of the contractile protein alpha smooth muscle actin (αSMA).[40] As it is governed by growth factors secreted during the inflammatory stage, the duration and magnitude of the proliferative stage is determined by the duration and intensity of the inflammatory phase. Thus, the amount of collagen deposited within the wound, and the degree to which myofibroblast-mediated tissue contraction occurs can be reduced by attenuating the inflammatory stage of wound healing.

In humans, attenuation of the inflammatory phase has been accomplished for over 100 years using an immuno-suppressive strategy. For example, corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs), among others, are modalities of inflammation control that disrupt the generation of pro-inflammatory mediators. This strategy ignores the fact that the resolution of inflammation is initiated by several of these very “pro-inflammatory” mediators that are suppressed.[27] The result is a dampening of clinical inflammatory signs due to a lack of pro-inflammatory stimuli, however it may also induce a state of “frustrated resolution” – defined as the absence RM signaling after inflammation,

which hinders the ordered return to homeostasis.[28] The discovery of RMs has brought to the forefront a novel strategy in which to shorten the duration and temper the intensity of inflammatory responses.[17] Finding therapeutic means to stimulate the body's own endogenous mechanisms of resolution will be the first of the immuno-resolvent interventions and have the potential to mitigate inflammation by working synergistically with human physiology as opposed to against it.

1.1.3 Remodeling Stage of Wound Healing

The remodeling stage usually begins about two to three weeks after the initial insult. Local inflammatory cell infiltrates are greatly reduced and eventually return to baseline levels.[38] The remodeling stage begins during the resolution of inflammation and it can span for months to years after injury. During this phase fibroblasts and myofibroblasts respond to autocrine, paracrine and physical ECM cues to reorganize the extracellular matrix in an attempt to recover normal tissue architecture.[41–44] Tension gradients within the matrix dictate the balance of matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs) eliciting degradation and re-synthesis of the ECM such that the stiffness and density of collagen fibers is increased.[44] The degree of stiffening and increased density is directly proportional to the amount of cellular infiltrates as well as the duration and intensity of the preceding inflammatory and proliferative stages.[45,46] Indeed, a novel class of RM termed resolvin conjugates in tissue regeneration (RCTRs) have been shown to improve regenerative wound healing by decreasing the duration and magnitude of inflammatory signaling during the inflammatory

phase.[22] Overall, the impact of RMs appear to be beneficial at all stages of the normal wound healing response, and their absence appears to lead to a state of frustrated resolution with less than optimal wound healing outcomes.

1.2 Glaucoma and Glaucoma Surgery

Glaucoma is the leading cause of irreversible blindness worldwide, with the global burden expected to rise to 111.8 million people by the year 2040.[47] Unfortunately, no definitive treatment for glaucoma exists. Currently, the only therapy known to slow the disease's progression is the lowering of intra-ocular pressure (IOP). Traditionally, first line therapy has been pharmacotherapy [48], despite a significant number of well documented challenges. Non-compliance,[49–52] difficulty with administration,[53,54] local irritation of the ocular surface,[55,56] as well as significant systemic side effects[57,58] are well known barriers to successful medical management. Surgical interventions are often undertaken once medical management and laser-based procedures have failed to control IOP or are no longer tolerated by the patient (Figure 1-3).

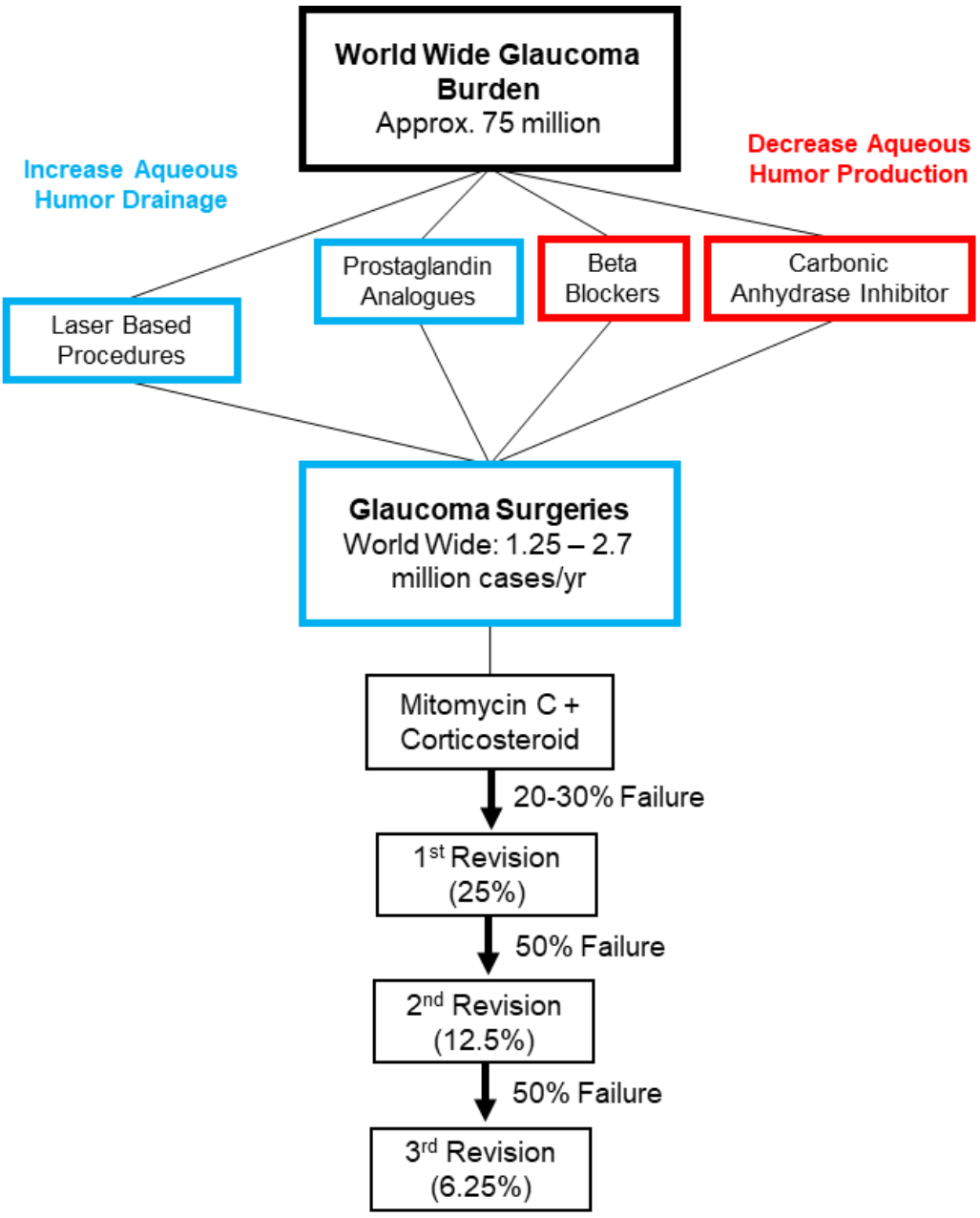
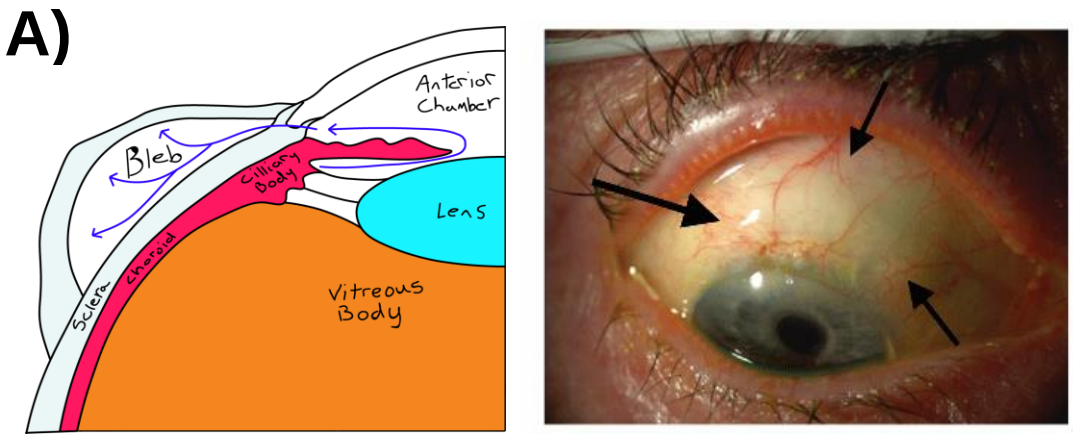


Figure 1-3: Schematic representing the treatment algorithm for patients with glaucoma. Patients usually initiate therapy medically then progress to more invasive methods of IOP management. The final treatment modality is IOP lowering surgical intervention. Surgical failure rates with use of the current wound modulatory adjuvants are indicated. Failure rates were obtained from previous work[65,66] and extrapolated to estimate the percentage of overall patients who require 1st, 2nd and 3rd revision surgeries.

Glaucoma filtration surgery (GFS) functions to lower IOP through the creation of a fistula from the anterior chamber of the eye into a surgically created drainage space. A microshunt can be implanted at the time of surgery to guard the fistula that connects to the anterior chamber to the drainage structure. If draining into the subconjunctival space, these drainage structures are termed filtration blebs (Figure 1-4A). If draining into the potential space between the ciliary body and the sclera, they are termed superciliary lakes (Figure 1-4B).[59] It is through these novel anatomic structures that aqueous exits the anterior chamber, collects within the surgically created space, diffuses through the interstitum and is removed from the eye by the venous and lymphatic systems – thus lowering a patient's IOP.[60,61] Therefore, successful GFS depends on the incomplete healing of the surgical wound and establishment of a controlled, chronic wound within the subconjunctival or superciliary tissues.[3,4] Maintaining the functional anatomy of this novel outflow pathway long-term in the face of acute surgical/chronic inflammation and aqueous humor driven wound healing, is a major challenge.



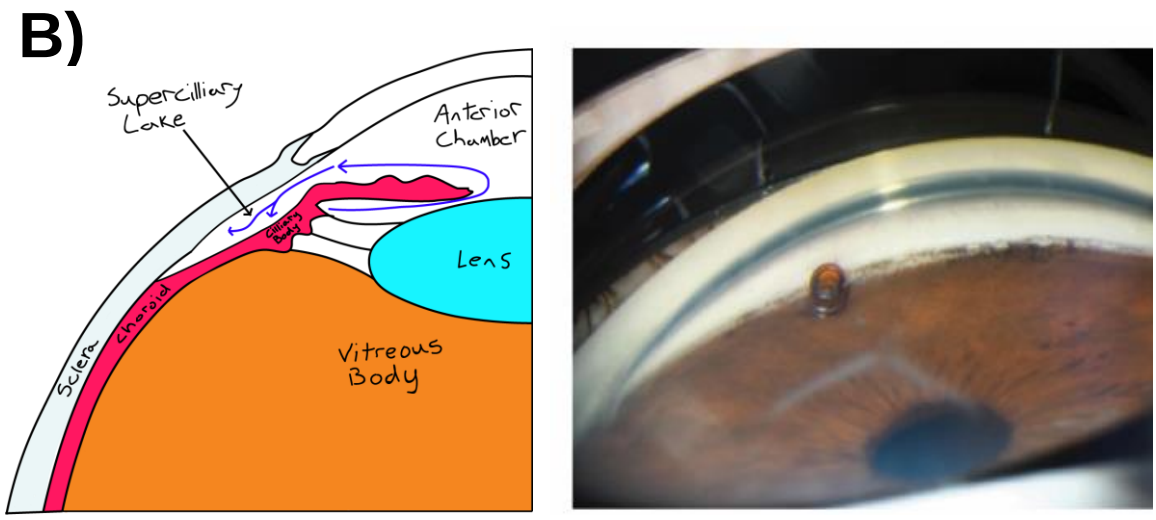


Figure 1-4: A) sub-conjunctival filtration bleb, modified from Gardiner et al. (2010).[59] B) superciliary lake, shown with the Cypass aqueous shunt implanted within, modified from Alcon Canada, Inc.

1.3 Consequences of Dysregulated Wound Healing in Glaucoma Surgery

The overwhelming majority of surgical failures occur through an inflammatory and fibroproliferative mechanism within the tissues surrounding the surgically created drainage pathway. Clinically, the events preceding failure are characterized as an overactive wound healing response.[2,62] The consequence is a fibroproliferative or contracture mediated obstruction of the surgical outflow tract - thereby hindering aqueous humor egress and ultimately the magnitude of IOP reduction achieved (Figure 1-5).[3,4,63] Glaucoma surgeries performed without current anti-fibrotic adjuvants fail at a lifetime rate approaching 100%.[64] Using current anti-fibrotic adjuvants, the lifetime surgical failure rate is approximately 30-50%.[65,66]

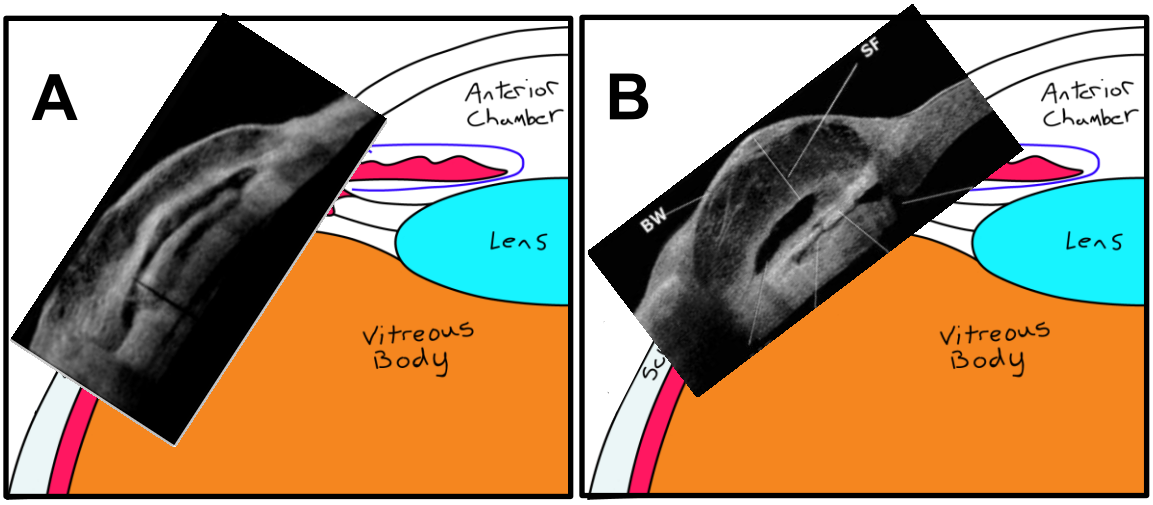


Figure 1-5: A) Depicts an anterior segment optical coherence tomography (AS-OCT) image of a functional subconjunctival filtering bleb superimposed over an anatomic diagram of the eye. B) Depicts a failing filtering bleb. Note the increased reflectance surrounding the fistula connecting the bleb to the anterior chamber, the tissue contracture, and the hypertrophic walls of the bleb. The patient subsequently underwent revision surgery. Modified from Shin 2010.[63]

Failure occurs most frequently within the first three weeks of surgery,[67] at approximately the same time as the inflammatory and proliferative phases of post-operative wound healing are occurring.[3,46] As the outflow tract loses patency and the patient's IOP begins to return to pre-surgical levels, the failure of GFS is managed in a progressive manner. In the early postoperative period, digital massage, scleral flap suture lysis and releasable suture removal can be attempted in order to restore the flow of aqueous. Glaucoma medical therapy is often subsequently reinstituted to control the patient's rising IOP. Tragically, these drugs themselves can lead to increased inflammation at the surgical site and can accelerate the deterioration of aqueous outflow capacity.[68–72] In up to 30% of glaucoma surgery patients, outflow capacity decreases sufficiently to require a second glaucoma surgery.[66,73] Subsequent surgeries experience even lower

odds of success - approximately 50% of these patients do not achieve their target IOP post-operatively.[64] Repeated attempts at surgery, until success is achieved, is the only remaining treatment path for these patients – who face blindness as an alternative.

The complication rate associated with scarring after glaucoma surgery is not only a burden to patients and surgeons but is also a major healthcare economic issue. The 5-year total treatment cost for glaucoma patients is similar when treated medically or surgically.[74] The ongoing cost of drugs is the major contributor to medical management costs, which average approximately $6500 USD over a 5-year span. The surgical treatment of the same patient would cost an average of approximately $6300 USD. Even with their increased expense and undesirable side effect profile, medications are the first line therapy due to the high risk of glaucoma surgery failure. This risk profile also contributes significantly to the overall cost – with management of post-surgical complications accounting for 60% of surgical treatment costs.[74] Reducing the risk of complications and/or revision surgery will dramatically reduce the total cost of glaucoma surgical interventions, and the reduced risks / costs could shift surgical interventions earlier in the treatment paradigm and would free patients from the burden of daily glaucoma medications – which would be a significant improvement to their quality of life.[75–77]

To avoid function altering scarring, normal wound healing requires the ordered and appropriate interaction of a complex molecular signaling network – those involved in inflammation, its resolution, and proliferation / remodeling. In the

subconjunctival tissues of glaucoma surgery patients there exist several mechanisms known to disrupt these signaling networks. Pre-existing chronic inflammation due to a patient's topical glaucoma medication burden,[55,78] co-morbid inflammatory ocular disease,[79] and/or the novel interaction of aqueous humor growth factors (AHGFs) with the mesenchymal subconjunctival tissues of the filtration bleb[80–83] all contribute to the post-surgical scarring and fibrosis observed (Figure 1-6). In particular, the contribution of AHGFs to the failure of aqueous filtration cannot be ignored, as they include several iconic molecular instigators of inflammation and fibroproliferation.[84] TGFβ1, TGFβ2, VEGF, CCN2, IL-1β, TNFα and INFɣ are all found in the aqueous humor, and further, are present at increased levels in glaucoma patients undergoing revision surgery compared to patients undergoing their first glaucoma surgery[83–88] – providing a physiological explanation for the increased likelihood of failure in these patients. Therefore, wound healing phenomena within the filtration structure's microenvironment must be modulated pharmacologically for both the short- and long-term success of GFS.

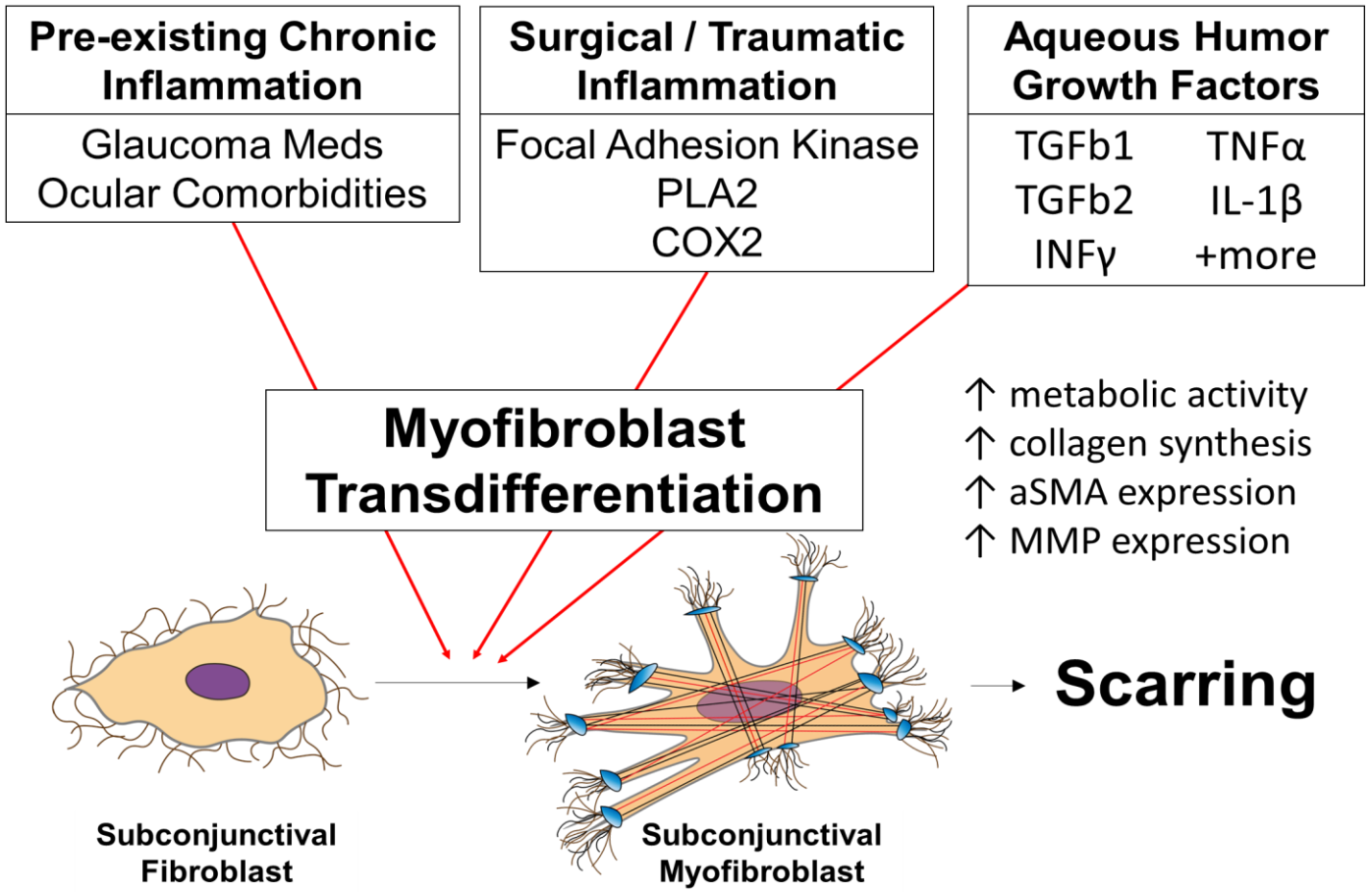


Figure 1-6: Many factors potentiate scarring within the subconjunctival tissues of glaucoma surgery patients. Pre-existing ocular inflammation or disease, surgical trauma / inflammation and the novel interaction of AHGFs with the subconjunctival tissues all promote myofibroblast transdifferentiation, fibroproliferation, contracture and ultimately surgical failure. COX2: cyclooxygenase 2; PLA2: phospholipase A2

1.4 Current Glaucoma Surgery Adjuvants

Currently, the strategy used to mitigate post-operative scarring involves the intra-operative application of cytotoxic agents, with or without the application of peri-operative anti-inflammatory drops (Figure 1-7).[89–91] Cytotoxic agents, such as mitomycin C (MMC) or 5-fluorouracil are dosed empirically, exerting their efficacy through the destruction of local fibroblasts through DNA intercalation. Thus, mitigating the post-operative wound healing response elicited by this cellular population within the surgical site. Due to the cytotoxic mechanism of action, these drugs are associated with complications that can be sight threatening.[92]

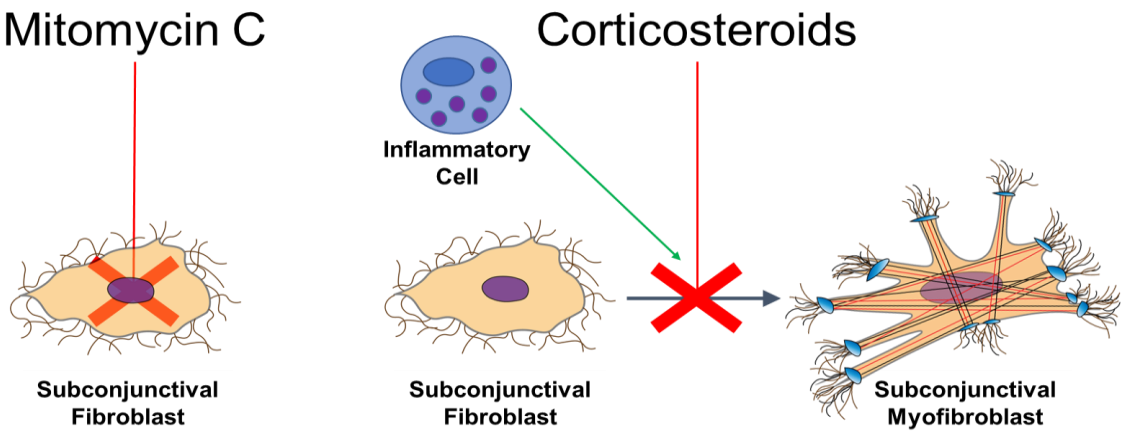


Figure 1-7: Mitomycin C intercalates DNA and is lethal to all cell types. It functions as an anti-scarring adjuvant through its cytotoxic actions on the local subconjunctival population. Corticosteroids prevent transcription of PLA2, which is responsible for generating the substrates for all lipid mediators of inflammation and resolution. By dampening the inflammatory response, fewer inflammatory cells are recruited to the subconjunctiva to activate local fibroblasts. Further, corticosteroids have been shown to directly act on fibroblasts to inhibit their activity.

To illustrate the variability inherent to the application of MMC, one need only reference the therapeutic range that is currently employed: 0.2-0.4mg/ml is applied by an MMC soaked sponge intra-operatively on the freshly dissected conjunctiva, for one to four minutes (at surgeon discretion), at the start of surgery.[93] Over application can lead to tissue necrosis, wound leak, corneal decompensation, hypotony and blindness. Under application results in loss of efficacy and causes the post-operative failure rate to approach 100%.[64,94]

Anti-inflammatory drops such as corticosteroids are used post-operatively to attenuate the inflammatory phase of wound healing and provide the clinician with a titratable means to control subconjunctival wound healing phenomena over a patient's post-operative course. Corticosteroids function indirectly to mitigate inflammation-induced activation of sub-conjunctival fibroblasts by preventing local inflammatory cell activation and recruitment.[65] They have also been shown to exert direct anti-proliferative actions on sub-conjunctival fibroblasts, as well as illicit a decrease in fibroblast wound healing associated biomarkers.[95] However, steroids are associated with adverse events such as cataract development, elevation of intraocular pressure (IOP) and increased infection risk.[96–103] These risks add to the unpredictability of post-operative wound healing and fuel the search for viable alternatives to control post-operative inflammation and scarring.

1.5 Therapeutic modulation of ocular wound healing: current evidence and contemporary strategies

The suppression of vascular endothelial growth factor (VEGF) signaling has become recently widespread in ophthalmology.[104] Anti-VEGF therapy as an anti-fibrotic intervention appears to be theoretically based on suppressing the proliferative stage of wound healing by blocking angiogenesis as well as interfering with VEGF's direct stimulatory effects on fibroblasts.[105] Several studies have evaluated subconjunctival injections of anti-VEGF antibodies as anti-fibrotic GFS adjuvants.[105–107] The results from initial animal investigations demonstrated improvements in wound healing related outcomes after GFS and several small human trials were subsequently undertaken. A recently published meta-analysis of their results indicated uncertainty as to the efficacy of anti-VEGF adjuvant therapy in human GFS patients.[108] Anti-VEGF therapy's lack of anti-scarring efficacy fits well with findings from its official use in another ocular disease. Up to 45% of patients receiving regular anti-VEGF injections for age related macular degeneration will experience progressive sub-retinal scarring, despite therapy.[109,110]

Transforming growth factor beta (TGFβ) isoforms are perhaps the most canonical proteins associated with wound healing and fibroproliferation.[111–114] Histological studies demonstrate significant elevation of TGFβ isoforms within the subconjunctival tissues of GFS patients post-operatively.[115–117] In animal studies, exogenous TGFβ was found to significantly increase IOP and the failure rate of

GFS.[118] Significant resources were spent at the *in vitro*, animal[119,120] and human level[121,122] to assess an anti-TGFβ2 antibody known as CAT-152 as an adjuvant for GFS. Ultimately the intervention failed, possibly attributable to the specificity of the CAT-152 antibody for the TGFβ2 isoform only. Interventions that incorporate a more broad blockade of both TGFβ1 and TGFβ2 isoforms, or the receptor that is shared by both are underway and have seen some success at pre-clinical stages.[118,123]

Matrix metalloproteinases and their inhibitors (TIMPs) are fundamental to the remodeling stage of wound healing. In the subconjunctiva of GFS patients, the expression of these molecules is upregulated and contributes to scar formation.[124] *In vitro*, an MMP inhibitor (ilomastat), was found to inhibit fibroblast-mediated collagen contraction by subconjunctival fibroblasts.[125] In two different animal models, ilomastat improved wound healing after GFS, prolonging bleb survival, lowering intraocular pressure, and reducing the amount of subconjunctival scar tissue to a similar degree as MMC.[126,127] This appears to be one of the more promising avenues of wound modulation research at the current time.

Ultimately, scarring and fibrosis appear to have redundant mechanisms for bypassing interventions targeting a single pathway. Suppressive strategies against specific mediators that each individually contribute to a redundant process is destined for difficulty. Such difficulties may be quite familiar to the cancer biologist, as similarly aberrant tumor growth is often fueled by multiple redundant intracellular signaling pathways.[128] This can potentially explain the

current position of nonspecific anti-proliferative drugs and steroidal anti-inflammatory agents as the gold standard adjuvants for GFS. More specific inhibitors of TGFβ signaling, MMP inhibitors, Nuclear Factor-κB signaling inhibitors, anti-oxidants and PPARγ agonists have all demonstrated promising wound modulating effects at various levels of *in vitro*, pre-clinical and clinical development.[4] However, none of these applications have thus far been translated into clinical use. Ideally, a point of biochemical intervention that has multiple downstream effects and counters many of the redundant pathways involved in scarring and fibrosis could be identified.

1.6 Thesis Overview

Sixty years ago, Dr. Epstein first noticed what he then coined: the fibrosing response to aqueous.[84] Since then dramatic improvements have been made in surgical success due to wound modulatory adjuvants. The aim of this thesis was to investigates potential means of improving these success rates still further – ideally in a more efficacious and/or safer manner than current standards of care.

The investigative work begins in Chapter 2, with a retrospective cohort study. This was the first study of its kind that was adequately powered to assess perioperative risk factors associated with revision GFS – as opposed to the failure of IOP control. We uncovered the surprising finding that perioperative NSAID exposure was more strongly associated with surgical success than corticosteroids, the current standard of care. These results inspired the subsequent *in vitro* investigation in Chapter 3, which found that these drugs exert

differential effects on wound healing phenomena elicited by subconjunctival fibroblasts. Data from both chapters were used to write a successfully funded grant for a randomized controlled trial assessing the merits of NSAID use in human GFS patients (Appendix I). The results of this trial are outside the scope of this thesis, however it felt appropriate to “close the translational loop” and do what was within reach to bring the knowledge attained from our retrospective and *in vitro* work – back to human patients.

While investigating the differences between NSAIDs and corticosteroids, a second manner in which to end this thesis presented itself. Based on the theory that corticosteroids more completely inhibit the endogenous generation of RMs than is observed with NSAIDs, I hypothesized that modulating the production of lipid mediators to resolve inflammation – rather than suppress it – is a better means to mitigate post-operative scarring. Such an approach works more synergistically with the body’s endogenous systems of inflammation and resolution, rather than against them. The following two chapters, Chapter 4 and Chapter 5, pursue this hypothesis and present data to support a novel therapeutic strategy which resolves inflammation and mitigates wound healing phenomena *in vitro*.

Taken together, these investigations highlight novel strategies to combat scarring after glaucoma surgery. The first suggests a shift in the utilization of currently used ophthalmic drugs to improve patient outcomes. The subsequent strategies would require more lengthy safety approvals – however they demonstrate even more promising efficacy *in vitro*. The knowledge gained from this thesis is

uniquely powerful, as it should enjoy rapid translation to human patients at the same time as significantly advancing our understanding of how LMs and more specifically RMs influence wound healing phenomena.

Chapter 2

2 Risk Factors for Secondary Surgical Intervention after Primary Glaucoma Filtration Surgery[1]

This study focused on surveying the current contributors to GFS failure risk in order to establish theoretical grounds for future *in vitro* work. We examined patient factors, surgical history, the type of GFS performed, and most importantly, we investigated various perioperative drugs exposures for association with GFS failure rates. This study consisted of the following:

1. Defining a cohort of filtration surgery patients within Ontario medical records spanning 2003 to 2015
2. Following this cohort for 1-year after GFS
3. Investigating candidate baseline / exposure variables for association with subsequent revisions procedures

[1] Parts of this chapter have been published: **Armstrong, J.J.**, Welk, B.K., Reid, J.N.S., Kansal, V., Hutnik, C.M.L. Secondary surgical intervention after primary glaucoma filtration surgery: an Ontario population-based study. Canadian Journal of Ophthalmology. 2018; 54(2): 212-222; DOI: https://doi.org/10.1016/j.jcjo.2018. 04.004.

2.1 Introduction

The failure of glaucoma filtration surgery is managed in a progressive manner. In the early postoperative period, digital massage, scleral flap suture lysis and releasable suture removal can be attempted. Medical therapy is often subsequently reinstituted, which itself may lead to increased inflammation at the surgical site and accelerate deterioration of aqueous outflow capacity.[68,70–72,129] Some of these patients are refractory to medical treatment and a secondary surgical intervention is the only remaining option.

Several studies have reported rates of secondary surgical intervention after primary glaucoma filtration surgery.[130–133] The Tube Versus Trabeculectomy study reported secondary surgical intervention rates of 2.8%[130] within the first post-operative year and Cankaya *et al.* reported a rate of 8.5% in a randomized controlled trial of 59 patients.[131] However, the event rate within these samples was too low to investigate factors associated with increased rates of secondary surgical intervention. Young age,[134–138] previous intraocular surgery,[135,139] chronic exposure to topical medications,[68,70–72,129] and comorbid systemic disease[136,138] have all been previously associated with failure of intra-ocular pressure (IOP) control. However, few studies have reported on patient factors associated with increased rates of secondary surgical intervention.

Such information is critical to establishing individualized patient treatment plans, as patients at high risk for secondary surgical intervention may be advised to delay surgery, to elect for any of the available less invasive surgical options

and/or to have modified perioperative adjunctive medical therapies. Further, even recent reports of secondary surgical intervention rates may not be representative of current surgical practices. The rapidly evolving surgical techniques, adjuvants and their place in the glaucoma treatment paradigm may confer different risks than those of past practice.[140,141] To address these issues, our objective was to determine the rate of secondary surgical intervention after primary filtration surgery within the first post-operative year in a large cohort of older adults undergoing their first glaucoma filtration surgery. We hypothesized that patient and surgical factors, pre-existing filtration surgery in the contralateral eye, as well as exposure to peri-operative and anti-glaucoma medications, would influence the rate of secondary surgical intervention.

2.2 Methods

2.2.1 Design and Setting

A retrospective, population-based cohort study was conducted using administrative healthcare data from the province of Ontario, Canada. As Canada's most populated province, Ontario provides all citizens with universal health coverage through a single health care system. In addition, individuals ≥65 years of age have universal access to a variety of prescription medications approved for coverage by the Ontario Drug Benefit (ODB) Plan, including most glaucoma medications. Individual patient level data were linked using a unique encoded identifier and were analyzed at the Institute for Clinical Evaluative Sciences (ICES) Western. Research approval is through the institutional review

board at Sunnybrook Health Sciences Centre, Toronto, Ontario. Study reporting followed the STROBE/RECORD checklist (Appendix A).[142]

2.2.2 Data Sources

Several linked population based administrative databases were used: the Ontario Health Insurance Plan (OHIP) which captures greater than 95% of Ontario physician services;[143] the Registered Persons Database (RPDB), which captures demographic data and vital statistics on all Ontario residents (approximately 13 million people);[144] the Canadian Institute for Health Information (CIHI) Discharge Abstract Database (DAD) and the Same Day Surgery (SDS) database, which contains detailed diagnostic and procedural information regarding all surgical procedures and hospital admissions;[144] and finally the Ontario Drug Benefits plan database (ODB), which accurately captures all prescriptions filled by outpatients aged 65 and older.[145] To identify certain systemic co-morbidities, we used databases derived from validated case definitions: the Hypertension Database (HYPER)[146], the Ontario Diabetes Database (ODD)[147] and the Ontario Asthma (ASTHMA) dataset.[148] All these databases provide accurate data on the covariables assessed in this study and have been validated for many outcomes, exposures and diagnoses.[144,145,149] All codes used for assembly of cohorts, as well as detection of covariables, are referenced in Appendix B-G.

2.2.3 Cohort Assembly

Ontario residents who underwent a primary glaucoma filtration surgical procedure (no previous incisional glaucoma surgery in both eyes) between April 1, 2003 to March 30, 2015 were identified. A patient's OHIP and CIHI records both had to indicate a glaucoma filtration procedure to be included in this study. Excluding any patients with conflicting records was a measure to reduce misclassification of patients and to increase the specificity of the cohort. Full coding definitions for cohort creation are referenced in Appendix B. In short: first, a physician billing record for a filtration procedure was required (from the OHIP database): a single OHIP surgeon fee code for "glaucoma filtration procedure" (E132) or "glaucoma filtering procedure and cataract extraction (same eye)" (E214). Second, a Canadian Classification of Health Interventions (CCI) procedural code for "drainage, anterior chamber (of eye)" (1.CJ.52.LA, 1CJ.52.LA-SJ, 1.CJ.52.LA-QB and 1.CJ.52.WJ) was required for the same patient in their CIHI-SDS/DAD hospital records. The date of this procedure was considered the index date.

Notable patient exclusions were: patients less than 66 years of age and greater than 105 years of age were excluded (n=7,072) to retain all patients with an ODB database record (and therefore medication utilization information) at least 1 year in length; patients with evidence of prior filtration surgery were excluded (n=1,332) to remove revision filtration surgeries that may have been miss-coded as primary surgeries; and finally, patients who had evidence of a revision surgery on the same day as the primary filtration surgery were excluded (n=767) as these were not considered to be secondary surgical interventions. If patients underwent

additional filtration surgery on the contralateral eye during the 365-day follow-up (n=2,510), they were then censored from the original cohort and placed into a separate cohort. This sequential-bilateral surgery cohort was used to investigate the possibility of a different outcome rate among these patients.

2.2.4 Outcome Measures

Patients were followed for one year after the date of primary filtration surgery, to a maximum of March 30, 2016. Primary outcomes were (a) revision filtering surgery and (b) any procedure (excluding needling) encompassing conjunctival manipulation. Revision filtering surgery was defined by OHIP fee codes E983 or E984 and CCI intervention codes 1.CJ.52.LA, 1CJ.52.LA-SJ, 1.CJ.52.LA-QB, and 1.CJ.52.WJ (accompanied by the intervention attribute “revision”). OHIP fee codes E212 and E213 and/or CCI intervention codes 1.CS.80, 1.CS.72, 1.CS.84, 1.CS.87, 1.CS.52, 1.CC.80, 1.CD.80 revision, 1.CJ.54, 1.CJ.55, 1.CJ.80, 1.CJ.87, and 1.CD.52.LA revision were used to identify patients who required conjunctival manipulation (excluding needling). See Appendix B for complete definition.

2.2.5 Covariables

Patient factors assessed included demographic data (age, sex, year of cohort entry and income quintile), medical comorbidity, ophthalmic surgical history, eye drop medication history and eye drop preservative exposure. Income quintile was derived from Statistics Canada census data on the average income per single-

person equivalent by postal code.[150] Medical comorbidity was estimated using a Charlson-Deyo comorbidity index with a 5-year review of CIHI-DAD.[151,152] Patients with no CIHI-DAD record were assigned a score of 0 indicating negligible comorbidity. Previously validated algorithms were used to identify patients with diabetes,[147] hypertension,[146] asthma,[148] stroke[153] and sleep apnea[154] (Appendix C). Ophthalmic surgical history was determined through OHIP billing codes (Appendix D) with a 5-year lookback window. Surgical procedures were grouped for analysis based on anatomic location. Data for eye drop prescription history were assessed for the 1-year period prior to the index event for glaucoma medications (Appendix E) and 30 days prior to index event for all other ocular medications (Appendix F) captured by the ODB.

Exposure to the ophthalmic eye drop preservative, benzalkonium chloride (BAK), was assessed for a subset of the main cohort based on data availability. Drugs with known BAK concentrations are listed in Appendix G. All patients who received a prescription for an ophthalmic drug with unknown BAK concentration were excluded from this portion of the analysis. One-year cumulative BAK exposure was calculated as the volume of medication dispensed to a patient during the 365 days before the index event, multiplied by each medication's BAK content (%vol). The resulting number was used to evaluate a patient's 365-day cumulative exposure to BAK.

2.2.6 Statistical Analysis

Data are reported as means and standard deviations unless otherwise indicated. Groups of patients <6 in size are not released in accordance with ICES privacy regulations, so some results are reported as “approximately or ≤5”. Significant differences between groups were initially assessed using Students t-test and chi square. Significant variables were then included in a multivariable cox proportional hazards model. Patients were censored on the date of a primary outcome, death, enucleation, evisceration or exenteration. A second primary filtration procedure on the contralateral eye within the observation window qualified patients for inclusion in the sequential-bilateral surgery cohort for separate analysis. Hazard ratios (HRs) and 95% confidence intervals (95% CIs) were determined using a Cox proportional hazards model. The proportional hazards assumption was evaluated using Schoenfeld residuals, interaction with time covariables, and log-negative-log plots of the survival function.[155] If the proportional hazards assumption was violated, then the hazard ratio at day zero of the 365 day follow up was reported along with their graphed functions. Immortality bias was assessed through a competing risk analysis with death during the follow-up period. Both the cause-specific and subdistribution hazard ratios were calculated, where the cause-specific model is the equivalent of our cox regression model (censoring death) for the primary outcome. All analyses were performed with SAS enterprise guide version 7.12 (SAS Institute, Cary, North Carolina) for UNIX.

2.3 Results

A total of 10,097 primary filtration surgeries were identified after 12,787 patients were excluded based on predetermined criteria (Figure 2-1). The yearly volume of primary filtration surgeries varied over the study period, ranging from a minimum of 1,335 cases for the year April 1, 2013 to March 31, 2014 to a maximum of 1,916 between April 1, 2005 to March 31, 2006. The complete cohort consisted of 4,287 male (42.46%) and 5,810 female (57.54%) patients with a mean ± standard deviation (SD) age of 76.69 ± 6.45 years, and interquartile range (IQR) 72-81 years (**Table 2-1**).

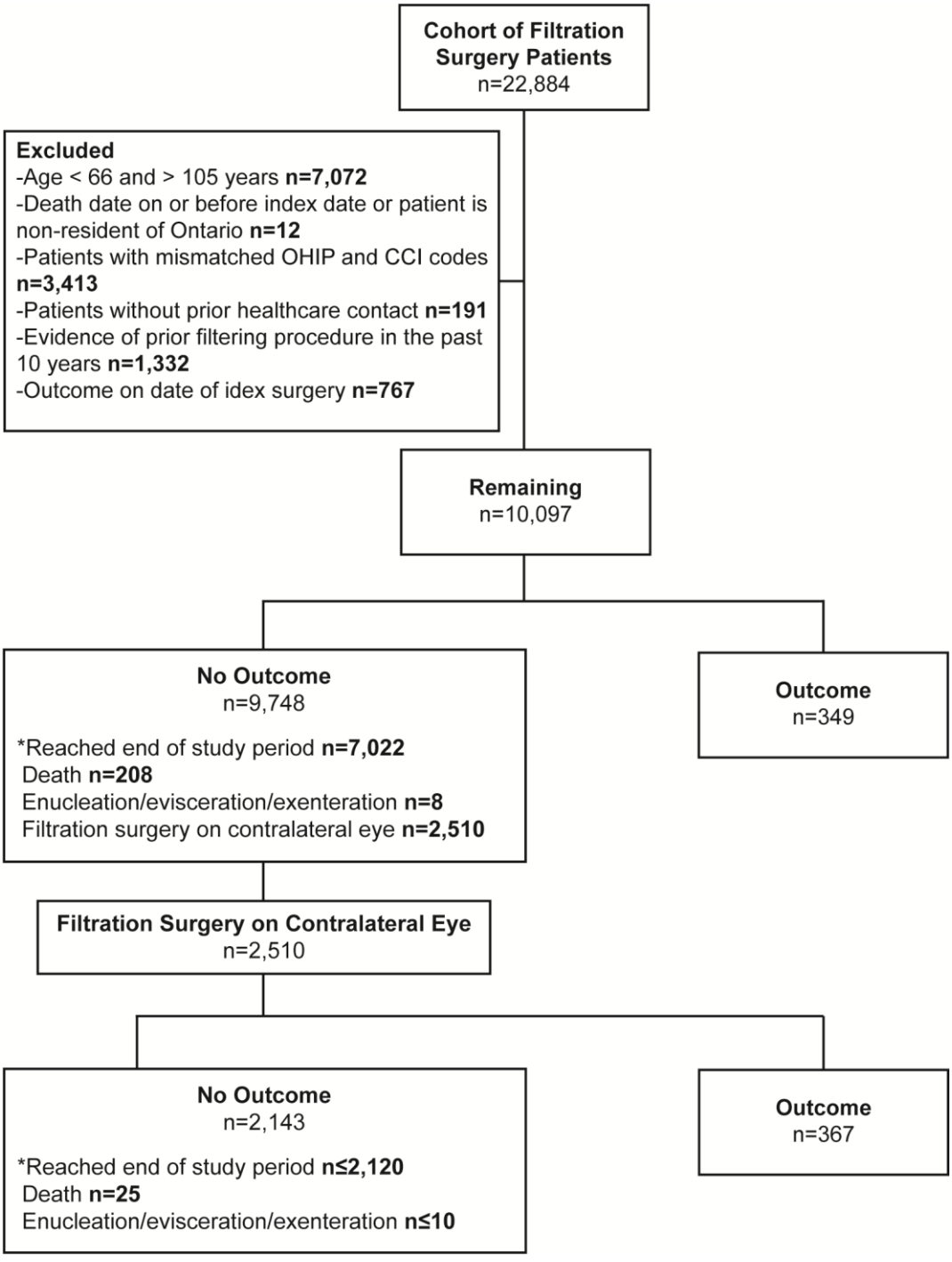


Figure 2-1: Flow Diagram of Cohort Selection

Table 2-1: Cohort Characteristics (Overall, Secondary Surgical Intervention and No Outcome)*

Variable	Entire Cohort n=10,097	Secondary Intervention n=349	No Outcome N=9,748	*P* Value
Demographics				
Age				
Mean ± SD	76.7 ± 6.5	75.8 ± 6.3	76.7 ± 6.5	
66-74	4,014 (39.8%)	162 (46.4%)	3,852 (39.5%)	**0.01**
75-90+	6,083 (60.2%)	187 (53.6)	5,932 (60.4%)	
Sex				
Male (%)	4,287 (42.5%)	147 (42.1%)	4,140 (42.5%)	0.90
Female (%)	5,810 (57.5%)	202 (57.9%)	5,608 (57.5%)	
Year of Primary Filtration Surgery				
2003-2004	1,604 (15.8%)	49 (14.0%)	1,555 (16.0%)	0.09
2005-2006	1,916 (19.0%)	74 (21.2%)	1,842 (18.9%)	
2007-2008	1,666 (16.5%)	76 (21.8%)	1,590 (16.3%)	
2009-2010	1,661 (16.5%)	52 (14.9%)	1,609 (16.5%)	
2011-2012	1,732 (17.2%)	49 (14.0%)	1,683 (17.3%)	
2013-2014	1,335 (13.2%)	42 (12.0%)	1,293 (13.3%)	
2015	183 (1.8%)	7 (2.0%)	176 (1.8%)	
Income Quintile				
1st (lowest)	2,024 (20.1%)	58 (16.6%)	1,966 (20.1%)	0.23
2nd	2,061 (20.4%)	84 (24.1%)	1,977 (20.3%)	
3rd	2,012 (19.9%)	69 (19.8%)	1,943 (19.9%)	
4th	1,879 (18.6%)	71 (20.3%)	1,808 (18.6%)	
5th (highest)	2,121 (21.0%)	67 (19.2%)	2,054 (21.1%)	
Charlson Comorbidity Index				
Mean ± SD	0.6 ± 1.2	0.7 ± 1.1	0.6 ± 1.2	0.35
0	6,915 (68.5%)	221 (63.3%)	6,694 (68.6%)	0.18
1	1,372 (13.6%)	57 (16.3%)	1,315 (13.5%)	
2	1,147 (11.4%)	47 (13.5%)	1,100 (11.3%)	
≥3	663 (6.5%)	24 (6.9%)	639 (6.6%)	
Diabetes				
Yes	2,024 (20.0%)	66 (18.9%)	1,958 (20.1%)	0.60
No	8,073 (80.0%)	283 (21.1%)	7,790 (79.9%)	
Hypertension				
Yes	6,041 (59.8%)	211 (60.5%)	5,830 (59.8%)	0.81
No	4,056 (40.2%)	138 (39.5%)	3,918 (40.2%)	
Asthma				
Yes	1,136 (11.3%)	44 (12.6%)	1,092 (11.2%)	0.41
No	8,961 (88.70%)	305 (87.4%)	8,656 (88.8%)	
Stroke				
Yes	704 (7.0%)	31 (8.9%)	673 (6.9%)	0.15
No	9,393 (93.0%)	318 (91.1%)	9,075 (93.1%)	
Sleep Apnea				
Yes	394 (3.9%)	11 (3.1%)	383 (3.9%)	0.46
No	9,703 (96.1%)	338 (96.9%)	9,365 (96.1%)	

*Data are expressed as n (%) unless otherwise indicated. SD, standard deviation; IQR, inter-quartile range.
†Institute for Clinical and Evaluative Sciences (ICES) privacy policies preclude the publication of data cells containing fewer than 5 patients.

Table 2-1. continued. Cohort Characteristics (Overall, Secondary Surgical Intervention and No Outcome)*

Variable	Entire Cohort n=10,097	Secondary Intervention n=349	No Outcome N=9,748	*P* Value
Prior Ocular Surgery (5 years)				
Yes	7,265 (72.0%)	255 (73.1%)	7,010 (72.0%)	0.64
No	2,832 (28.0%)	94 (26.9%)	2,738 (28.0%)	
Prior Conjunctival Disrupting Surgery (5 years)				
Yes	532 (5.3%)	20 (5.7%)	502 (5.3%)	0.69
No	9,565 (94.7%)	329 (94.3%)	9,236 (94.7%)	
Scleral				
†Yes	≤60 (0.6%)	≤5 (1.4%)	56 (0.6%)	0.48
†No	≥10,037 (99.4%)	≥344 (98.6%)	9,629 (99.4%)	
Vitreous				
Yes	444 (4.4%)	17 (4.4%)	427 (4.4%)	0.66
No	9,653 (95.6%)	332 (95.6%)	9,321 (95.6%)	
Retinal				
†Yes	≤100 (1.0%)	≤5 (1.4%)	97 (1.0%)	0.43
†No	≥9,097 (99.0%)	≥344 (98.6%)	9,651 (99.0%)	
Conjunctival				
†Yes	25 (0.3%)	≤5 (1.4%)	20 (0.2%)	0.74
†No	10,072 (99.7%)	≥344 (98.6%)	9,728 (99.8%)	
Prior Conjunctival Sparing Surgery (5 years)				
Yes	7,230 (71.6%)	254 (72.8%)	6,976 (71.6%)	0.62
No	2,867 (28.4%)	95 (27.2%)	2,772 (28.4%)	
Lens				
Yes	2,609 (25.8%)	100 (28.6%)	2,509 (25.7%)	0.22
No	7,488 (74.2%)	249 (71.4%)	7,239 (74.23%)	
Corneal Transplant				
Yes	109 (1.1%)	8 (2.3%)	101 (1.0%)	**0.026**
No	9,988 (98.9%)	341 (97.7%)	9,648 (99.0%)	
Iris				
Yes	1,517 (15.0%)	35 (10.0%)	1,482 (15.2%)	**0.008**
No	8,580 (85.0%)	314 (90.0%)	8,266 (84.8%)	
Laser angle surgery				
Yes	4,916 (48.7%)	182 (52.2%)	4,734 (48.6%)	0.19
No	5,181 (51.3%)	167 (47.8%)	5,014 (51.4%)	
Photocoagulation				
Yes	620 (6.1%)	13 (3.7%)	607 (6.2%)	0.06
No	9,477 (95.9%)	336 (94.3%)	9,141 (93.8%)	
Interventions for retinal disease				
Yes	497 (4.9%)	13 (3.7%)	484 (5.0%)	0.29
No	9,600 (95.1%)	336 (96.2%)	9,264 (95.0%)	

*Data are expressed as n (%) unless otherwise indicated. SD, standard deviation; IQR, inter-quartile range; CAI, carbonic anhydrase inhibitor; PGA, prostaglandin analogue; NSAIDs, non-steroidal anti-inflammatory drugs.
†Institute for Clinical and Evaluative Sciences (ICES) privacy policies preclude the publication of data cells containing fewer than 5 patients.

Table 2-1 continued. Cohort Characteristics (Overall, Secondary Surgical Intervention and No Outcome)*

Variable	Entire Cohort n=10,097	Secondary Intervention n=349	No Outcome N=9,748	*P* Value
Glaucoma Medication History (1 year)				
Beta-Blocker				
Yes	5,379 (53.3%)	191 (54.7%)	5,188 (53.2%)	0.58
No	4,718 (46.7%)	158 (43.3%)	4,560 (46.8%)	
Mean ±SD days of meds/patient	136.3 ± 105.9	130.3 ± 108.2	136.5 ± 105.8	0.42
CAI				
Yes	2,253 (22.3%)	74 (21.3%)	2,179 (22.4%)	0.61
No	7,844 (77.7%)	275 (78.7%)	7,569 (77.6%)	
Mean ±SD days of meds/patient	124.4 ±111.6	117.0 ± 124.0	123.7 ± 111.1	0.65
Miotics				
Yes	2,595 (25.7%)	90 (25.8%)	2,505 (25.7%)	0.97
No	7,502 (74.3%)	259 (74.2%)	7,243 (74.3%)	
Mean days ±SD of meds/patient	108.0 ± 108.0	105.1 ± 113.4	108.1 ± 107.8	0.80
PGA				
Yes	8,987 (89.0%)	323 (92.5%)	8,664 (88.9%)	**0.03**
No	1,110 (11.0%)	26 (7.5%)	1,084 (11.1%)	
Mean ±SD days of medspatient	159.4 ± 107.3	153.4 ± 102.6	159.7 ± 107.5	0.32
Sympathomimetics				
Yes	4,538 (44.9%)	172 (49.3%)	4,366 (44.8%)	0.14
No	5,559 (55.1%)	177 (50.7%)	5,382 (55.2%)	
Mean ±SD days of meds/patient	131.6 ± 108.1	124.4 ± 93.0	131.8 ± 108.6	0.47
Other Ophthalmic Medications (30 days)				
Antibiotic				
Yes	851 (8.4%)	46 (13.2%)	805 (8.3%)	**0.001**
No	9,246 (91.6%)	303 (86.8%)	8,943 (91.7%)	
Corticosteroid				
Yes	4,519 (44.8%)	169 (48.4%)	4,350 (44.6%)	0.16
No	5,578 (55.2%)	180 (51.6%)	5,398 (55.4%)	
Aminoglycoside				
Yes	135 (1.3%)	15 (4.3%)	120 (1.2%)	**<0.001**
No	9,962 (98.7%)	334 (95.7%)	9,628 (98.8%)	
Mydriatic				
Yes	426 (4.2%)	33 (9.5%)	393 (4.0%)	**<0.001**
No	9,671 (95.8%)	316 (90.5%)	9,355 (96.0%)	
NSAIDs				
Yes	1,767 (17.5%)	36 (10.3%)	1,731 (17.8%)	**<0.001**
No	8,330 (82.5%)	313 (89.7%)	8,017 (82.2%)	

*Data are expressed as n (%) unless otherwise indicated. SD, standard deviation; IQR, inter-quartile range; CAI, carbonic anhydrase inhibitor; PGA, prostaglandin analogue; NSAIDs, non-steroidal anti-inflammatory drugs.
†Institute for Clinical and Evaluative Sciences (ICES) privacy policies preclude the publication of data cells containing fewer than 5 patients.

2.3.1 Type of Primary Filtration Surgery

Most primary filtration surgeries were solo procedures, with 6,947 (68.80%) patients undergoing primary filtration surgery without cataract extraction or implantation of an indwelling drainage device (IDD). Filtration surgery was combined with cataract extraction in 1,745 (17.28%) patients. An IDD was used in approximately 860 (8.52%) cases, and approximately 550 (5.45%) cases were combined with cataract extraction and implantation of an IDD. Of these initial cases, 2,510 (25.8%) patients had their second eye undergo primary filtration surgery. Of these, 1,698 (67.7%) were solo-procedures, 504 (20.1%) were combined with cataract extraction, 134 (5.3%) involved an IDD and 174 (6.9%) cases were combined with an IDD and cataract extraction.

2.3.2 Primary Outcome

Secondary surgical intervention was required for 349 patients (3.46%) a median [IQR] of 21 [9-56] days following the initial filtering surgery. Life table is presented in **Table 2-2**. For these patients, 303 conjunctival manipulations occurred, approximately 5 implanted drainage devices were removed, and 72 revision filtration surgeries were performed (certain patients experienced multiple secondary surgical interventions within the 1-year follow-up period).

Table 2-2: Life table of primary outcome – unilateral cohort†

Time Interval (days)	Filtering procedure (solo-procedure)		Filtering Surgery +IDD		Filtering Surgery +cataract extraction		Filtering Surgery +IDD, +cataract extraction	
	N Outcome	N at Risk	N Outcome	N at Risk	N Outcome	N at Risk	N Outcome	N at Risk
0-182	270	5427	20	740	27	1354	≤5	427
182-365	20	4825	≤5	681	≤5	1184	≤5	357

†Institute for Clinical and Evaluative Sciences (ICES) privacy policies preclude the publication of data cells containing fewer than 5 patients

Solo-filtration surgeries had a secondary surgical intervention rate of 1.43 per 10,000 person-days. With use of an IDD, the rate was approximately 1.00 per 10,000 person-days (vs solo-filtration, p = 0.0.035). When filtering surgery was combined with cataract extraction the rate was 0.61 per 10,000 person-days (vs solo-filtration, $p < 0.0001$). Patients who underwent filtration surgery combined with cataract extraction and an IDD experienced secondary surgical interventions at a rate of less than 0.25 per 10,000 patient-days (vs solo-filtration, $p < 0.0001$).

In the sequential-bilateral filtration surgery cohort, following patients for an additional 365-days after primary intervention on the contralateral eye revealed higher rates of secondary surgical intervention compared to patients with monocular surgical interventions (**Table 2-3**). Secondary surgical intervention was undertaken in 367 patients and had an incidence rate of 7.44 per 10,000 person-days, which was higher than that of the unilateral surgery cohort – 1.08 per 10,000 person-days ($p < 0.0001$). Considering a per-eye event rate equal to half that observed in the sequential-bilateral cohort, there was additional risk observed on a per-eye basis.

Table 2-3: Primary outcome by cohort and type of filtration surgery

	Unilateral Cohort			Sequential Bilateral Cohort			
Type of Primary Surgery	N Outcomes	Person-days of follow up	Event Rate*	N Outcomes	Person-days of follow up	Event Rate*	P value**
Filtering procedure (solo-procedure)	290	2,028,425	**1.43**	235	338,050	**6.95**	<0.0001
Filtering surgery + IDD†	≤30	272,971	**≤1.00**	59	17,099	**34.50**	<0.0001
Filtering surgery + cataract extraction	31	507,594	**0.61**	60	102,812	**5.84**	<0.0001
Filtering surgery + cataract extraction + IDD†	≤5	157,397	**≤0.25**	13	35,432	**3.67**	<0.0001
Total	321	2,966,387	**1.08**	367	493,393	**7.44**	<0.0001

*Event rate per 10,000 person-days; **p-value was derived using a poison regression model to compare incidence rates in unilateral and bilateral groups; †Institute for Clinical and Evaluative Sciences (ICES) privacy policies preclude the publication of data cells containing fewer than 5 patients

2.3.3 Covariables

Patient demographics, medical comorbidities, ophthalmic surgical history, eye drop medication history and eye drop preservative exposure are listed in **Table 2-1** for the complete cohort (n=10,097), those who required secondary surgical intervention (n=349) and those who did not experience an outcome within the first post-operative year (n=9,748).

Analysis of patients who underwent unilateral glaucoma filtration surgery revealed that the initial type of filtration surgery was significantly associated with secondary surgical intervention rates. Surgeries that included an IDD, phacoemulsification, or both had decreased risks compared with solo-filtration procedures without an IDD (HR=0.58 (p=0.013), HR=0.33 ($p < 0.0001$), and HR=0.087 ($p = 0.0001$) respectively). In the adjusted multivariable Cox proportional hazards model, several factors in a patient's medical history were identified to have a significant association with secondary surgical intervention (**Table 2-4**). Patients under the age of 75 were more likely to undergo secondary surgical intervention than those over 75 years of age (HR=1.35, p=0.005). A history of iris surgery (laser iridotomy, iridectomy) was associated with a reduced risk of secondary surgical intervention (HR=0.70, p=0.04). Filling a prescription for aminoglycoside or mydriatic eye drops within the month preceding filtration surgery was associated with an increased risk of secondary surgical intervention, HR=3.19 ($p < 0.0001$) and HR=2.32 (p=0.0002) respectively. Hazard functions that violated the proportionality assumption, and are variable over time, are

displayed in Figure 2-2 for the unilateral cohort and in Figure 2-3 for the bi-lateral cohort.

Table 2-4: Multivariable Cox Proportional Hazards Model for Secondary Surgical Intervention – Unilateral and Bilateral

Significant Covariates	Unilateral Cohort Hazard Ratio (95%CI)	P Value	Bilateral Cohort Hazard Ratio (95% CI)	*P* Value
Age (younger vs older than 75y)	1.35 (1.10-1.66)	**0.005**	0.92 (0.75-1.14)	0.45
Surgical History				
Corneal transplant	*1.22 (0.46-3.22)	0.70	4.77 (2.58-8.83)	**<0.0001**
Surgeries on the Iris	0.70 (0.49-0.99)	**0.04**	*0.86 (0.62-1.19)	0.35
Prescriptions filled in the month before surgery				
Topical antibiotics	*1.18 (0.79-1.74)	0.42	0.85 (0.59-1.22)	0.37
Topical aminoglycosides	3.19 (1.89-5.36)	**< 0.0001**	*1.32 (0.52-3.35)	0.56
Topical mydriatics	*2.32 (1.49-3.61)	**0.0002**	4.03 (3.02-5.37)	**<0.0001**
Topical NSAIDs	0.62 (0.44-0.88)	**0.007**	0.93 (0.71-1.22)	0.60
Type of primary filtration surgery				
Filtering surgery w/IDD	*0.58 (0.37-0.89)	**0.01**	3.24 (2.42-4.35)	**<0.0001**
Combined w/cataract extraction only	*0.33 (0.21-0.52)	**<0.0001**	0.87 (0.65-1.15)	0.33
Combined w/cataract extraction and IDD	*0.09 (0.03-0.31)	**0.0001**	0.56 (0.32-0.97)	**0.04**

*Proportionality assumption violated and therefore the model was adjusted with an "interaction with time" covariate, and the HR at time zero is reported. The hazard functions for covariates violating the proportionality assumption, and whose associated hazard varies over the follow-up period are displayed in Figures 2 and 3.

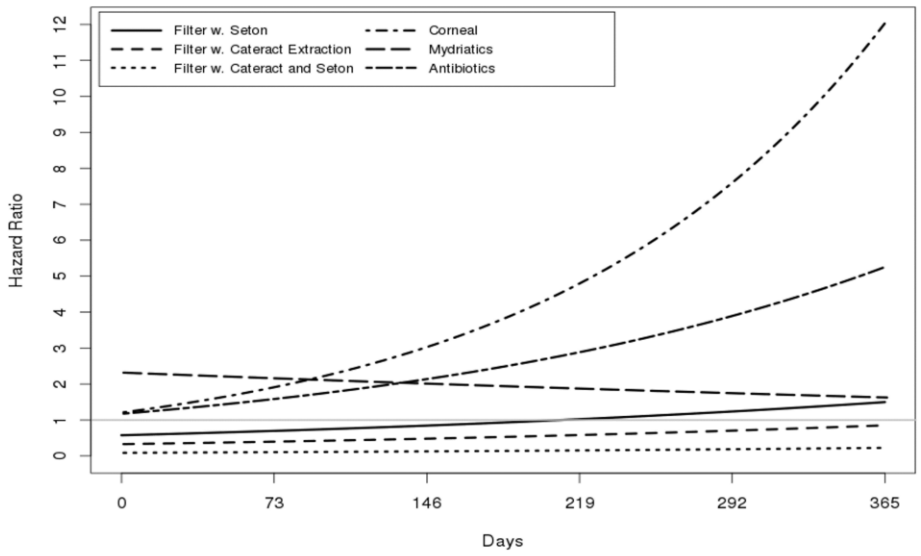


Figure 2-2 Hazard functions that violated the proportionality assumption are displayed for the unilateral cohort. The proportional hazards assumption was evaluated using Schoenfeld residuals, interaction with time covariables, and log-negative-log plots of the survival function.

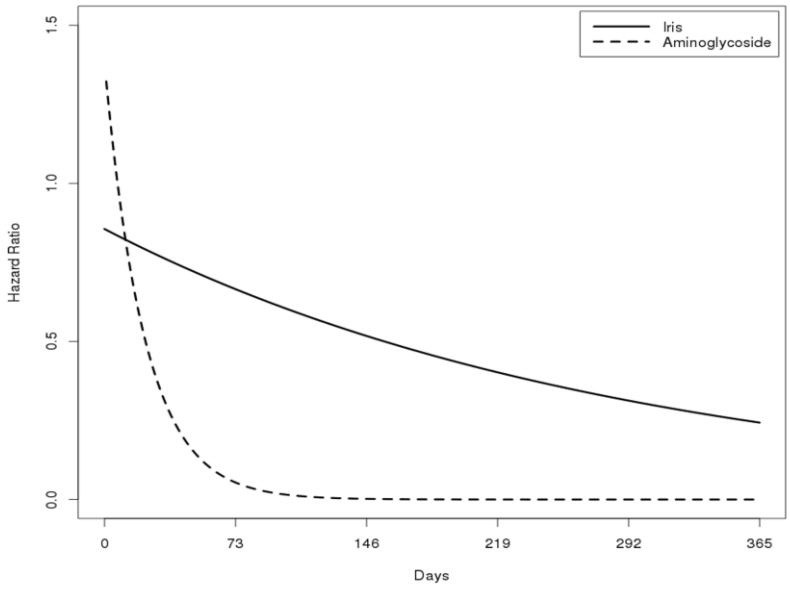


Figure 2-3: Hazard functions that violated the proportionality assumption are displayed for the cohort of patients who had filtration surgery on both eyes. Iris: prior surgeries on the iris.

Within the sequential-bilateral surgery cohort, several covariables were significantly associated with the rate of secondary surgical intervention (**Table 2-4**). Surgeries involving an IDD were associated with higher rates of secondary surgical intervention (HR=3.24, $p < 0.0001$), whereas surgeries combined with cataract extraction and an IDD were associated with reduced risk (HR=0.56, $p < 0.04$). History of corneal transplant was associated with more frequent secondary surgical interventions (HR=4.77, $p < 0.0001$) as was perioperative exposure to mydriatic eye drops (HR=4.03, $p < 0.0001$). Hazard functions that violated the proportionality assumption (surgeries of the iris and aminoglycoside exposure), and vary over time, are displayed in Figure 2-3.

The competing risk of death was considered not to have confounded results (**Appendix H**), as the cause-specific hazard ratios were almost equivalent to the subdistribution hazard ratios - due to the rarity of the competing risk event (death).[156] Further, the cumulative incidence function for death was not significant ($p=0.7403$) across all types of initial filtration surgeries. Therefore, immortality bias was not a major concern.

2.3.4 Benzalkonium Chloride Exposure Cohort

Patient demographics, medical comorbidities, ophthalmic surgical history, eye drop medication history and eye drop preservative exposure are listed in **Table 2-5** for the BAK-cohort (n=8,676), those who required secondary surgical intervention (n=315) and those who did not experience an outcome within the first post-operative year (n=8,361). Within the BAK-cohort, only 48 patients were exclusively prescribed preservative free medications within the 365 days prior to surgery. None of these patients experienced an outcome within the follow-up period. However, cumulative 365-day BAK exposure preceding surgery was not associated with secondary surgical intervention, with patients requiring secondary surgical intervention exposed to a median [IQR] of 11 [5-23] vs. 12 [5-26] ml for patients who did not (p = 0.64).

Significant covariables were unchanged from the main study cohort except in the case of PGA exposure. The group of patients who filled a prescription for PGA eye drops within the year preceding primary filtration surgery experienced a post-operative secondary intervention rate of 3.6% (p=0.031). However, in the BAK cohort a history of PGA had no significant association with secondary intervention (p=0.066).

Table 2-5: BAK Exposure Cohort Characteristics (Overall, Secondary Surgical Intervention and No Outcome)*

Variable		BAK Cohort n=8,676	Secondary Intervention n=315	No Outcome N=8,361	*P* value
Demographics					
Age					
	Mean ± SD	76.7 ± 6.5	75.7 ± 6.4	76.7 ± 6.4	
	66-69.9	3,474 (40%)	150 (47.6%)	3,324 (39.8%)	**0.005**
	70-74.9	5,202 (60%)	165 (52.4%)	5,037 (60.2%)	
Sex					
	Male (%)	3,654 (42.1%)	130 (41.3%)	3,524 (42.2%)	0.76
	Female (%)	5,022 (57.9%)	185 (58.7%)	4,837 (57.8%)	
Year of Primary Filtration Surgery					
	2003-2004	1,202 (13.9%)	44 (14.0%)	1,158 (13.9%)	0.14
	2005-2006	1,712 (19.7%)	67 (21.3%)	1,645 (19.7%)	
	2007-2008	1,601 (18.5%)	75 (23.8%)	1,526 (18.3%)	
	2009-2010	1,613 (18.6%)	52 (16.5%)	1,561 (18.7%)	
	2011-2012	1,449 (16.7%)	41 (13.0%)	1,408 (16.8%)	
	2013-2014	957 (11.0%)	30 (9.5%)	927 (11.1%)	
	2015	142 (1.6%)	6 (1.9%)	136 (1.6%)	
Income Quintile					
	1st (lowest)	1,740 (20.1%)	50 (15.8%)	1,690 (20.2%)	0.20
	2nd	1,772 (20.4%)	76 (24.1%)	1,696 (20.3%)	
	3rd	1,719 (19.8%)	67 (21.3%)	1,652 (19.8%)	
	4th	1,612 (18.6%)	61 (19.4%)	1,551 (18.6%)	
	5th (highest)	1,833 (21.1%)	61 (19.4%)	1,772 (21.1%)	
Charlson Comorbidity Index					
	Mean ± SD	0.64 ± 1.2	0.68 ± 1.1	0.64 ± 1.2	0.58
	0	5,940 (68.5%)	205 (65.0%)	5,735 (68.6%)	0.54
	1	1,166 (13.4%)	46 (14.6%)	1,120 (13.4%)	
	2	1,001 (11.5%)	43 (13.7%)	958 (11.5%)	
	≥3	569 (6.6%)	21 (6.7%)	548 (6.5%)	
Diabetes					
	Yes	1,713 (19.7%)	57 (18.1%)	1,656 (19.8%)	0.45
	No	6,963 (80.3%)	258 (81.9%)	6,705 (80.2%)	
Hypertension					
	Yes	5,173 (59.6%)	190 (60.3%)	4,983 (59.6%)	0.79
	No	3,503 (40.4%)	125 (39.7%)	3,378 (40.4%)	
Asthma					
	Yes	1,023 (11.8%)	43 (13.6%)	980 (11.7%)	0.29
	No	7,653 (88.2%)	272 (86.4%)	7,381 (88.3%)	
Stroke					
	Yes	612 (7.1%)	28 (8.9%)	584 (7.0%)	0.19
	No	8,064 (92.9%)	287 (91.1%)	7,777 (93.0%)	
Sleep Apnea					
	Yes	344 (3.9%)	8 (2.5%)	336 (4.0%)	0.19
	No	8,332 (96.0%)	307 (97.5%)	8,025 (96.0%)	

*Data are expressed as n (%) unless otherwise indicated. SD, standard deviation; IQR, inter-quartile range.
†Institute for Clinical and Evaluative Sciences (ICES) privacy policies preclude the publication of data cells containing fewer than 5 patients.

Table 2-5 continued. BAK Exposure Cohort Characteristics (Overall, Secondary Surgical Intervention and No Outcome)*

Variable	BAK Cohort n=8,676	Secondary Intervention n=315	No Outcome N=8,361	*P* value
Prior Ocular Surgery (5 years)				
Yes	6,247 (72.0%)	226 (71.7%)	6,021 (72.0%)	0.92
No	2,429 (28.0%)	89 (28.3%)	2,340 (28.0%)	
Prior Conjunctival Disrupting Surgery (5 years)				
Yes	458 (5.3%)	19 (6.0%)	439 (5.3%)	0.54
No	8,218 (94.7%)	296 (94.0%)	7,922 (94.7%)	
Scleral				
†Yes	≤55	≤5	51 (0.6%)	0.51
†No	≥8,621	≥310	8,310 (99.4%)	
Vitreous				
Yes	383 (4.4%)	16 (5.1%)	367 (4.4%)	0.56
No	8,293 (95.6%)	299 (94.9%)	7,994 (95.6%)	
Retinal				
†Yes	≤85	≤5	82 (1.0%)	0.54
†No	≥8,591	≥310	8,279 (99.0%)	
Conjunctival				
†Yes	≤20	≤5	18 (0.2%)	0.70
†No	≥8,656	≥310	8,343 (99.8%)	
Prior Conjunctival Sparing Surgery (5 years)				
Yes	6,216 (71.7%)	225 (71.4%)	5,991 (71.7%)	0.93
No	2,460 (28.3%)	90 (28.6%)	2,370 (28.3%)	
Lens				
Yes	2,234 (25.8%)	87 (27.6%)	2,147 (25.7%)	0.44
No	6,442 (74.2%)	228 (72.4%)	6,214 (74.3%)	
Corneal Transplant				
Yes	97 (1.1%)	8 (2.5%)	89 (1.1%)	**0.02**
No	8,579 (98.9%)	307 (97.5%)	8,272 (98.9%)	
Iris				
Yes	1,318 (15.2%)	32 (10.2%)	1,286 (15.4%)	**0.01**
No	7,358 (84.8%)	283 (89.8%)	7,075 (84.6%)	
Laser angle surgery				
Yes	4,232 (48.8%)	163 (51.7%)	4,069 (48.7%)	0.28
No	4,444 (51.2%)	152 (48.3%)	4,292 (51.3%)	
Photocoagulation				
Yes	544 (6.3%)	12 (3.8%)	532 (6.4%)	0.07
No	8,132 (93.7%)	303 (96.2%)	7,829 (93.6%)	
Interventions for retinal disease				
Yes	427 (4.9%)	12 (3.8%)	415 (5.0%)	0.35
No	8,249 (95.1%)	303 (96.2%)	7,946 (95.0%)	

*Data are expressed as n (%) unless otherwise indicated. SD, standard deviation; IQR, inter-quartile range; CAI, carbonic anhydrase inhibitor; PGA, prostaglandin analogue; NSAIDs, non-steroidal anti-inflammatory drugs; BAK, benzalkonium chloride.

Table 2-5 continued. BAK Exposure Cohort Characteristics (Overall, Secondary Surgical Intervention and No Outcome)*

Variable	BAK Cohort n=8,676	Secondary Intervention n=315	No Outcome N=8,361	*P* value
Glaucoma Medication History (1 year)				
Antibiotic				
Yes	716 (8.23%)	38 (12.1%)	678 (8.1%)	**0.01**
No	7,960 (91.7%)	277 (87.9%)	7,683 (91.9%)	
Corticosteroid				
Yes	3,951 (45.5%)	154 (48.9%)	3,797 (45.4%)	0.22
No	4,725 (54.5%)	161 (51.1%)	4,564 (54.6%)	
Aminoglycoside				
Yes	119 (1.4%)	13 (4.1%)	106 (1.3%)	**< 0.001**
No	8,557 (98.6%)	302 (95.9%)	8,255 (98.7%)	
Mydriatic				
Yes	373 (4.3%)	30 (9.5%)	343 (4.1%)	**< 0.001**
No	8,303 (95.7%)	285 (90.5%)	8,018 (95.9%)	
NSAIDs				
Yes	1,580 (18.2%)	34 (10.8%)	1,546 (18.5%)	**< 0.001**
No	7,096 (81.8%)	281 (89.2%)	6,815 (81.5%)	
BAK Exposure (1 year)				
BAK Containing Drops				
Yes	8,628 (99.5%)	315 (100%)	8,628 (99.5%)	**0.02**
No	48 (0.5%)	0 (0%)	48 (0.5%)	
Cumulative BAK Load (percentile)				
1st to 25th	2,166 (25.0%)	73 (23.2%)	2,093 (25.0%)	0.64
25th to 50th	1,956 (22.5%)	82 (26.0%)	1,874 (22.4%)	
50th to 75th	2,042 (23.5%)	82 (26.0%)	1,960 (23.5%)	
75th to 100th percentile	2,512 (29.0%)	78 (24.8%)	2,434 (29.1%)	

*Data are expressed as n (%) unless otherwise indicated. SD, standard deviation; IQR, inter-quartile range; CAI, carbonic anhydrase inhibitor; PGA, prostaglandin analogue; NSAIDs, non-steroidal anti-inflammatory drugs; BAK, benzalkonium chloride.

2.4 Discussion

This large administrative data study of more than 10,000 cases identified the proportion of filtration surgery patients that required secondary surgical intervention (3.46%) within the first year of follow-up. The rate was higher in solo filtration surgery procedures, while combined surgeries (IDD, cataract extraction) experienced reduced rates of secondary surgical intervention. The rate was unchanged between men and women, however older age was associated with reduced rates of secondary surgical intervention. No significant differences were found in annual rates from 2003 to 2015 and there was no significant association with socioeconomic status. Overall, prior intraocular surgery had minimal association with secondary surgical intervention rates. Age, prior iris surgery as well as perioperative aminoglycoside and mydriatic exposure were all significant variables in the adjusted Cox multivariate model.

The sequential-bilateral cohort revealed a dramatically increased rate of secondary surgical intervention, with several different covariables reaching significance compared to the unilateral cohort. The difference in risk may be due to patients requiring sequential-bilateral interventions having potentially more advanced glaucoma, and thus placing them at a higher risk for secondary surgical intervention – even when considered on a risk-per-eye basis.[157]

Analysis of the BAK-cohort revealed no major changes in significant covariables from the main cohort. BAK exposure had no effect on secondary surgical intervention rates. No trends were evident for any other types of prior ocular

surgeries, any of the systemic comorbidities assessed nor any specific class of glaucoma medication, save the prostaglandin analogs which showed a significant association with secondary surgical intervention in the main study cohort.

This population-based analysis is strengthened by the nature of the public healthcare system in Ontario. Filtration surgery, as well as all risk factors discussed, are insured services and for physicians to be reimbursed for their services, billing records must be submitted to the Ontario Health Insurance Plan. Patient records are continuous, even patients who have switched surgeons within Ontario are captured in our datasets. The number of patients that are lost to follow-up is low. Only if a patient elects to pay out-of-pocket for surgery in another province, country or in an independent surgical center would that data not be captured. Finally, our analysis of BAK exposure as a risk factor used a cumulative 365-day pre-operative exposure, which allowed us to model it as a continuous outcome in order to maximize the chance of detecting a significant effect on surgical failure rates. However, all data relies on the accuracy and reliability of billing practices and medical records coding. Further, ODB only tracks prescriptions filled by patients greater than 65 years old, necessitating exclusion of patients under the age of 66 from our study in order to obtain and analyze prescription information. Medically treated surgical failures were not captured – only secondary surgical interventions. Further limitations to these data should also be clarified. We could not determine the type of IDD used in combined procedures, patient compliance with prescribed medications, receipt of

non-recorded “sample” medications from physician offices, nor could we determine any risk factors for IOP-related outcomes.

It is our understanding that, to date, this study is the largest to investigate rates of secondary surgical intervention within the first post-operative year after glaucoma filtration surgery, considering single eye interventions and sequential-bilateral interventions separately. The observed rate within the present study’s unilateral cohort was similar to those reported previously, ranging from three to eight percent in unilateral interventions.[131–133,158] Patients who underwent glaucoma surgery in the contralateral eye were more than twice as likely to experience secondary surgical interventions. In agreement with these data, Mietz and colleagues previously analyzed 138 trabeculectomy patients and found that additional interventions occurred more frequently in the second operated eye.[159] The work of Iwasaki et. al. further supports this trend and found that as the length of time between surgery on the first and second eye increased, so did the risk of failure.[160]

Our unilateral cohort showed significantly lower secondary surgical intervention rates when undergoing glaucoma procedures combined with cataract extraction and/or an IDD. This is in contrast to previous reports indicating similar safety profiles for both filtration surgery and phaco-filtration surgery – however it is possible that our larger sample size has allowed us to statistically elucidate this association.[161,162] As Ontario does not yet have device-specific billing codes, data specifying the type of IDD used were unavailable. Thus, the IDD procedures captured within this study were likely a heterogeneous mixture of various shunt

devices including both traditional as well as micro-invasive glaucoma surgical (MIGS) drainage devices. As MIGS devices are currently only indicated to be combined with phacoemulsification, patients receiving these devices would likely have been captured in the cataract extraction w/IDD group. However, this precludes attributing any of the observed risk factors to a specific type of IDD. Data must be interpreted within the context of its collection however, and the included heterogeneity may allow for greater generalizability (i.e. what risk is attributable to a foreign body in the eye vs no foreign body and is it associated with secondary surgical intervention).

In patients who underwent glaucoma surgery on their second eye, combined procedures were also protective, except for the insertion of an IDD alone. In this IDD group, a much larger hazard was associated with IDDs than observed in the unilateral cohort. The severity of glaucomatous disease is likely greater in patients who had surgeries in both eyes, and perhaps these IDDs were more likely to be traditional valves and shunts, not novel MIGS devices, which could help to explain these findings. Nevertheless, care should be taken when inserting any foreign body into an eye with advanced disease.

In the present study, older age significantly reduced the risk of secondary surgical intervention in the unilateral cohort, however the significance was lost if patients underwent sequential-bilateral procedures. Several studies have reported similar results for unilateral procedures.[134,139] Previous intraocular surgery has been well explored as a risk factor for filtration failure.[135,139] Broadway and Chang found that fibroblast and inflammatory cell numbers were increased in the conjunctiva of

patients with a positive history, providing a potential physiological mechanism for this risk factor.[71,72] However, data from the present study did not reveal any clear association between prior ocular surgery and rates of secondary surgical intervention in the real world setting. No significant effect was observed for prior conjunctival disrupting surgeries, nor for conjunctival sparing surgeries, save for the protective effect of prior surgery on the iris in the unilateral cohort and prior corneal transplant in the sequential-bilateral cohort.

Exposure to topical antiglaucoma medications and the preservative benzalkonium chloride (BAK) has been associated with filtration surgery failure.[68,70–72] Our results indicated that neither the type, save prostaglandin analogs in the main study cohort, nor the amount of dispensed glaucoma medications in the year prior to surgery were significantly associated with secondary surgical intervention rates. Neither was cumulative BAK exposure. Previous work has supported BAK exposure as a risk factor for early failure, however it was also noted that significance was independent of the number of BAK containing medications used.[68] As just 48 patients received prescriptions for exclusively preservative free medications during the study period, and none of these patients experienced an outcome during the observation period, it remains possible that the complete absence of BAK exposure does result in a lower risk of secondary surgical intervention.

Prescriptions for certain topical ocular medications, filled within 30 days prior to surgery, were significantly associated with secondary surgical intervention. Topical NSAIDs were significantly associated with decreased rates of secondary

surgical intervention in the unilateral cohort, however significance was lost within the sequential-bilateral cohort. Interestingly, dexamethasone was not significantly associated with intervention rates. Previously, a randomized clinical trial investigated the potential benefit of pre-operative topical NSAIDs versus steroids before trabeculectomy.[163] Patients were treated with either drug 4 times daily for one month before surgery and both drugs were found to significantly reduce needling rates. These findings were also mirrored in a study involving Ahmed glaucoma valves.[90] These results together with the incidence of steroid induced IOP spikes[97] suggests NSAIDs could serve as preferential adjunctive medications to these surgical procedures.

Filling a prescription for an antibiotic, particularly aminoglycosides, prior to surgery was significantly associated with increased rates of secondary surgical intervention in the unilateral cohort. One in nine patients who received a prescription for aminoglycosides required a secondary surgical intervention. Aminoglycosides are known to be toxic in the ear and induce toxicity and scarring in the kidney.[164,165] However, it is unclear if this study's observation is a consequence of these drugs acting on subconjunctival tissues, or if the infection being treated by these medications may be negatively influencing surgical outcomes. An ocular infection and accompanied inflammation just prior to filtration surgery could prime the conjunctiva for aggressive post-surgical wound healing. These novel findings warrant further investigation.

In conclusion, the rate of secondary surgical intervention within the first post-operative year after glaucoma filtering surgery is modest within the Ontario.

Approximately a quarter of patients undergo sequential-bilateral filtering procedures, and secondary surgical interventions occur much more frequently in this group of patients – even on a per-eye basis. Surgeries involving IDDs, combination with cataract surgery or both experienced fewer surgical interventions in the unilateral cohort. Surgeries combined with both an IDD and cataract extraction experienced fewer secondary surgical interventions in the sequential-bilateral cohort, whereas surgeries with IDDs alone experienced secondary surgical interventions more frequently. Further investigation into the effects of mydriatic and aminoglycoside use prior to surgery should be undertaken and caution may be warranted when patients undergoing filtration surgery have experienced recent ocular infections. Exploring the potential physiological basis of these associations could provide novel strategies for perioperative wound management and guide the development of novel adjuvant therapies. Results indicate that filtration surgery combined with an IDD and/or phacoemulsification may be considered as a primary surgical intervention and patients undergoing sequential-bilateral glaucoma procedures should be counseled regarding their greatly increased surgical risk.

Chapter 3

3 Differential Effects of Dexamethasone and Indomethacin: Implications for Glaucoma Surgery[2]

In the previous chapter, surprisingly, perioperative exposure to NSAIDs was found to be more strongly associated with GFS success than were corticosteroids. Due to the favorable actions of RMs during wound healing, and the fact that much of RM biosynthesis is abrogated by corticosteroids, we hypothesized that NSAIDs may better impede post-operative wound healing phenomena than corticosteroids.

In Chapter 3, these ideas are pursued *in vitro*, comparing the effects of steroidal vs. NSAID anti-inflammatory exposure on wound healing phenomena elicited by primary human subconjunctival fibroblasts within a 3D collagen culture system.

[2] Parts of this chapter have been published: Armstrong, J.J., Denstedt, J., Trelford, C.B, Li, E. and Hutnik, C.M.L. Differential effects of dexamethasone and indomethacin on Tenon's capsule fibroblasts: Implications for glaucoma surgery. Experimental Eye Research. 2019; 182(Jan); 65

3.1 Introduction

Antimetabolites dramatically increase glaucoma surgery success rates, but may be associated with complications relating to overly suppressed wound healing - i.e. bleb leakage, blebitis, endophthalmitis, bleb dysesthesia and hypotony.[92,166–168] The use of perioperative, anti-inflammatory drops permits further control of inflammation-driven wound healing. This strategy allows the surgeon to adjust dosing in response to changing bleb morphology over the post-operative period, a clinical luxury absent when relying on anti-metabolites as the sole modality of wound modulation.[169] To this end, topical corticosteroids are commonly used to control inflammation-driven scarring after glaucoma surgery. Typically, they are applied peri-operatively, oftentimes for several weeks or months, before[89]and/or after glaucoma surgery.[170] This includes trabeculectomy, phacotrabeculectomy, traditional tube shunt or many of the novel micro-invasive glaucoma surgical (MIGS) devices or procedures. However, steroids are associated with adverse events such as cataract development, elevation of intraocular pressure (IOP) and increased infection risk.[96–103] These risks add to the unpredictability of post-operative wound healing and fuel the search for viable alternatives to control post-operative inflammation and scarring.

Several clinical trials have compared topical NSAIDs to topical steroids for perioperative inflammation control in glaucoma surgery.[90,163,171–173] A recent meta-analysis synthesized the results from several of these smaller trials and determined that there was insufficient evidence to recommend one modality of inflammation control over the other.[174] When examining the individual studies of

this meta-analysis, most found topical NSAIDs to produce equivalent IOP outcomes to those attained with topical steroids.[90,163,171,175] However, in a randomized trial of 42 patients undergoing phacotrabeculectomy, patients receiving post-operative topical diclofenac required fewer glaucoma medications after surgery compared to those who received topical dexamethasone.[175] Another study reported a reduced likelihood of post-operative needling in a group of 54 patients who received topical ketorolac before trabeculectomy compared to the group receiving topical fluorometholone.[163] A further two studies found that NSAIDs after Ahmed glaucoma valve insertion lead to significantly lower post-operative IOPs – albeit with a higher risk of wound healing related complications such as hypotony and bleb leak.[90,173] Finally, we recently conducted a retrospective analysis of over 10,000 glaucoma surgery patients and found that those who filled a prescription during the perioperative period for a topical NSAID experienced significantly fewer interventions directed against bleb failure compared to patients who did not fill a prescription for an NSAID during the perioperative period.[67] These clinical findings suggest that NSAIDs and steroids exert differential effects on the quality of subconjunctival wound healing when given perioperatively for glaucoma surgery.

Steroids mitigate inflammation by blocking transcription of phospholipase A2[176] as well as the activity of the cyclooxygenase and lipoxygenase biosynthetic pathways, thereby suppressing the production of lipid-derived autacoids – of which the prostaglandins and leukotrienes are the best known pro-inflammatory examples.[177,178] However, steroids also abrogate synthesis of another two

classes of lipid-derived autacoids, synthesized by the lipoxygenase enzymes and known to actively resolve inflammation – the specialized pro-resolving mediators (SPMs) and the lipoxins.[17,179] Non-steroidal anti-inflammatory drugs (NSAIDs) are known to be very effective at reducing blood aqueous barrier breakdown and preventing cystoid macular edema after cataract surgery through inhibition of cyclooxygenase activity and prostaglandin synthesis.[180–182] NSAIDs do not significantly inhibit phospholipase A2 nor the lipoxygenase enzymes. This subtle difference in mechanism of action leaves the endogenous enzymatic machinery responsible for the resolution of inflammation intact – not the case after treatment with steroidal anti-inflammatories.

NSAIDs are not in routine use after glaucoma filtration surgery, and their effects relative to steroidal treatment on critical post-operative wound healing phenomena remain unknown. Two dimensional monolayer cell culture experiments have demonstrated the anti-proliferative effects of several NSAIDs to be greater than that of dexamethasone on human ocular fibroblasts.[95,183] However, their effects on ocular fibroblast mediated wound healing activity have yet to be compared within a three-dimensional collagen-based culture system. Such a culture system permits the study of cell-matrix interactions, allowing one to study the influence of experimental treatment on cell-mediated tissue contraction and matrix remodeling

Successful filtering surgery depends on the incomplete healing of the surgical wound and establishment of a controlled, chronic wound within the subconjunctival or superciliary tissues.[3,4] Therefore, it is of critical importance to

understand how these two classes of anti-inflammatory drug effect the wound healing functions of human ocular fibroblasts. The purpose of this study was to compare cell-mediated collagen contraction and remodeling in a three-dimensional collagen-based culture system.[184] Through macroscopic and microscopic assessment of the human Tenon's capsule fibroblast (HTCF) containing collagen matrices that are produced by this culture method, we present *in vitro* evidence, complementary to previous *in vivo* trials, for the differential effects of these drugs on collagen remodeling and wound healing phenomena orchestrated by HTCFs.

3.2 Methods

3.2.1 Isolation and Culture of HTCFs

This study followed the tenants of the Declaration of Helsinki and was approved by the office of Human Research Ethics at Western University (REB# 106783). Primary HTCF cell lines were derived from 2-4 mm^3 surgically resected segments of Tenon's capsule. After the acquisition of informed consent, tissue specimens were obtained from male and female glaucoma patients undergoing primary trabeculectomy at St. Josephs Hospital, London, Canada. The samples were obtained by ophthalmic surgeons who removed segments of Tenon's capsule and placed them into primary culture growth media containing Dulbecco modified Eagle's minimal essential medium (DMEM), 10% Fetal Bovine Serum (FBS), 1% penicillin/streptomycin, and 1% amphotericin, all from Sigma-Aldrich (Oakville, Canada). After, specimens were placed in fibronectin (Sigma-Aldrich) coated 6-well culture plates, submerged in primary culture media, and subcultured upon

confluency for further use. Cell cultures were used for experimentation prior to passage five. **Table 3-1** provides a summary of donor characteristics for the HTCF cell lines used in experiments.

Table 3-1: Donor information for the HTCF cell lines used for experimentation

Patient no.	Age/Sex	Glaucoma Diagnosis	Surgery	Glaucoma Medications (at time of surgery)
1	69/F	POAG	Trabeculectomy	Bimatoprost, Dorzolamide-timolol
2	78/M	POAG	Trabeculectomy	Brimonidine, Timolol, Latanoprost
3	71/M	POAG	Seton Implant	Bimatoprost, Brimonidine, Timolol
4*	63/F	OAG	Seton Implant	Timolol, Metoprolol
5	83/F	POAG	Trabeculectomy	Bimatoprost, Dorzolamide-timolol
6#,*	71/F	OAG	Trabeculectomy	Timolol, Metoprolol

#Histologically processed sections not available for fluorescent microscopy experiments; *Protein samples not available for western blot experiments; POAG: primary open-angle glaucoma; OAG: open-angle glaucoma

3.2.2 Collagen Contraction Assay

The delayed release fibroblast populated collagen lattice model,[185] based on the model first proposed by Bell,[186] was used to assess the effects of dexamethasone and indomethacin on HTCF-mediated gel contraction. In brief, HTCFs were mixed within an extracellular matrix (ECM) mixture containing 400µl of type I collagen (1.8mg/ml; A1048301, Gibco), 80µl of neutralizing solution (equal parts Waymouth media (Sigma-Aldrich) and 0.275M NaOH) and 20µl of HTCF conditioned media (concentrated to 25x to obtain a 1x final concentration within the 500µl construct volume) in order to achieve a final cell density of 2.5 x 10^5 cells/mL within the solution. Cell free collagen lattices were prepared identically, albeit without the inclusion of HTCFs, as negative control. The

solution was pipetted gently to ensure homogenous distribution of HTCFs while avoiding the production of air bubbles, then 500µl were pipetted into each well of a 24-well tray. Collagen constructs were allowed to polymerize at 37°C and 5% CO2 for 45 minutes before adding low serum culture media containing DMEM, 2% Fetal Bovine Serum (FBS), 1% penicillin/streptomycin.

After incubation at 37°C and 5% CO_2 for 72 hours, a sterile spatula was used to detach each collagen construct from the edges of the culture well. Plates were then immediately scanned on a flatbed laser scanner (Scanjet 8200, Hewlett-Packard) to record baseline area, and then every subsequent 24 hours for 7 days. The surface area of each collagen construct was measured using ImageJ[187] and standardized against the baseline surface area measurement to express changes in area as a percentage of original surface area.

3.2.3 Anti-Inflammatory Treatment Protocol

After release of collagen constructs from the sides of the culture wells, the media was removed twice daily (8am and 6pm) and replaced with one of the three treatment solutions for a duration of 15 minutes. This schedule continued from time of release for 7 days at which point the experiment concluded. Dexamethasone (0.05, 0.1 and 0.2% wt/vol in DMEM with dimethyl sulfoxide (DMSO: D2650, Sigma-Aldrich) 0.1% vol/vol), indomethacin (0.03, 0.1, 0.3 %wt/vol in DMEM with 0.1% DMSO) and vehicle control (DMSO, 0.1% vol/vol in DMEM) treatment solutions were prepared from purified form (dexamethasone: D4902; indomethacin: I8280, both from Sigma-Aldrich) and then filtered through a 0.2µm syringe filter to remove particulate and sterilize. These concentrations of

indomethacin and dexamethasone were chosen to mirror doses in clinically available topical preparations. After each treatment, the collagen constructs were washed with PBS to remove any remaining treatment solution and fresh culture media was added.

3.2.4 Extracellular Matrix Remodeling

Upon conclusion of the contraction assay, collagen constructs were fixed in 4% paraformaldehyde overnight. After fixation, collagen constructs were dehydrated in ethanol, embedded in paraffin blocks, sectioned (5µM) and mounted on glass microscope slides using standard methods. For histological staining, sections were deparaffinized and hydrated using standard protocols. Sections were stained with picrosirius red. Briefly, a solution of 0.1% Sirius red (Sigma-Aldrich) in saturated picric acid (Sigma-Aldrich) was applied for 60 min, followed by 2 x 0.5% acetic acid washes. Collagen birefringence, used to determine collagen fibrillar hue,[188] was assessed by circularly polarized light microscopy of picrosirius red stained sections. Images were taken with an Abrio quantitative birefringence imaging system (Hinds Instruments, Portland, Oregon) mounted on an Olympus BX-51 microscope (Olympus Corporation, Tokyo, Japan). Specifically, a constant light intensity, a fixed 45° angle to the polarizing filter, a 20x objective and the same analyzer were used to facilitate consistent comparisons between each sample.

When viewed under polarized light, the color of the collagen fibers stained with picrosirius red depends upon fiber thickness and spatial orientation; with the color changing from blue to yellow to orange to red as fiber thickness and density

increase.[189,190] This method has been used previously in rabbit experimental filtration surgery to examine subconjunctival fibrosis, and red/orange staining was associated with bleb dysfunction.[105] Using ImageJ, this property was leveraged to determine the relative proportions of different color fibers within the collagen constructs. This quantitative method has been previously described by Rich and Whittaker.[191] In short, relative color content of the images is obtained by separating the digital images into their hue, saturation and value components. The hue component contains information on the color of each pixel within the image. Every pixel can have one of 256 possible colors. To identify the red, orange, yellow and blue pixels within a given image the following hue definitions were used within ImageJ: red 2-9 and 230-256, orange 10-38, yellow 39-51 and blue 52-128.[191] The number of pixels within each hue range is calculated and expressed as a proportion of the total number of pixels within each field representing collagen. Ten random frames using a 20x objective were taken per tissue section, with three tissue sections imaged per patient cell line and treatment group.

3.2.5 Fluorescent Microscopy

Collagen constructs were cast with equal cell density, therefore there should be equal variance in cell density between treatment groups after the 7-day incubation period. This was assessed through fluorescent microscopy. Briefly, deparaffinized and hydrated sections were permeabilized for 30 minutes with 1% Triton X-100 (Sigma-Aldrich) in PBS (Sigma-Aldrich). Slides were then stained with 4,6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich) for 10 minutes and

imaged with a laser-scanning confocal microscope (A1R HD; Nikon Instruments Inc., Tokyo, Japan). Relative cell density was determined by cell (nucleus) count standardized to area of collagen autofluorescence (in pixels) within each section and measured using ImageJ. Ten random frames were taken with the 40x objective per tissue section, with three tissue sections imaged per patient cell line and treatment group. The laser intensity settings were kept consistent between slides to facilitate consistent comparison between replicates.

Expression of the contractile protein alpha smooth muscle actin (αSMA) by HTCFs within collagen constructs was assessed through immunohistochemistry. Deparaffinized and hydrated sections were permeabilized for 30 minutes with 1% Triton X-100 in PBS. After blocking of nonspecific sites with 1% BSA in PBS, sections were incubated for 40 minutes with Alexa Fluor 488–conjugated primary antibody against αSMA (Abcam, ab202295). Finally, slides were stained with DAPI for 10 minutes. For each tissue section, ImageJ was used to measure the total area staining positive for αSMA. This value was then normalized to the total number of nuclei counted within that same frame and compared between treatment groups. Since fibroblasts and myofibroblasts are mononuclear this approximates the αSMA expression per cell. Ten random frames were taken with the 40x objective per tissue section, with three tissue sections imaged per patient cell line and treatment group. The laser intensity settings were kept consistent between slides to facilitate consistent comparison between replicates. Thresholding of images to minimize background autofluorescence of the collagen

matrix was accomplished using identical cut off values for each laser channel and image analyzed prior to analysis.

3.2.6 Cell Culture and Western Blot

HTCFs were grown in 6-well culture plates in DMEM with 10% FBS at 37°C and 5% CO_2 until 80-90% confluent. The cultures were then starved of serum for 24hrs, after which they were treated with vehicle (DMEM), TGFβ1 (2ng/ml, Sigma-Aldrich) or TGFβ1 co-incubated with one of the experimental anti-inflammatory treatment solutions. After 48hrs incubation, western blot was used to assess relative protein expression between the experimental treatment groups. Briefly, cells were lysed in lysis buffer (PhosphoSafe Extraction Reagent, Novagen) containing a protease inhibitor cocktail (P2714, Sigma-Aldrich) and the crude protein lysate (10µg) was resolved using a Novex WedgeWell 4-20% tris-glycine gel (Invitrogen). Using an iBlot Gel Transfer Device (IB1001, Invitrogen), the separated protein was transferred to a nitrocellulose membrane (IB301001, iBlot Transfer Stack, Invitrogen) which was then blocked with 5% (*w/v*) bovine serum albumin (Sigma- Aldrich) in Tris buffered saline (TBST) for 1 hour at room temperature. The membranes were incubated overnight at 4°C with primary antibody diluted in TBST containing 5% BSA (w/vol). Primary antibodies used were as follows: collagen 1 (ab138492, Abcam), αSMA (ab5694, Abcam) and GAPDH (Santa Cruz Biotechnology, Inc.). After incubation with primary antibodies, the blots were washed and hybridized with 1:3000 (*v/v*) dilutions of goat anti-rabbit IgG conjugated with horseradish peroxidase (Santa Cruz Biotechnology, Inc.). Visualization was accomplished by applying WesternBright

Quantum chemiluminescent reagent (Advansta, Inc.), with GAPDH used as a protein loading control. Imaging and relative densiometric quantification was accomplished using a ChemiDoc MP System (Bio-Rad Laboratories, Inc.) connected to Image Lab (Version 6, Bio-Rad Laboratories, Inc.).

3.2.7 Statistical Analysis

All data are presented as means ± SEM. For reporting purposes, all experimental replicates using multiple cell lines are denoted using 'N = *x'*, and the number of experimental repeats performed using each cell line will be denoted using 'n = *y*'. Collagen contraction assay data were subject to two-way analysis of variance followed by, if necessary, the Tukey-Kramer test with use of statistical and graphing software Prism 7 (Version 7.03, GraphPad Software Inc.). For collagen remodelling assay data, the relative area of collagen stained blue, yellow, orange or red, under different treatment conditions were assessed by one-way ANOVA with Dunnett's multiple comparisons *post hoc* test with a single pooled variance, if necessary. The same methods were used for western blot and fluorescent microscopy data. For all experiments, a *P* value of less than 0.05 was considered statistically significant.

3.3 Results

3.3.1 Collagen Contraction Assay

An *in vitro* assay utilizing HTCFs seeded within delayed-release collagen matrices was employed to measure the effects of drug exposures on HTCF mediated collagen contraction. Compared to vehicle control, exposure to all tested indomethacin and dexamethasone treatment solutions (except dex 0.05%

at all timepoints) significantly inhibited collagen contraction over the seven-day incubation period (**Table 3-2**). Tukey-Kramer multiple comparisons test indicated that 0.3% wt/vol. indomethacin inhibited HTCF mediated collagen contraction to a significantly greater degree than 0.05% wt/vol. dexamethasone at all timepoints (Figure 3-1, $N = 6$, $n = 6$, $p<0.05$). Indomethacin 0.1% wt/vol. also inhibited contraction to a significantly greater degree than 0.05% wt/vol. dexamethasone at all timepoints ($N = 6$, $n = 6$, $p<0.05$) except on day six where the *P* value was 0.07.

Table 3-2: Fibroblast-mediated collagen contraction (% baseline area, mean and SD, N = 6, n = 6)

	Vehicle		Dex 0.05%		Dex 0.1%		Dex 0.2%		Indo 0.03%		Indo 0.1%		Indo 0.3%	
Day	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	100.0	0.0	100.0	0.0	100	0	100	0	100.0	0.0	100.0	0.0	100.0	0.0
1	55.1	13.4	62.8	12.3	67.3	11.1	*73.0	13.3	69.4	11.2	*79.4	16.1	*82.7	14.0
2	50.0	13.8	59.8	12.7	65.8	12.3	*70.3	15.1	*69.2	11.6	*76.2	17.5	*80.8	14.7
3	46.1	14.8	55.4	13.2	*64.1	13.2	*68.7	13.8	*68.0	12.2	*73.3	18.6	*77.2	14.0
4	43.5	14.2	54.0	14.4	*62.9	14.6	*67.0	15.3	*69.2	13.0	*70.5	18.1	*75.3	15.3
5	40.8	14.1	52.8	14.6	*60.7	15.4	*63.8	15.6	*68.0	13.3	*69.1	20.3	*73.7	15.6
6	38.8	14.5	52.8	16.1	*60.1	15.9	*64.0	16.2	*65.0	14.1	*67.9	22.6	*72.1	15.9
7	37.1	13.8	51.7	15.8	*60.0	17.4	*64.6	17	*63.3	14.9	*68.8	16.4	*70.2	22.6

*$p < 0.05$ vs. vehicle control

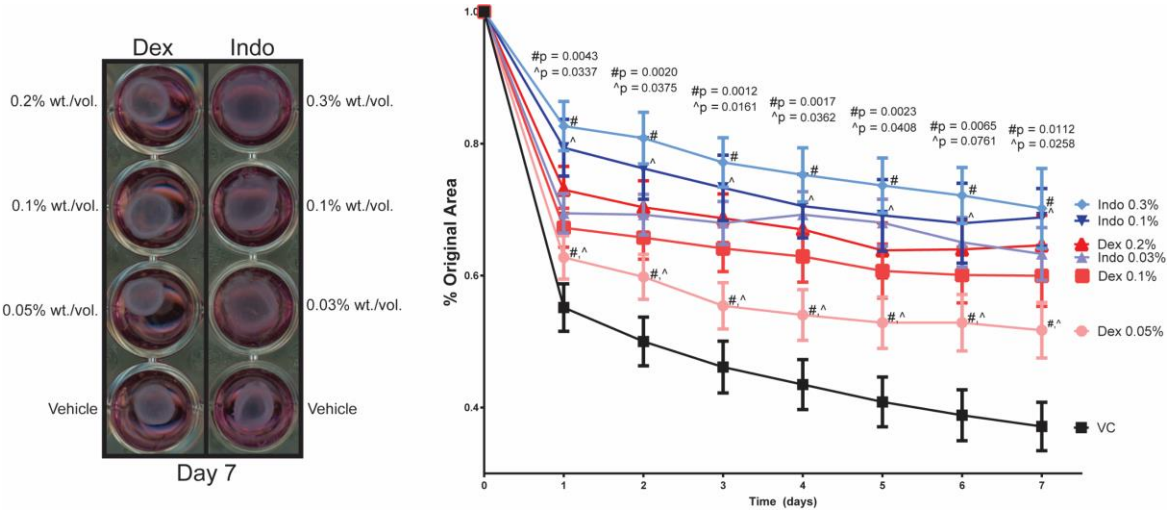


Figure 3-1: Scan of HTCF-collagen matrices at experiment conclusion and graph illustrating the effects of indomethacin (blue lines: 0.03, 0.1, 0.3% w/vol.) or dexamethasone (red lines: 0.05, 0.1, 0.2% w/vol.) on the contraction of HTCF-populated collagen lattices compared to vehicle treated (black line). A Tukey-Kramer multiple comparisons test demonstrated that indomethacin dosed at 0.3% and 0.1% had a significantly greater inhibitory effect on contraction than 0.05% dexamethasone at the indicated time points. Data shown are mean ± SEM (N = 6, n=6, #indicates significance level for Indo 0.3% vs. Dex 0.05%, ^indicates significance level for Indo 0.1% vs. Dex 0.05%).

3.3.2 Extracellular Matrix Remodeling

Picrosirius red staining was used in combination with circularly polarized light as a highly sensitive means to visualize collagen fibers. Fibrillar hue was used to assess structural changes induced by HTCFs in the collagen matrix compared to cell free matrices (Figure 3-2). Cell free matrices, incubated for seven days under identical experimental conditions, contained significantly greater proportions of blue staining compared to constructs containing HTCFs, which revealed a predominance of densely packed, mature red/orange staining collagen fibers (Figure 3-2). These changes are inferred to be cell mediated, structural changes to the collagen matrix.

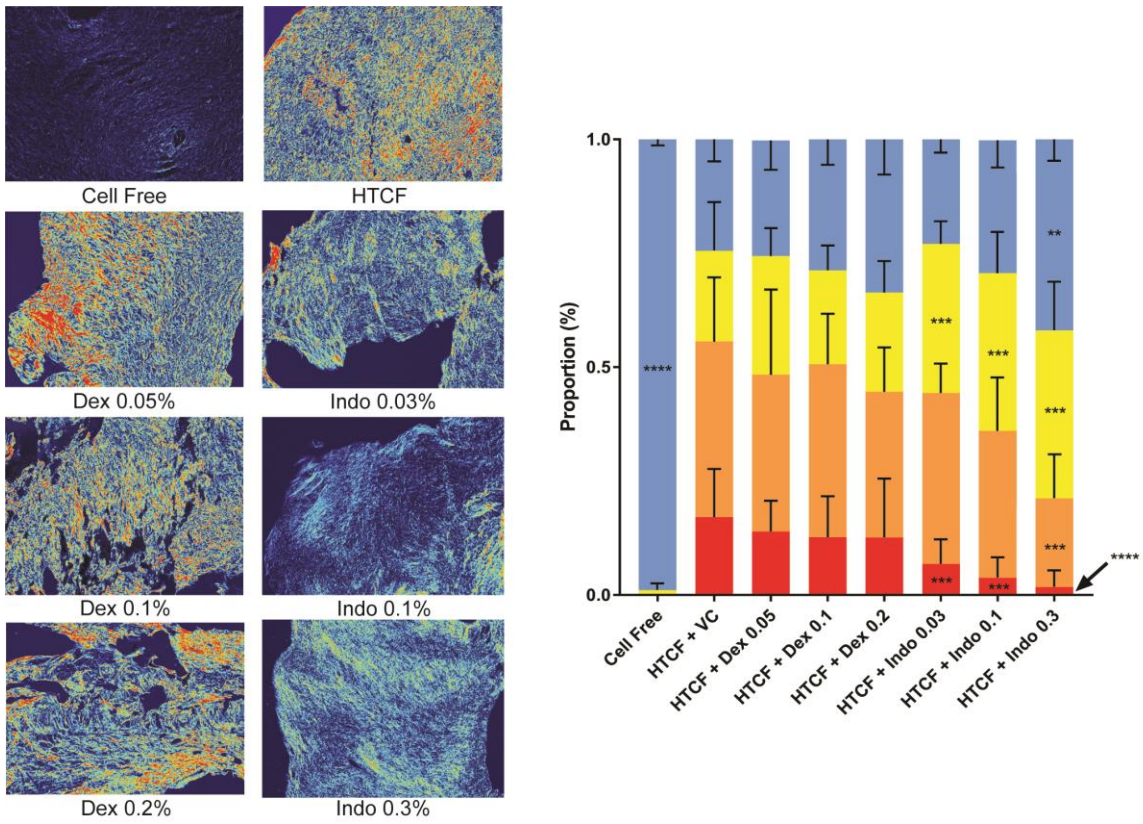


Figure 3-2: Picrosirius red and polarized light microscopy enabled assessment of cell-mediated collagen remodeling. Collagen matrices were prepared with and without HTCFs then cultured under normal conditions for two weeks, fixed, sectioned and stained with picrosirius red. HTCF remodeling activity on the collagen matrix is revealed by comparing cell free matrices, which stain uniformly blue, to those cultured with HTCFs. Those cultured with HTCFs exhibit regions staining yellow, orange and red where cell-mediated matrix modifications are self-evident. This remodeling activity was semi-quantitatively assessed in the presence of steroidal (dexamethasone) and non-steroidal (indomethacin) anti-inflammatory drugs by comparing the mean relative color content of each image within a treatment group and using one-way ANOVA with Dunnett's Multiple comparisons test (N = 6, n = 3, five random high powered frames at 20x magnification imaged per tissue section; **p<0.03, ***p<0.01, ****p<0.001 vs. HTCF+VC experimental group).

The effects of anti-inflammatory exposure on this remodeling activity was assessed (Figure 3-2). Sections of dexamethasone treated constructs displayed a similar fibrillar hue composition at all treatment doses compared to vehicle treated sections (N = 6, n = 3, $p>0.05$). Indomethacin treated sections exhibited significantly reduced red staining compared to vehicle treated sections and sections treated with any dose of dexamethasone. Indomethacin treated sections exhibited significantly greater areas of blue/yellow staining collagen fibrils compared to vehicle and dexamethasone treated sections.

3.3.3 Fluorescent Microscopy

The number of DAPI stained nuclei per unit area of collagen matrix was calculated to evaluate the final number of HTCFs within the collagen constructs after seven days of experimental conditions. The effects of anti-inflammatory treatment on final cell number are illustrated in Figure 3-3. Dexamethasone had no significant effect compared to vehicle control on the density of HTCFs within the collagen matrix at any concentration. The two highest concentrations of indomethacin (0.1 and 0.3%) significantly reduced the number of HTCFs per unit area of matrix after seven days of experimental conditions compared to vehicle control. Expression of αSMA by HTCFs within the collagen constructs was assessed using immunohistochemistry. Those treated with 0.2% dexamethasone as well as those incubated with 0.1 and 0.3% indomethacin showed significantly reduced αSMA staining per nuclei compared to vehicle control (Figure 3-3).

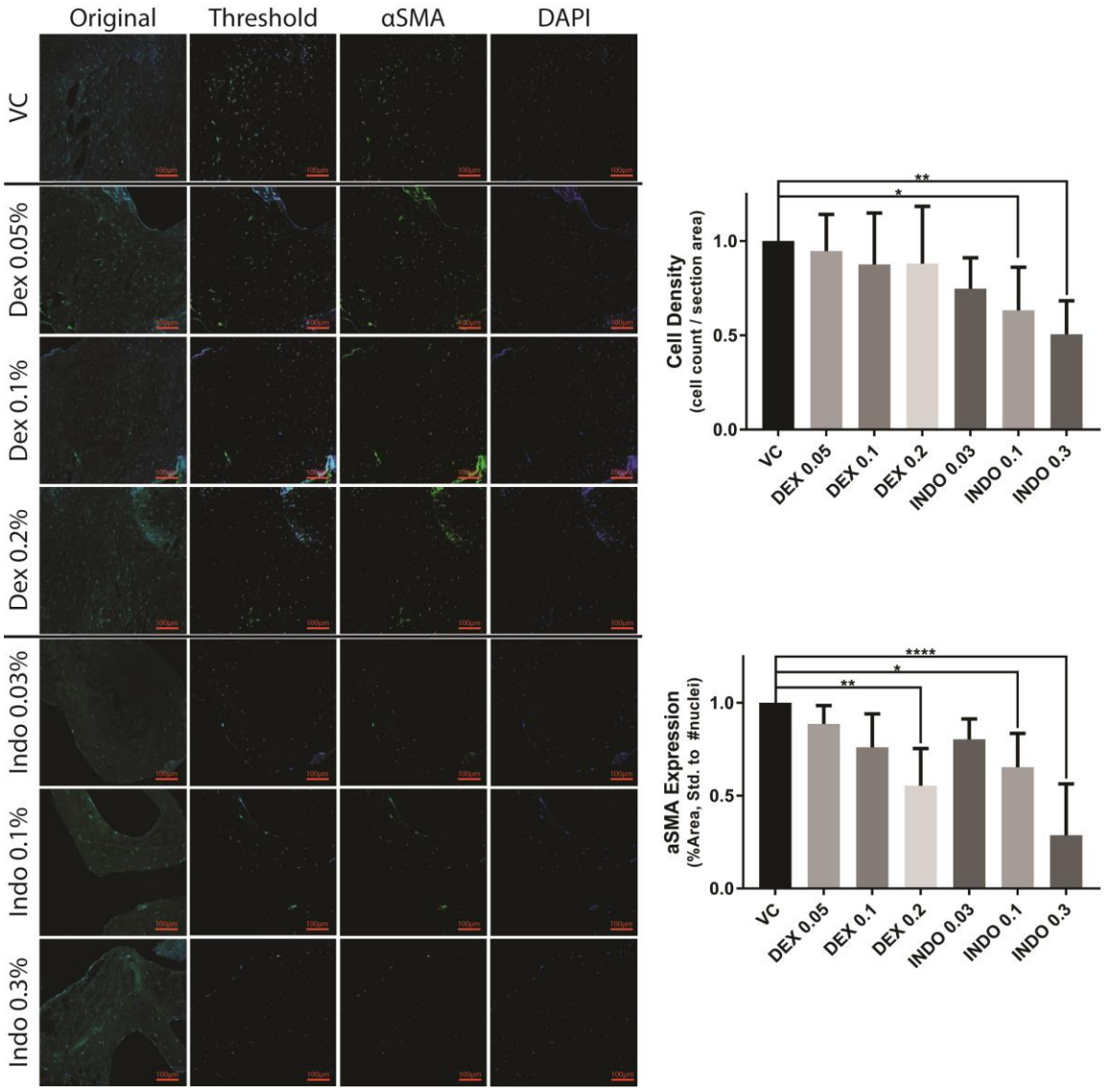


Figure 3-3 Representative confocal laser scanning microscope images for each treatment group. Cellular density was calculated as the number of nuclei (blue) standardized to collagen matrix area (background collagen autofluorescence – isolated by thresholding) within each given frame. Only indomethacin significantly affected cellular density relative to vehicle control (N = 5, n = 3, *p<0.05, **p<0.01). αSMA expression (green) within HTCF collagen constructs was calculated as the area of αSMA staining (green) standardized to the number of nuclei stained with DAPI (blue) within each given frame. Dexamethasone 0.2% and indomethacin 0.1 and 0.3% significantly reduced the area of αSMA staining per nuclei compared to vehicle control (N = 5, n = 3, *p<0.05, **p<0.01, ****p<0.0001).

3.3.4 Western Blot

Western blot analysis of crude protein lysate revealed that, after 48hrs of culture, indomethacin significantly impaired TGFβ1-induced collagen 1 and αSMA expression such that it resembled the expression level of TGFβ1 naïve cells at all concentrations tested (Figure 3-4, N = 4, *p<0.05, **p<0.01). Dexamethasone exhibited a dose dependent impairment of TGFβ1-induced collagen 1 expression, however this trend only reached statistical significance relative to the TGFβ1 control group at the highest concentration tested. The inhibition of αSMA observed with dexamethasone was statistically significant in the 0.1 and 0.2% (w/vol) treatment groups (Figure 3-4, N = 4, *p<0.05, **p<0.01, ***p<0.001).

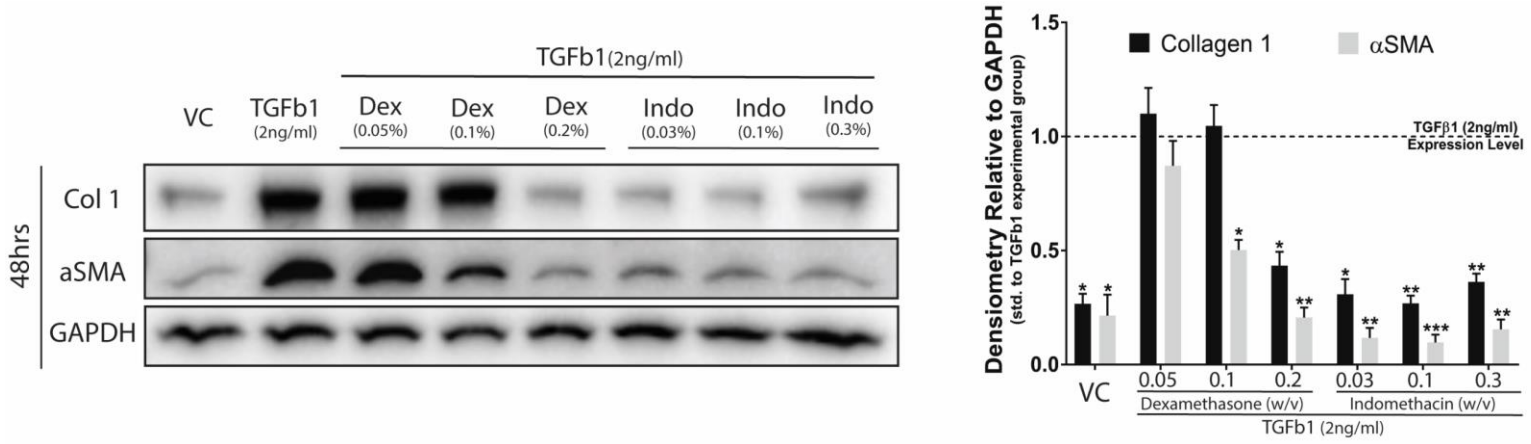


Figure 3-4 Effects of anti-inflammatory treatment on TGFβ1-induced expression of collagen 1 and αSMA. HTCFs were cultured for 48hrs with one of: 1) vehicle control (DMEM with 0.1% DMSO), 2) TGFβ-1 (2ng/ml), 3) TGFβ-1 (2ng/ml) with dexamethasone (0.05, 0.1 and 0.2% (w/vol) in DMEM with 0.1% DMSO) or indomethacin (0.03, 0.1 and 0.3% (w/vol) in DMEM with 0.1% DMSO). Representative western blot with densiometric analysis of multiple cell lines reported as mean ± SEM (N = 4, *p<0.05, **p<0.01, ***p<0.001).

3.4 Discussion

Tissues undergo repair in three stages: inflammation, proliferation and remodeling. Normal tissue repair requires an orderly and efficient transition between these stages and events that disrupt these transitions, such as pre-existing chronic inflammation due to a patient's medication burden or the novel interaction of aqueous humor with soft tissues at the surgical site, can cause scarring and fibrosis. This complication is responsible for the majority of glaucoma filtration surgery failures. Pharmacologic suppression of the inflammatory response and modulation of fibroblast activity are thus essential to the success rate of glaucoma filtering surgery.

Myofibroblast mediated tissue contraction is believed to contribute to bleb encapsulation and a loss of patency in the surgical fistula that connects the AC to the drainage structure, both of which can limit the structure's outflow capacity.[3,4,192] Histological specimens of Tenon's capsule obtained from failed trabeculectomy blebs have been compared to control specimens obtained from patients in surgically naïve eyes undergoing filtration surgery, retinal reattachment or cataract surgery.[193] These control sections demonstrate loose connective tissue with few cellular infiltrates, and few cells positive for αSMA. In contrast, sections from failed filtration structures tend to demonstrate αSMA positive cell-rich mesenchymal proliferations containing densely packed collagen fibrils which stain predominantly red/orange with picrosirius red.[105,115,117,192–194]

The intent of the presented *in* vitro model was to mimic the *in vivo* exposure patient subconjunctival tissues might experience after topical application of

steroid (dexamethasone) or NSAID (indomethacin) post-operatively. Collagen constructs were immersed in treatment solutions twice daily for 15 minutes after detachment of collagen constructs from culture wells, similar to the topical application of medications after surgical tissue manipulation. Both dexamethasone and indomethacin significantly inhibited HTCF-mediated collagen contraction compared to control, with indomethacin exhibiting significantly greater inhibition than dexamethasone at the extremes of the doses assessed. Expression of the myofibroblast marker and contractile protein, αSMA, is necessary for cell mediated tissue contraction to occur.[112,195] Immunohistochemical staining demonstrated that both drugs had an inhibitory effect on the expression of αSMA, however this effect was again slightly greater with higher doses of indomethacin compared to dexamethasone. The effects of treatment solutions on TGFβ1-induced αSMA expression was also assessed through western blot. These results were corroborative of the trends seen in the immunohistochemical experiments. These findings suggest that both drugs may function *in vivo* to prevent decreases in outflow capacity due to the contraction of tissues at or around the filtration site, however at the level of the fibroblast, NSAIDs may better impair collagen contraction and associated biomarkers.

Picrosirius red staining revealed that only indomethacin significantly mitigated HTCF-mediated collagen remodeling. Western blot after monolayer cell culture revealed significant inhibition of TGFβ1-induced collagen 1 expression at all tested indomethacin concentrations and certain dexamethasone concentrations. The observed trend in collagen 1 expression is supportive of the trend seen in the

picrosirius collagen remodeling assay. Indomethacin's effects on collagen remodeling may further be explained by the observation that treatment with indomethacin significantly reduced the number of cells within collagen constructs at experimental conclusion leaving fewer to remodel the matrix relative to the control and dexamethasone groups. These observations are further strengthened by previous monolayer cell culture experiments comparing the antiproliferative effects of NSAIDs to that of steroids. NSAIDs showed comparable, or better, anti-proliferative effects on ocular fibroblasts than did steroids.[95] Another possible explanation comes from the work of Sakaki et al, who cultured human gingival fibroblasts and found that indomethacin reduced expression of matrix metalloproteinases (MMPs).[196] MMPs play an important role in extracellular matrix remodeling and tissue repair, and MMP inhibitors have previously reduced scarring outcomes in an *in vitro* model similar to the one presented here.[125] Further, dexamethasone was previously demonstrated to decrease glycosaminoglycan synthesis in cultured fibroblasts.[197,198] Glycosaminoglycans increase the hydration capacity of collagen matrixes, permitting looser arrangement of fibers,[199] thus providing further explanation for the denser matrices produced by HTCFs when exposed to dexamethasone above that provided by our collagen 1 western blot results. Further work is necessary to characterize the cellular mechanisms underpinning the significant differential effect these anti-inflammatory modalities exert on HTCF-mediated collagen remodeling.

In the present study, the combined anti-contractile and anti-remodeling effects of indomethacin, in addition to its effects on cellular density, collagen 1 and αSMA expression, suggest a capacity to mitigate many of the histopathological findings associated with failed filtration blebs. Several previous glaucoma surgical trials have compared perioperative treatment with NSAIDs to steroids and assessed the impact on surgical outcomes.[90,163,173,175,200] Patients treated post-operatively with NSAIDs exhibited a clinical tendency to do better than those treated with steroidal anti-inflammatory modalities in terms of the number of post-operative glaucoma medications required, final IOP and bleb morphology.[175] Favorable bleb morphology with NSAID treatment in a clinical setting may be analogous to the *in vitro* effects NSAIDs exhibited on HTCF-mediated collagen contraction and remodeling in the present study.

Two previous studies evaluated NSAID versus steroid treatment after Ahmed valve implantation and found a more favorable post-op IOP reduction with NSAID treatment.[90,173] However, complications relating to inadequate healing (such as wound leak, hypotony, conjunctival retractions, etc.) were observed at higher rates in the NSAID arm of one study.[90] These findings support the conclusion that indomethacin may better impede collagen remodeling orchestrated by HTCFs. Remodeling, however, is an essential aspect of healthy wound healing and critical, to a degree, in order to prevent bleb leaks, infection and hypotony. This highlights the importance of modulating the existing positive and negative wound healing stimuli that may influence the activity of HTCFs and, ultimately, surgical outcomes. The glaucoma surgeon and patient will benefit from the understanding

that NSAIDs and steroids are both effective at mitigating wound healing during the post-operative phase. However, understanding the subtle differences in efficacy and cellular mechanisms of action will allow for greater control of the maturing bleb *in vivo*, allowing for finer adjustments to bleb maturation in response to post-operative clinical observations.

Most of our current understanding of cellular functions such as migration, differentiation and reaction to extra-cellular forces has been derived from studying cells in two-dimensional monolayer cultures.[184] *In vivo*, wound healing and pathological scarring processes are a complex interaction between cells and the extracellular matrix – the outcome of which is largely dependent on the immediate microenvironment. The presented 3D collagen-based culture model enables assessment of cell-mediated changes in collagen architecture as well as differences in markers of cellular phenotype.[201] It also accounts for the three-dimensional cell-matrix interactions and signal transduction pathways, present *in vivo*, that are unaccounted for in two-dimensional monolayer culture models.[184] Our conclusions are strengthened by the complementary nature of the macroscopic collagen contraction assay and the microscopic assessment of collagen architecture as well as cellular morphology and protein expression.

A limitation of this *in vitro* model is the absence of inflammatory cells and immune system. The infiltration of immune cells is thought to contribute to the failure of glaucoma filtration surgery.[71] It is highly likely that the anti-inflammatory properties of steroids and NSAIDs act to limit the effects of these cells *in vivo* as well, which in turn might diminish any negative impact on local fibroblast activity.

Future studies could be undertaken incorporating inflammatory cells within a co-culture system to investigate the impact these drugs have on HTCF wound healing activities in the presence of immune cells.

3.5 Conclusions

In conclusion, both steroidal and non-steroidal anti-inflammatory treatment can influence HTCF-mediated collagen contraction and αSMA expression. Indomethacin alone was observed to mitigate HTCF-mediated changes in collagen remodeling. This may be the result of different intracellular secondary messengers being stimulated by the two drugs or a difference in relative potency at the clinically available concentrations tested. These findings support the use of this *in vitro* model to help understand observations from clinical trials involving *ab externo* glaucoma surgery. Further, this model may be useful to investigate the effects of other wound modulating agents, as well as the influence of surgically implanted minimally invasive glaucoma (MIGS) devices. Given the rapidity and frequency by which MIGS devices are emerging, this may be useful in providing evidence to help optimize the success rates of implanted devices and peri-operative wound management practices.

Chapter 4

4 Acetylsalicylic Acid Mitigates Cytokine Induced Myofibroblast Transdifferentiation and Activity within Human Ocular Fibroblasts

Given the significant inhibitory effects on wound healing phenomena that are observed with competitive COX inhibition, acetylsalicylic acid (ASA) and its unique covalent inhibition of COX enzymes and PG production struck me as potentially more powerful than what could be achieved with competitive inhibition. Delving deeper, I learned of the relatively recent finding that ASA-acetylated COX2 remains functional *in situ* – with the acetyl-COX2 enzyme now capable of producing RMs from PUFA precursors, but not PGs. The ability to quickly and simply acetylate this enzymatic engine of inflammation propagation into an engine of resolving mediator production has the potential to be an immuno-resolvent intervention. Chapter 5 pursues this idea and examines the effects of ASA on RM generation, and the effects these ASA-triggered RMs exert on the *in vitro* wound healing phenomena of subconjunctival fibroblasts.

4.1 Introduction

Glaucoma represents a group of conditions involving progressive damage to the optic nerve and subsequent vision loss. Glaucoma is one of the leading causes of irreversible blindness, affecting over 70 million people across the world.[202] There is currently no cure, and the only known modifiable risk factor is the reduction of intraocular pressure (IOP). Patients are prescribed medications to control their intraocular pressure. However, due to physiological tolerance to the medications, as well as poor treatment compliance, patients may undergo trabeculectomy.[203] Trabeculectomy, also known as filtration surgery, involves the creation of a pocket under the conjunctiva called a filtering bleb. Excess aqueous humor drains into this bleb, thus lowering the intraocular pressure. Filtration surgery may be considered the gold standard surgical intervention for refractory glaucoma patients.[202] However, the surgical failure rate is high: with a 10% failure rate per year and up to a 50% failure rate in 5 years. This rate may be due to a fibroproliferative response in the trabecular meshwork, the fistula between the anterior chamber and the bleb, and/or the Tenon's capsule, leading to subconjunctival scarring after surgery and increased resistance to aqueous outflow.[3,4]

The Tenon's capsule is a collagen rich layer of connective tissue that lies under the conjunctiva. This region contains fibroblasts; cells that transdifferentiate into myofibroblasts,[1] which function in wound healing by producing extracellular matrix and contractile force.[32,204] Under normal physiological conditions, the Tenon's capsule does not encounter aqueous humor. However, after filtration

surgery, there is an influx of aqueous humor to this region of the eye. Numerous cytokines are present in the aqueous humor, such as transforming growth factor beta 1 (TGFβ1) and TGFβ2,[86,205] tumor necrosis factor-alpha (TNFα), interferon-gamma (IFN-γ), and interleukin-1b (IL-1β).[85,206,207] These inflammatory and wound healing molecules function at the level of the fibroblast to promote wound healing process, both directly and vicariously through immune cell recruitment. These processes are required to some degree to avoid bleb leaks. However, if the inflammatory phase of the wound healing response fails to resolve and re-establish homeostasis in the eye, chronic inflammation ensues.[16,20,202] The TGFβ isoforms are well-established as one of the main drivers of scarring after glaucoma surgery.[208–210] Myofibroblasts within the subconjunctival and Tenon's capsule tissues of failing glaucoma surgeries display excess proliferation, wound contraction, and extracellular matrix protein production.[1,4,211] These cellular processes ultimately result in subconjunctival scar formation, increased resistance to aqueous outflow and failure of IOP control.

Within the pro-inflammatory microenvironment of the filtration bleb, phospholipids in the plasma membrane of HTCFs are converted to arachidonic acid by phospholipase.[12] Cyclooxygenase (COX) enzymes convert arachidonic acid into pro-inflammatory mediators such as prostaglandins, which can recruit inflammatory cells and subsequently vicariously activate local fibroblasts such that they express fibrosis associated proteins such as alpha-smooth muscle actin (α-SMA) and matrix metalloproteinases (MMPs).[33,34] α-SMA is a structural protein involved in scar contraction during the wound healing process. MMPs encompass

a group of enzymes that degrade extracellular matrix and are involved in collagen remodeling in wound healing.[212] Previous work has demonstrated the involvement of MMP9 in fibrosis after glaucoma surgery[125,127,213] and MMP9 is also upregulated during inflammatory processes.[214] A healthy wound healing response involves the build-up of pro-inflammatory mediators within the fibroblast,[16,215] which triggers the activity of lipoxygenases (LOX).[35,216] The LOX enzymes are the group enzymes whose actions produce most pro-resolving lipid mediators, which then function to ultimately resolve inflammatory processes.[17,20,22]

Anti-scarring agents in current use after glaucoma surgery include mitomycin C — an antimetabolite that inhibits cell proliferation[217] — and corticosteroids — broad-acting anti-inflammatory agents.[65,218,219] However, these options have had limited success. Mitomycin C may increase patients' risk of developing cataracts[220], infection and hypotony.[100,221] It is dosed empirically and has a very narrow therapeutic range. As well, the use of corticosteroids have many adverse effects, such as, dramatically increasing intraocular pressure in a subset of patients, infection and cataract.[69,97,222] Therefore, the efficacy of other agents needs to be explored.

As an alternative, nonsteroidal anti-inflammatory drugs (NSAIDs) have shown promise in a large retrospective study,[67] *in vitro*[223] and in a recent meta-analysis of several small clinical trials.[174] Acetylsalicylic acid (ASA), is unique among all NSAIDs in that it does not competitively inhibit the COX2 enzyme. Instead, it acetylates COX2 at Ser516 and changes its function.[224,225] The acetylated COX2

enzyme no longer produces prostaglandins, but instead produces pro-resolving lipid mediators akin to those produced by the LOX enzymes. Examples of ASA-triggered lipid mediators include: 5*R*-hydroxyeicosatetraenoic acid (HETE), 15(R)-HETE[226–228], 18-hydroxyeicosapentaenoic acid (HEPE)[229] and 17*R*-hydroxy docosahexaenoic acid (OHDHA).[6,230–232] Thus, ASA facilitates the transition within cells from the pro-inflammatory biolipid synthesis pathway to the pro-resolving biolipid synthesis pathway by triggering the production of pro-resolving lipid mediators from COX2 at the same time as mitigating PG synthesis from the same enzyme. The downstream effects of ASA on TGFβ1-induced myofibroblastic changes in human Tenon's capsule fibroblasts are unknown. In this study, we hypothesize that ASA will decrease TGFβ1-induced cell metabolic activity and protein expression in HTCFs through the induction of pro-resolving lipid mediators.

4.2 Materials and Methods

4.2.1 Isolation and Culture of Human Tenon's Capsule Fibroblasts

This study was approved by the Human Research Ethics board at Western University (REB# 106783). Human Tenon's capsule tissue, 2-4 mm^3 in size, were resected from patients at St. Joseph's Hospital, London, Canada. Specimens were placed in a 1.5 mL microtube containing Dulbecco's Modified Eagle Medium (DMEM) with 10% (*v/v*) Fetal Bovine Serum (FBS) and 1% (*v/v*) Penicillin and Streptomycin (PS), then placed into culture plates coated with fibronectin (Sigma-Aldrich, USA) at 37℃, 5% CO_2. Cells from outgrowth were passaged prior to reaching 80% confluence and all experiments were conducted with cells that

were passaged fewer than 5 times. Donor information for the cell lines used in experimentation are listed in **Table 3-1**.

HTCFs were cultured in separate 75 cm^2 flask in DMEM with 10% FBS and 1% PS (Gibco, Thermo Fisher Scientific, Canada). For all experiments, cells were seeded at 10^5 cells/mL in 10% FBS-1% PS DMEM for 24 h or until 80% confluence was reached. Next, cells were serum-starved for 24h. Vehicle control HTCFs were treated with 0% FBS-1% PS DMEM and positive control HTCFs was treated with 2 ng/mL of TGFβ1 (Thermo Fisher Scientific, Canada) in 0% FBS-1% PS DMEM. To assess the effects of ASA (Thermo Fisher Scientific, Canada) on HTCFs under non-inflammatory conditions, 3200 µg/mL ASA was fully dissolved for 15 minutes in 0% FBS DMEM at 37℃, then serially diluted by a factor of 2 to the lowest concentration of 100 µg/mL ASA, obtaining a total of seven ASA concentrations. To assess the effects of ASA on TGFβ1-induced HTCFs, cells were treated with the same set of ASA concentrations co-incubated with 2 ng/mL of TGFβ1. Plates were incubated for 48 h at 37℃ and 5% CO_2 prior to conducting MTT/LDH assays, western blot, and immunohistochemistry.

4.2.2 Lipid Mediator Secretion Assay

Relative quantification of secreted pro-inflammatory and pro-resolving lipid mediators was achieved using liquid chromatography tandem mass spectrometry (LC-MS/MS). Specifically, we focused our analyses on the COX2 derived lipid mediators, kPGF1a and PGE2; as well as the acetyl-COX2 derived mediators, 5-HETE, 15-HETE, 17-OHDHA and 18-HEPE; and their PUFA precursors, AA, DHA and EPA. Known quantities of deuterated internal standards of the analyzed

the lipid mediators were added to each sample to enable relative quantification between samples (Item Numbers: #10007737, #320110, #314010, #11182, #315210, #319030, #390030, #10006410, #10006199, #334230, and #10008040, Cayman Chemical). This is a well-established method to determine the relative activity of lipid biosynthetic pathways [233,234]. In brief, the ratios of the integrated areas of the chromatographic peaks corresponding to each analyte and the integrated areas of the peaks corresponding to each analyte's internal standard (with known absolute quantity) are used to determine the relative quantity of each analyte in a given sample. After supernatant sample collection, 250µl of methanol containing 100pg of each deuterated internal standard was added. Samples were then vigorously vortexed and centrifuged at 10,000g for 10min before loading onto solid-phase extraction cartridges (Strata-X 33um Polymeric Reversed Phase, 10mg, Phenomenex 8B-S100-AAK), that were previously activated with 2ml methanol and rinsed with 2ml water. Samples were diluted upon loading so that the final concentration of methanol was between 10 and 15% of total volume. After washing with 5ml water, extracts were eluted with 1ml methanol. Solvent was then evaporated under vacuum in a SpeedVac centrifuge, and the extract was resuspended in 100µl acetonitrile/water 60:40 (v/v). An aliquot of 20µl was injected into the LC-MS/MS system for analysis. A Sciex ExionLC Integrated System was used with a 0.3ml/min flow rate, initial mobile phase of 10% water / 0.1% formic acid followed by 100% acetonitrile / 0.1% formic acid on a Kinetex 2.6um C18 100 Å 100x2.1mm, Phenomax column (OOD-4462-AN). A Sciex Triple Quad 6500+ mass spectrometer with multiple reaction monitoring was used in negative ion mode. The chromatographic profile

of the ion count for each *m/z* transition was monitored, and the area under the peaks (ion intensity vs elution time) was integrated using commercial software (MultiQuant, Sciex). Total cellular protein concentration was used for normalization of the supernatant samples.

4.2.3 Collagen Contraction Assay

The delayed release fibroblast populated collagen lattice model,[185] based on the model first proposed by Bell,[186] was used to assess the effects of experimental treatments on cell-mediated gel contraction. In brief, HTCFs were mixed within an extracellular matrix (ECM) mixture containing 80% type I collagen (1.8mg/ml; A1048301, Gibco), 16% neutralizing solution (equal parts Waymouth media (Sigma-Aldrich) and 0.275M NaOH) and 20µl of ocular fibroblast conditioned media (concentrated to 25x to obtain a 1x final concentration within the 500µl construct volume) in order to achieve a final cell density of 2.5×10^5 cells/mL within the solution. Cell free collagen lattices were prepared identically, albeit without the inclusion of ocular fibroblasts, as negative control. The solution was pipetted gently to ensure homogenous distribution of ocular fibroblasts while avoiding the production of air bubbles, then 500µl were pipetted into each well of a 24-well tray. Collagen constructs were allowed to polymerize at 37°C and 5% CO_2 for 45 minutes before adding low serum culture media containing DMEM, 2% Fetal Bovine Serum (FBS), 1% penicillin/streptomycin.

After incubation at 37°C and 5% CO_2 for 72 hours, a sterile spatula was used to detach each collagen construct from the edges of the culture well. Plates were then immediately scanned on a flat bed laser scanner (Scanjet 8200, Hewlett-

Packard) to record baseline area, and then periodically for the duration of the experiment. The surface area of each collagen construct was measured using ImageJ[187] and standardized against the baseline surface area measurement to express changes in area as a percentage of original surface area.

4.2.4 Collagen Remodeling Assay

Upon conclusion of the contraction assay, collagen constructs were fixed in 4% paraformaldehyde overnight. After fixation, collagen constructs were dehydrated in ethanol, embedded in paraffin blocks, sectioned (5μM) and mounted on glass microscope slides using standard methods. For histological staining, sections were deparaffinized and hydrated using standard protocols. Sections were stained with picrosirius red. Briefly, a solution of 0.1% Sirius red (Sigma-Aldrich) in saturated picric acid (Sigma-Aldrich) was applied for 60 min, followed by 2 x 0.5% acetic acid washes. Collagen birefringence, used to determine collagen fibrillar hue,[188] was assessed by circularly polarized light microscopy of picrosirius red stained sections. Images were taken with an Abrio quantitative birefringence imaging system (Hinds Instruments, Portland, Oregon) mounted on an Olympus BX-51 microscope (Olympus Corporation, Tokyo, Japan). Specifically, a constant light intensity, a fixed 45° angle to the polarizing filter, a 20x objective and the same analyzer were used to facilitate consistent comparisons between each sample.

When viewed under polarized light, the color of the collagen fibers stained with picrosirius red depends upon fiber thickness and spatial orientation; with the color changing from blue to yellow to orange to red as fiber thickness and density

increase.[189,190] This method has been used previously in rabbit experimental filtration surgery to examine subconjunctival fibrosis, and red/orange staining was associated with bleb dysfunction.[105] Using ImageJ, this property was leveraged to determine the relative proportions of different color fibers within the collagen constructs. This quantitative method has been previously described by Rich and Whittaker.[191] In short, relative color content of the images is obtained by separating the digital images into their hue, saturation and value components. The hue component contains information on the color of each pixel within the image. Every pixel can have one of 256 possible colors. To identify the red, orange, yellow and blue pixels within a given image the following hue definitions were used within ImageJ: red 2-9 and 230-256, orange 10-38, yellow 39-51 and blue 52-128.[191] The number of pixels within each hue range is calculated and expressed as a proportion of the total number of pixels within each field representing collagen. Ten random frames using a 20x objective were taken per tissue section, with three tissue sections imaged per patient cell line and treatment group.

4.2.5 Cellular Metabolic Activity Assay

To determine the effects of ASA on HTCF cell metabolic activity, MTT assays (Sigma-Aldrich, USA) were conducted in 24-well plates. Supernatant from each well was collected in individual microtubes for the LDH assay. Then, 0.5 mg/mL MTT working solution in 0% FBS-1% PS DMEM was added per well, followed by incubation for 3 h at 37℃, 5% CO_2. Afterwards, the MTT solution was removed and 500 µL of dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Canada) was added to

each well. Plates were mixed for 30s on an orbital shaker, then analyzed using a spectrophotometer at 575 nm. Optical densities (ODs) were corrected for background OD from the DMSO, outliers were removed, and the mean corrected optical densities of triplicate values were calculated. Cell metabolic activity was calculated using the following equation:

$$\text{Metabolic activity (\%)} = \frac{\text{mean corrected OD570 of experimental condition}}{\text{mean corrected OD 570 of negative control}} \text{ x } 100\%$$

4.2.6 Cytotoxicity Assay

Cytotoxicity was assessed using a Pierce LDH Cytotoxicity Assay Kit (ThermoFisher, Canada). For this colorimetric assay, DMEM culture media without phenol red (Gibco, Thermo Fisher Scientific, Canada) was used. In addition to the previously indicated experimental treatment groups, HTCFs were treated with 10% (*v/v*) TritonX-100 (Sigma-Aldrich, USA) diluted in 0% FBS-1% PS DMEM for 1 h and 48hr, as a positive necrotic cell death control. After experimental conclusion, supernatants were collected and the samples immediately centrifuged for 10 minutes at 1,800g and 4℃. Next, each sample was plated in duplicate in a 96-well plate with LDH standards. Output was measured by spectrophotometer at dual filter 490 nm and 655 nm. Optical densities were corrected for background, and the mean corrected optical densities of the sample duplicates were calculated. Percentage of cell death was calculated using the following equation:

$$\text{Cytotoxicity (\%)} = \frac{\text{mean corrected OD490/655 of experimental condition}}{\text{mean corrected OD490/655 of negative control}} \text{ x } 100\%$$

4.2.7 Western Blot

Once the effects of acetylsalicylic acid on TGFβ1-induced cell metabolic activity were determined, ASA concentration at 100 µg/mL, 400 µg/mL, 1600 µg/mL and 3200 µg/mL were selected to assess the effects of ASA on TGFβ1-induced protein levels. HTCFs were seeded in 6 well-plates at 10^5 cells/mL, then co-treated with 2 ng/mL TGFβ1 and the selected ASA concentrations for 48 h at 37℃, 5% CO_2. Afterwards, total protein was extracted with lysis buffer (PhosphoSafe Extraction Reagent, Novagen) containing a protease inhibitor cocktail (P2714, Sigma-Aldrich) and a cell scraper (Pierce, Thermo Fisher Scientific, Canada). Crude protein lysate (10 µg), determined using a Micro BCA Protein Assay Kit (Thermo Fisher Scientific, USA), was separated using a 10% acrylamide gel, alongside a SeeBlue Pre-stained Protein Standard (Thermo Fisher Scientific, Canada). After gel electrophoresis, proteins were transferred to a nitrocellulose membrane using an iBlot Gel Transfer Device (IB1001, Invitrogen). Membranes were blocked in 5% (*w/v*) bovine serum albumin (BSA) (Sigma- Aldrich, USA) dissolved in 10% (*v/v*) tris buffered saline (TBST) (Thermo Fisher Scientific, Canada) for 1 h at room temperature, then incubated in primary antibody overnight at 4℃, followed by incubation in secondary antibody conjugated with horseradish peroxidase for 1 h at room temperature on a benchtop shaker. Membranes were washed three times, 5 minutes each, with 10% TBST before and after the addition of each antibody. For analysis of alpha-smooth muscle actin (α-SMA) protein levels, membranes were incubated in 2 µL/mL rabbit anti-alpha smooth muscle actin antibody (ab5694, Abcam, Canada) in 3% BSA solution, washed, then incubated in 0.66 µL/mL secondary goat anti-

rabbit antibody (Bio-Rad Laboratories, Canada) in 3% BSA. For analysis of matrix metalloproteinase-9 (MMP-9) protein levels, the same procedure was followed but using rabbit anti-MMP9 antibody (ab38898, Abcam, Canada). All membranes were also incubated with 2 µL/mL mouse anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibody (ab8245, Abcam, Canada) in 3% BSA, followed by incubated in 0.66 µL/mL secondary anti-mouse antibody (Bio-Rad Laboratories, Canada) both for 1 h at room temperature on a benchtop shaker. Membranes were visualized by applying WesternBright Quantum chemiluminescent reagent (Advansta, Inc.), with GAPDH used as a protein loading control. Imaging and relative densiometric quantification was accomplished using a ChemiDoc MP System (Bio-Rad Laboratories, Inc.) connected to Image Lab (Version 6, Bio-Rad Laboratories, Inc.).

4.2.8 Immunohistochemistry

In order to examine the effects of ASA on TGFβ1-induced myofibroblast morphology and α-SMA levels, HTCFs were seeded in 24-well plates, each containing a 13 mm glass coverslip. Cells were treated in duplicate and analyzed after incubation for 48h in the same treatment conditions as indicated for the western blot experiments. Cells were washed with PBS two times, fixed and permeabilized with 4% formaldehyde for 15 minutes, then washed again with PBS two times. Cells were then incubated in 5% BSA dissolved in PBS for 1 h at room temperature, followed by incubation with rabbit anti-α-SMA antibody at a 1:200 (*v/v*) dilution in 5% BSA for 1 h at room temperature. Cells were washed 3 times with PBS, 5 minutes each, then incubated in secondary goat anti-rabbit IgG

(Alexa Fluor 555 Dye, Abcam, Canada) for 1 h, F-actin Staining Kit in Blue Fluorescence (ab112124, Abcam, Canada) for 30 minute, then stained with Hoechst solution (Thermo Fisher Scientific, Canada) for 15 minutes. Cells were washed with PBS two times and dH_2O two times prior to imaging using fluorescence microscopy at 60x objective. Images were analyzed using ImageJ by comparing, within each treatment group, the total area of α-SMA to the number of nuclei. Ten random frames were taken with the 40x objective per replicate, with three replicates imaged per treatment group within each patient cell line. The laser intensity settings were kept consistent between samples to facilitate consistent comparisons.

4.2.9 Exogenous Lipid Mediator Assay

The pro-resolving capabilities of ASA-triggered lipid mediators on TGFβ1-induced HTCFs were assessed by western blot. HTCFs were seeded in 6-well plates at 10^5 cells/ml, serum-starved for 24 h, then treated with 2ng/mL TGFβ1 alone or co-incubated with one of: 5(R)-HETE, 11(R)-HETE, 15(R)-HETE, and 17(R)-OHDHA (Cayman Chemicals, USA) at concentrations of 10, 100, and 1000 ng/ml. After 48 h incubation, total protein was collected, and samples were analyzed by western blot using the procedure described above. To include a non-ASA-triggered lipid mediator negative control, 11-HETE was used due to its derivation from cytochrome P450 *in vivo*.

4.2.10 Statistical Analysis

Data from all experiments are presented as mean ± SD. Experimental replicates are denoted as ‘N=*x*’ and technical replicates performed within each cell line are

denoted as ‘n=*y*’. Data collected from multiple treatment groups over time were assessed by two-way repeated measures ANOVA with Dunnett’s multiple comparison *post hoc* test. Data from the exogenous lipid mediator western blot were assessed by one-way ANOVA with Tukey’s multiple comparisons *post hoc* test. A p-value of less than 0.05 was considered statistically significant.

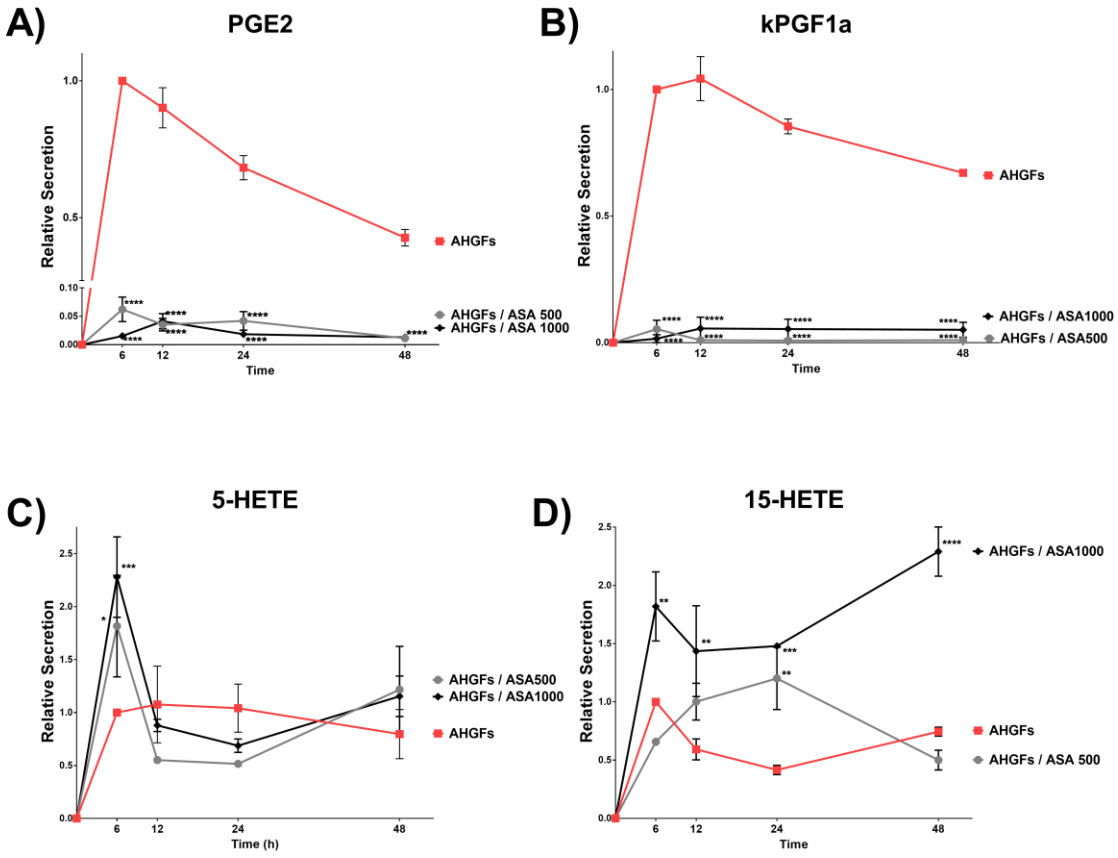
4.3 Results

4.3.1 Acetylsalicylic acid reduces prostaglandin production and induces resolving mediator production in HTCFs

First, we assessed the ability of ASA to illicit ASA-triggered lipid mediators within inflammation activated ocular fibroblasts. Cells induced with growth factors found within the aqueous humor (AHGFs - 1ng/ml each: TGFβ1, TNFα, INFɣ and IL-1β) exhibited high levels of prostaglandin production. Any dose of ASA exposure resulted in the near complete abrogation of PG production (Figure 4-1A and B). The relative production of ASA-triggered mediators 5-HETE, 15-HETE and 18-HEPE was significantly increased after ASA exposure in a dose dependent manner, up to 48hrs after exposure Figure 4-1C-E). No 17-OHDHA was detected in any sample analyzed.

Error! Reference source not found.F summarizes the relative biosynthetic activity of COX2 vs. acetyl-COX2 enzymes. The mean relative production of COX2 products (PGE2 and kPGF1a) was calculated, and from this figure the mean relative production of acetyl-COX2 products (5-HETE, 15-HETE and 18-HEPE) was subtracted. This way a value of zero would indicate equal relative biosynthetic activity between acetyl-COX2 and COX2 enzymes; positive values

would indicate COX2 dominated biosynthesis of lipid mediators; and negative values would indicate acetyl-COX2 dominated lipid mediator biosynthesis.



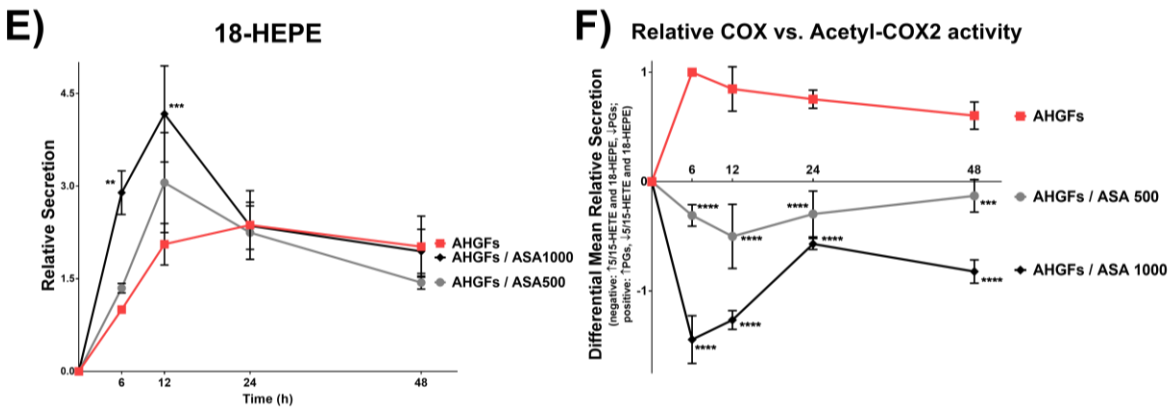
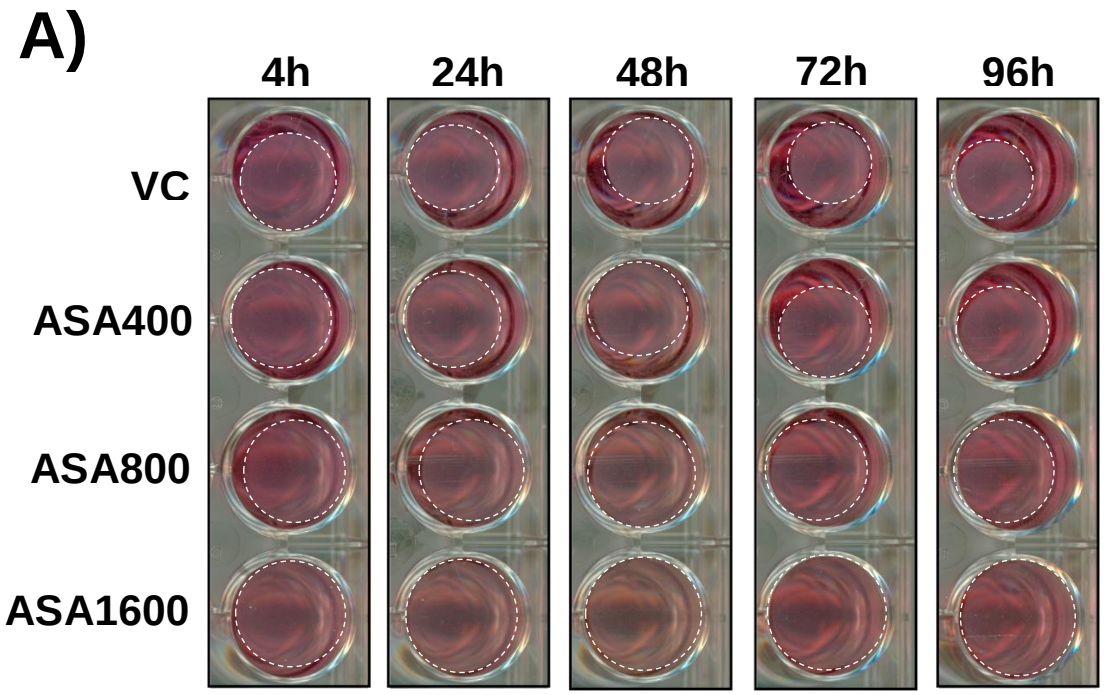


Figure 4-1 Relative AHGF-induced LM secretion was determined by supernatant sampling and LC-MS/MS analysis at the indicated timepoints after exposure of AHGF-induced ocular fibroblasts to the indicated experimental treatment. A-B) COX2 prostaglandin products: kPGF1a and PGE2. C-E) Acetyl-COX2 RM products: 5-HETE, 15-HETE and 18-HEPE. F) Displays the differential mean relative secretion between pro-inflammatory mediators (PGE2 and kPGF1a) and pro-resolving mediators (5-HETE, 15-HETE, 18-HEPE). A value of zero indicates equal relative biosynthetic activity between acetyl-COX2 and COX2; positive values indicate COX2 dominated biosynthesis of lipid mediators; and negative values indicate acetyl-COX2 dominated lipid mediator biosynthesis. A-F) Two-way ANOVA with Dunnett's *post hoc* test: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

4.3.2 Acetylsalicylic acid inhibits HTCF-mediated collagen contraction and remodeling

Cell-mediated collagen contraction was assessed in the presence of increasing concentrations of ASA (Figure 4-2). Compared to vehicle treated replicates, ASA elicited a significant and dose dependent inhibition of ocular fibroblast-mediated contraction over a period of four days. Contraction was almost completely inhibited at 1600µg/ml ASA.



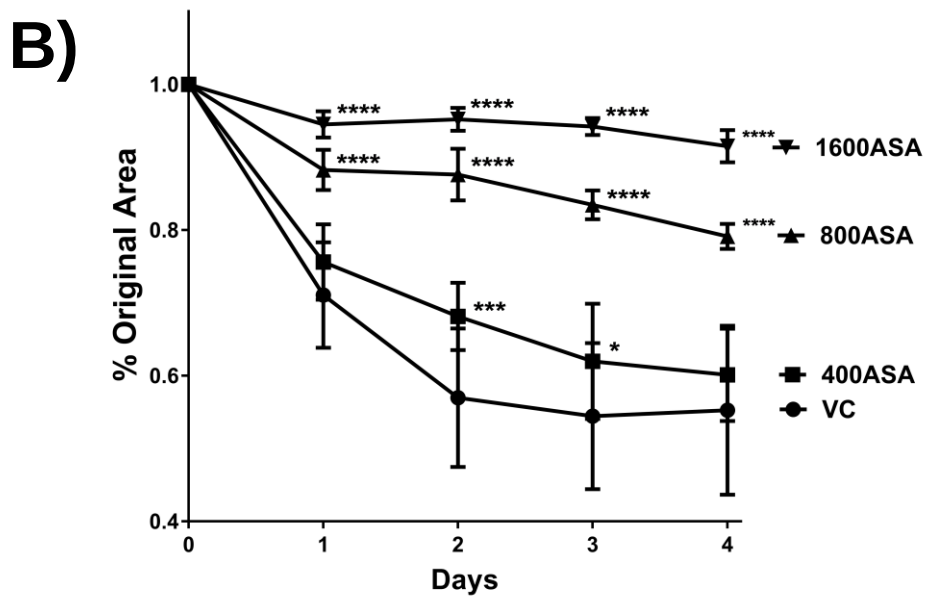


Figure 4-2 A) Representative scans of the same four contracting HTCF-populated collagen lattices with the indicated treatments, the first 96hrs after detachment from culture wells. B) Graph illustrating the effects of ASA (400, 800 and 1600 µg/ml in DMEM) on the contraction of HTCF-populated collagen lattices compared to vehicle treated. Data shown are mean ± SEM (N = 6, n=6). Two-way ANOVA with Dunnett's *post hoc* test: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Picrosirius red staining was used in combination with circularly polarized light as a highly sensitive means to visualize collagen fiber morphology. Fibrillar hue was used to assess structural changes induced by ocular fibroblasts in the collagen matrix compared to cell free matrices (Figure 4-3). Cell free matrices, incubated for seven days under identical experimental conditions, contained significantly greater proportions of blue staining compared to constructs containing HTCFs, which revealed a predominance of densely packed, mature red/orange staining collagen fibers (Figure 4-3). These changes are inferred to be cell mediated, structural changes to the collagen matrix. Exposure to 1600µg/ml ASA significantly inhibited the observed cell-mediated remodeling activity of ocular fibroblasts.

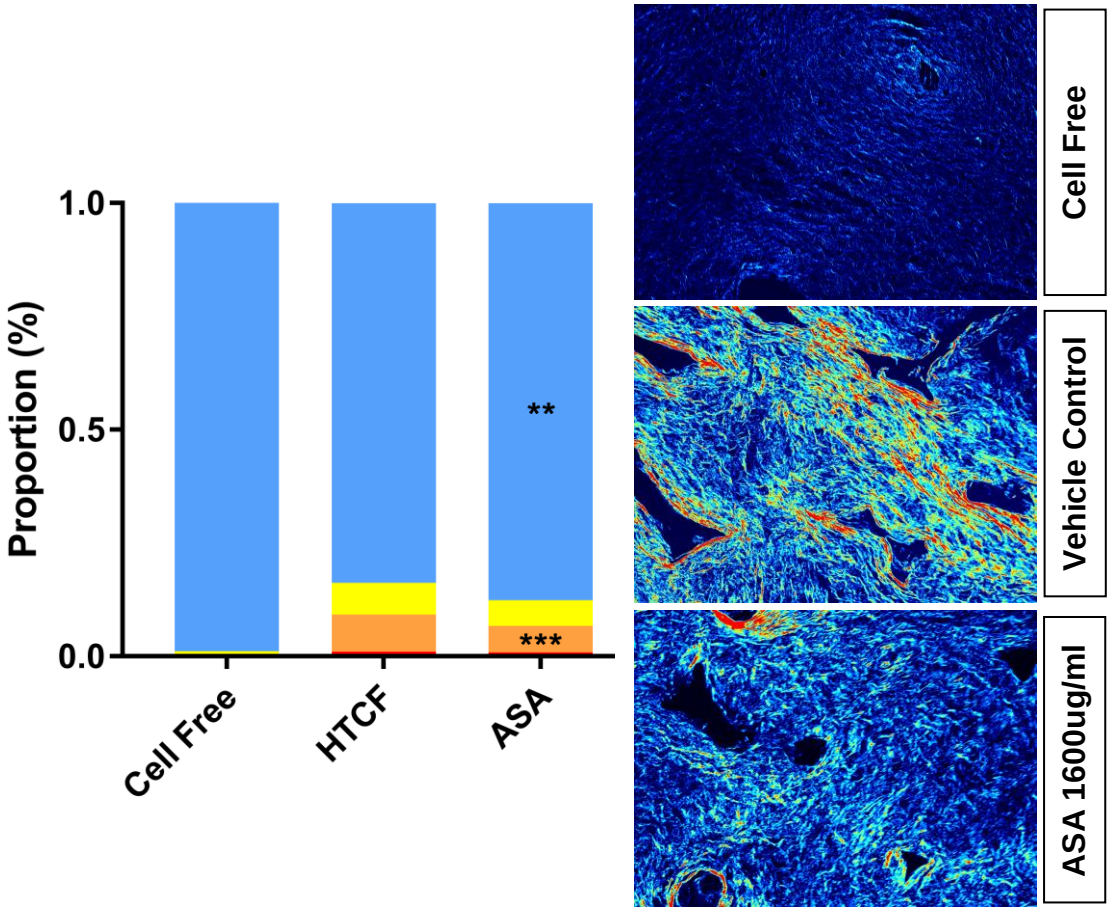


Figure 4-3 Picrosirius red and polarized light microscopy enabled assessment of cell-mediated collagen remodeling. Collagen matrices were prepared with and without HTCFs then cultured under normal conditions for two weeks, fixed, sectioned and stained with picrosirius red. HTCF remodeling activity on the collagen matrix is revealed by comparing cell free matrices, which stain uniformly blue, to those cultured with HTCFs. Those cultured with HTCFs exhibit regions staining yellow, orange and red where cell-mediated matrix modifications are self-evident. This remodeling activity was semi-quantitatively assessed over 7 days in the presence of 1600µg/ml ASA in DMEM. One-way ANOVA with Tukey's *post hoc* test: N=3, n=3, frames=10; **p<0.01, ***p<0.001.

4.3.3 Acetylsalicylic acid decreases TGFβ1 induced HTCF metabolic activity without increasing necrotic cell death

High levels of PG production are often associated with increased cellular metabolic activity. Thus, to better understand the mechanisms underlying ASA's effects on *in vitro* wound healing phenomena, we examined the effects of ASA on TGFβ1-induced HTCF cell metabolic activity. TGFβ1 increased HTCF cell metabolic activity compared to un-induced fibroblasts (Figure4-4A). TGFβ1-induced fibroblasts, treated with increasing concentrations of ASA, exhibited a dose-dependent decrease in metabolic activity. In contrast, ASA had minimal effects on HTCF metabolic activity in the absence of TGFβ1. A notable exception in both cases was ASA at 3200 µg/mL — where metabolic activity was lower than all other samples for both groups. Potentially reflecting an interference with COX1's homeostatic functions. Importantly, ASA at 1600 µg/mL returned TGFβ1-induced metabolic activity to that of the baseline, no-TGFβ1 control. Thus, ASA produced a significant and dose-dependent decrease in TGFβ1-induced HTCF metabolic activity.

Next, we assessed whether ASA was exerting its effects — in particular at higher concentrations — through cytotoxic mechanisms. We conducted an LDH assay using supernatant from HTCFs (N=4, n=3) treated with the same conditions as the MTT assay. HTCFs were also treated with 10% TritonX-100 for 1 h and 48 h as a positive cell death control. For HTCFs treated both in the presence and absence of TGFβ1, there was no difference in cell death – even at the highest dose assessed (Figure4-4B). When compared to cells treated with vehicle and those treated with TritonX-100, ASA treatments caused no significant increase in

cell death. These results suggest that ASA decreases TGFβ1-induced metabolic activity in HTCFs through a non-cytotoxic mechanism.

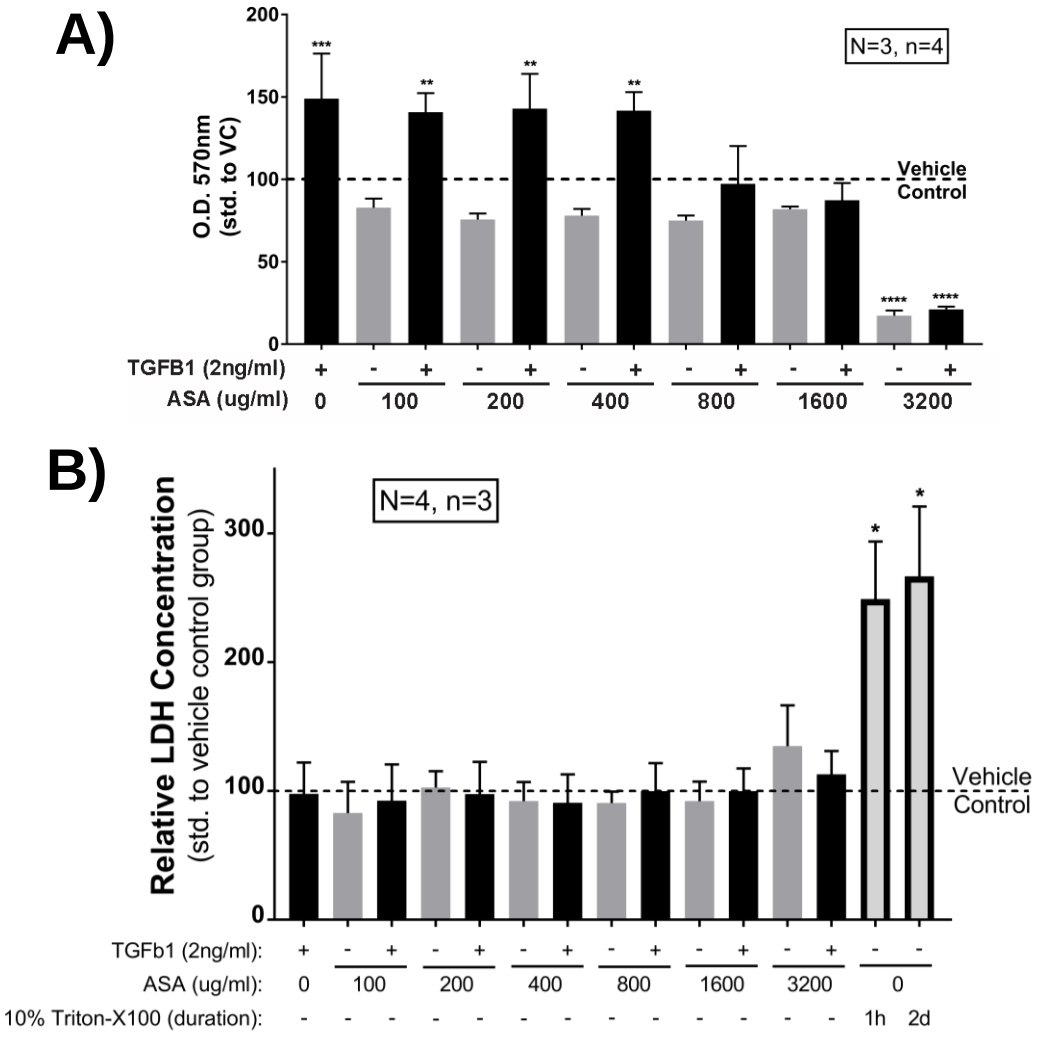
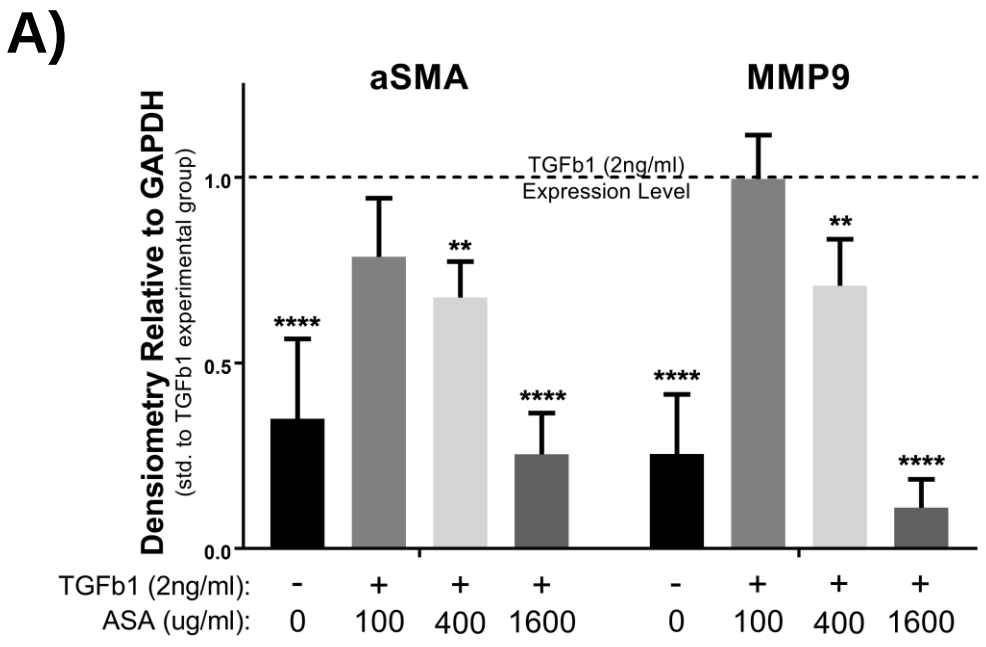


Figure 4-4 ASA decreases, A) cell metabolic activity, without significantly impacting, B) cell death. HTCFs were co-cultured in triplicate in 24-well plates at 1 x 10^5 cells/mL with TGFβ1 and ASA at 100-3200 µg/mL for 48 h at 37℃ in 5% CO_2. Cell metabolic activity (N=6, n=3) and cell death (N=4, n=3) were measured by MTT and LDH assays, respectively. TritonX-100 at 10% concentration was used to assess maximal cell death. Results are presented as mean ± SD. Mean and SD values were obtained at OD_{595} for MTT and $OD_{490/655}$ for LDH. Readings were corrected for background optical density, outliers were removed, and treatment group ODs were normalized to the vehicle control OD. ASA, acetylsalicylic acid; OD, optical density; VC, vehicle control. One-way ANOVA with Tuckey's *post hoc* test are indicated *$p < 0.05$, ***$p < 0.001$, ****$p < 0.0001$

4.3.4 Acetylsalicylic acid decreases TGFβ1-induced fibroproliferation-associated proteins

We assessed the effects of ASA on downstream protein expression within TGFβ1-induced HTCFs by western blot. HTCFs (N=3-4, n=1) were co-treated with TGFβ1 and ASA at 100, 400 and 1600 µg/mL for 48h. Relative levels of myofibroblast-associated proteins—αSMA and MMP9—were measured and normalized to GAPDH. HTCFs treated with TGFβ1 displayed an increase in αSMA and MMP9 protein levels compared with that of the no-TGFβ1 vehicle control (Figure 4-5A and B). A reduction in the levels of both proteins was observed after treatment with increasing concentrations of ASA, suggesting that ASA decreases the levels of TGFβ1-induced myofibroblast-associated proteins.



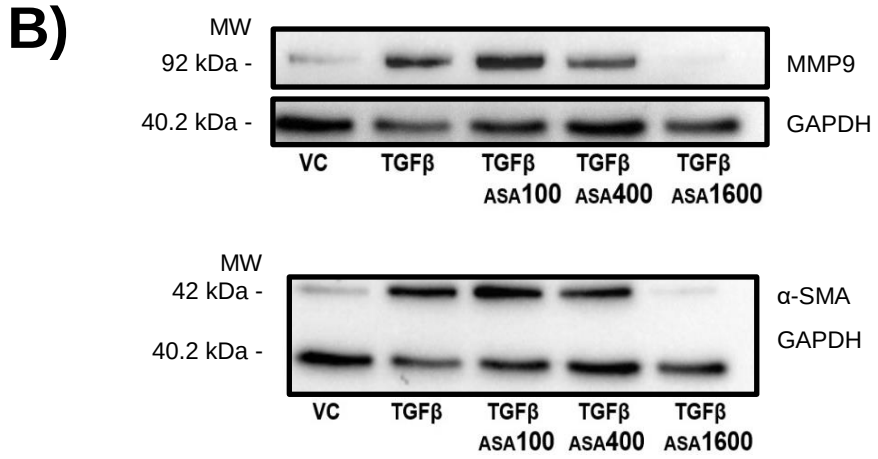
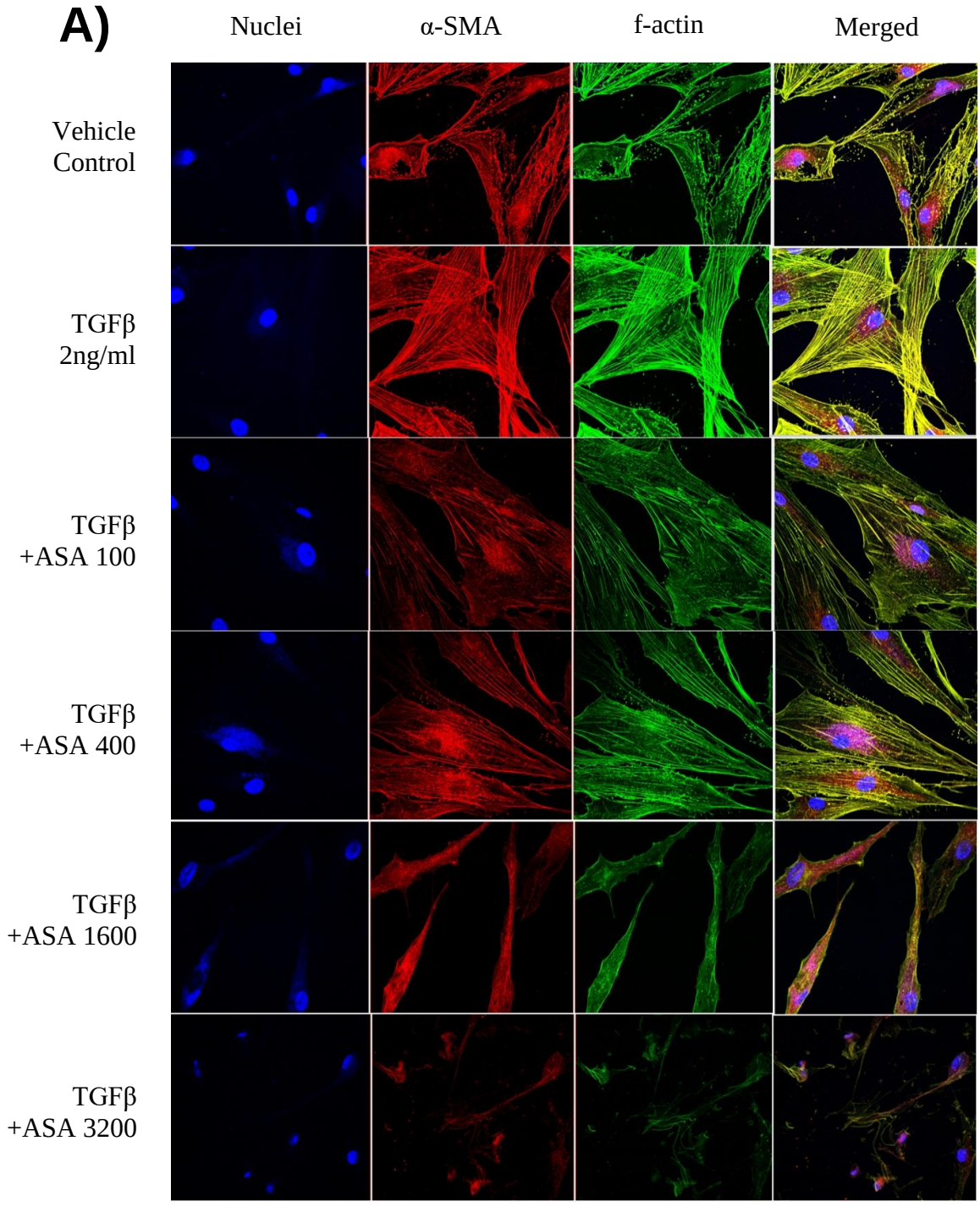


Figure 4-5 ASA decreases TGFβ1-induced protein levels. HTCFs (N=4) were co-cultured in 6-well plates at 10^5 cells/mL with TGFβ1 and ASA for 48 h at 37℃ in 5% CO_2. Relative protein levels were measured by western blot. A) Results of densiometric analysis are presented as mean ± SD normalized to GAPDH with one-way ANOVA with Tuckey's *post hoc* test are indicated *p < 0.05, ***p < 0.001, ****p < 0.0001. B) Images of representative blots for each protein. ASA, acetylsalicylic acid; α-SMA, alpha-smooth muscle actin; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; VC, vehicle control.

To supplement the western blot findings, we conducted immunohistochemistry to examine the effects of ASA on cell morphology and α-SMA levels. HTCFs treated with TGFβ1 exhibited an expanded cell shape with a noticeable increase in linear actin filaments when compared to vehicle control (Figure4-6A). After treatment with increasing concentrations of ASA, there was an observable reduction in this expanded and linear cell morphology. Furthermore, semi-quantitative analysis of α-SMA within this assay revealed increased expression after treatment with TGFβ1 when compared to vehicle control (Figure4-6B). A significant and dose dependent reduction in α-SMA expression was observed with ASA exposure. These findings follow a similar trend with those of the western blots, suggesting that ASA represses myofibroblast-associated proteins induced by TGFβ1.


A)
Nuclei
α-SMA
f-actin
Merged
Vehicle Control
TGFβ 2ng/ml
TGFβ +ASA 100
TGFβ +ASA 400
TGFβ +ASA 1600
TGFβ +ASA 3200

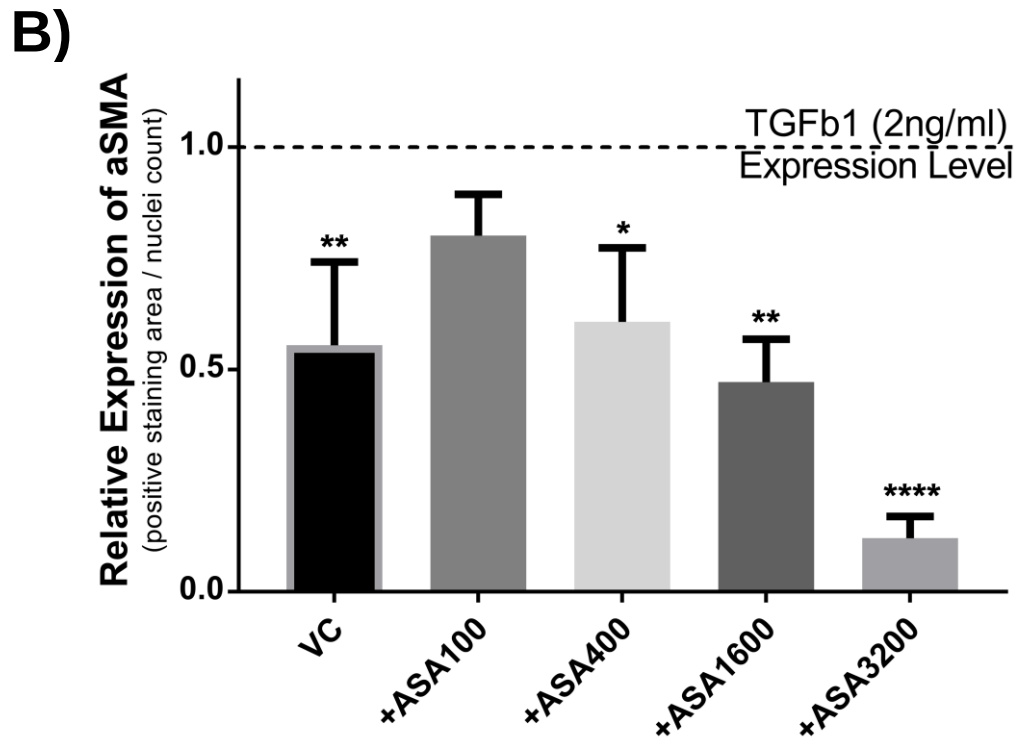


Figure 4-6 ASA decreases TGFβ1-induced myofibroblastic changes. HTCFs (N=3, n=2) were co-cultured in 24-well plates at 10^5 cells/mL with TGFβ1 and ASA for 48 h at 37℃ in 5% CO_2. A) Area of α-SMA was obtained through ImageJ analysis and normalized to nuclei number. α-SMA levels are presented as mean ± SD. Results from a one-way ANOVA with Tukey's *post hoc* test are indicated: **p* < 0.05, ****p* < 0.001, *****p* < 0.0001. B) α-SMA (red) and F-actin (green) expression were assessed by immunohistochemistry ASA, acetylsalicylic acid; α-SMA, alpha-smooth muscle actin; VC, vehicle control.

4.3.5 Acetylsalicylic acid triggered lipid mediators decrease TGFβ1 induced alpha-smooth muscle actin expression

We assessed whether exogenous treatment of ASA-triggered lipid mediators would yield the same effect as treatment with ASA on TGFβ1-induced HTCFs. We conducted western blots to assess relative levels of the myofibroblast-associated protein, α-SMA, after lipid mediator treatment. HTCFs (N=7, n=1) were co-treated for 48 h with TGFβ1 and lipid mediator 5(R)-HETE, 11(R)-HETE, 15(R)-HETE, and 17(R)-OHDHA at concentrations 10, 100, and 1000 ng/mL. HTCFs treated with TGFβ1 alone displayed a significant increase in α-SMA level compared to vehicle control (Figure 4-7). A significant reduction in α-SMA was observed after treatment with 5(R)-HETE, 15(R)-HETE and 17(R)-OHDHA at 100 and 1000 ng/ml, however, no significant difference was observed after treatment with 11(R)-HETE. These findings suggest that the downstream lipid mediators triggered by ASA can, themselves, repress myofibroblast-associated proteins.

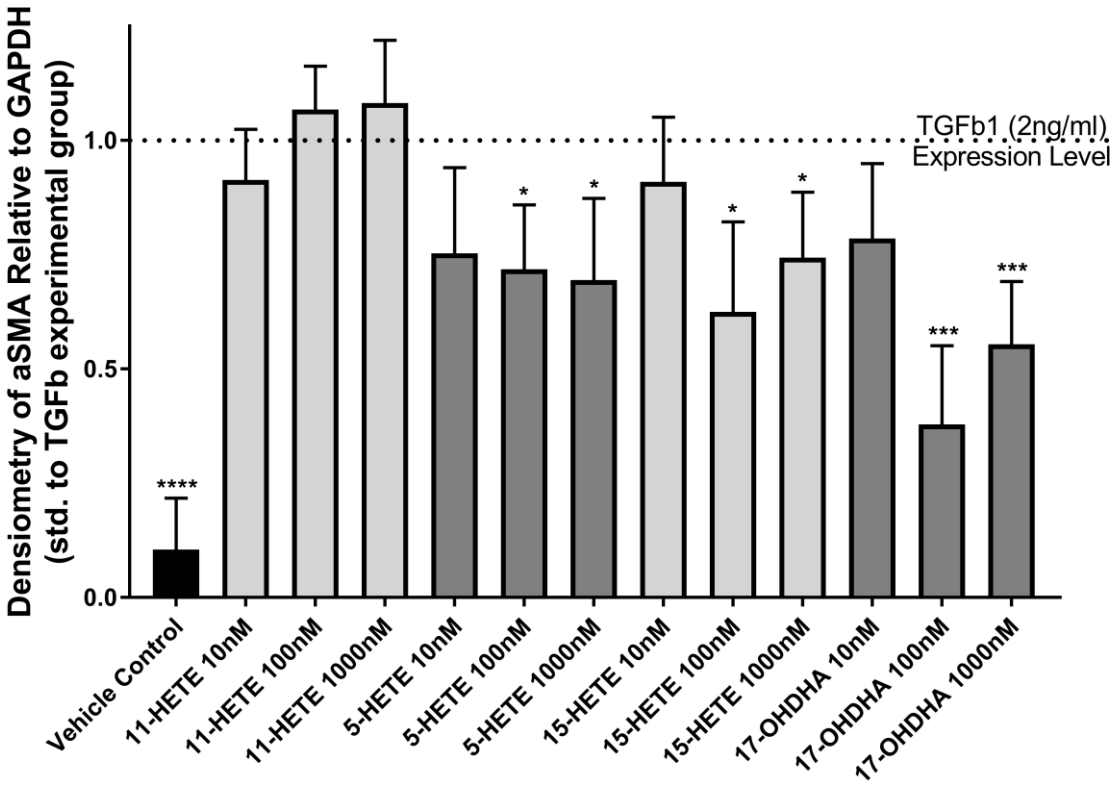


Figure 4-7 Exogenous lipid mediators decrease TGFβ1-induced α-SMA levels. HTCFs (N=6, n=2) were co-cultured in 6-well plates at 10^5 cells/mL with TGFβ1 and lipid mediators: 5*R*-hydroxyeicosatetraenoic acid (HETE), 11(R)-HETE, 15(R)-HETE and 17(R)-hydroxy docosahexaenoic acid (17-OHDHA) for 48 h at the indicated concentrations (nM). α-SMA levels were measured by western blot. Results are normalized to GAPDH and presented as mean ± SD. Dotted line represents α-SMA densitometry after treatment with TGFβ1 alone. One-way ANOVA with Tukey's *post hoc* test *$p < 0.05$, ***$p < 0.001$, ****$p < 0.0001$. GAPDH, glyceraldehyde 3-phosphate dehydrogenase; VC, vehicle control.

4.4 Discussion

We have demonstrated, for the first time, that inflammation and wound healing cytokines found within the aqueous humor illicit high levels of PG production within HTCFs. PG production and the associated heightened metabolic activity can be mitigated by ASA exposure, at the same time as inducing the production of ASA-triggered resolving mediators. Both ASA and the exogenous applications of ASA-triggered lipid mediators were found to repress TGFβ1-induced cellular metabolic activity and protein expression in human Tenon's capsule fibroblasts, suggesting that this signaling system is capable of impeding the propagation of both inflammatory and fibroproliferative processes. Indeed, results from the cell-mediated collagen contraction and remodeling assays demonstrate ASA's ability to impair cell mediated wound healing phenomena *in vitro*.

TGFβ1, as well as its other two isoforms, are present in aqueous humor[235] and are implicated in post-surgical scarring by inducing the transdifferentiation of fibroblasts into myofibroblasts.[1,4] Based on the MTT results, ASA seems to reverse the effects of TGFβ1 in a dose-dependent manner by decreasing TGFβ1-induced cell metabolic activity. This finding is most prominently observed at ASA concentration 1600 μg/mL, where TGFβ1-induced metabolic activity returns to the baseline control level. Previous studies have observed a similar decline in metabolic activity either after treatment at a single ASA concentration on non-human fibroblasts,[236,237] or after treatment at multiple concentrations of ASA on non-fibroblast cell lines.[238–241] Therefore, to our knowledge, our study is the first

to elucidate a dose-response to ASA in TGFβ1-induced human Tenon's capsule fibroblasts.

Furthermore, in the LDH assay, no notable increase in necrotic cell death was observed, even at increasing ASA concentrations. This finding indicates that the ASA concentrations tested are not cytotoxic to the fibroblasts, which aligns with work from other research groups that have demonstrated the protective effects of ASA against inflammation induced cellular damage.[242–244] Together, the MTT and LDH results suggest that ASA may be inactivating—rather than killing—TGFβ1-induced fibroblasts, which subsequently lowers their metabolic activity level to that of the pre-inflammation-induced state. On theoretical grounds, these results may translate to a desirable outcome *in vivo* by decreasing fibroblast scarring activity after glaucoma filtration surgery without inducing lethal effects on healthy tissues.

We have also examined the downstream effects of ASA on expression of TGFβ1-induced proteins, such as α-SMA and MMP-9. Western blot analysis demonstrates that ASA represses the levels of both scarring associated proteins, suggesting that ASA may mitigate the scar-forming potential these TGFβ1-induced fibroblasts. Immunohistochemistry imaging confirmed these findings and further demonstrates the ability of ASA to resolve the linear actin contractile fibers induced by TGFβ1. These results are strengthened by the work from other research groups, which have found a similar reduction in fibrosis-associated proteins expression after ASA treatment in human[236] and mouse[237] fibroblasts, as well as in other cell types.[238–241] ASA's ability to represses TGFβ1-induced

proteins that are implicated in fibrosis suggests that ASA may mitigate the ability of these cells to form scar tissue *in vivo* after filtration surgery.

After exogenous treatment of ASA-triggered lipid mediators, we have observed a decrease in α-SMA levels within TGFβ1-induced HTCFs. This suggests that downstream lipid mediators generated by ASA may have a role in transducing the intracellular effects of ASA exposure. These findings align with those of previous studies, which have demonstrated the ability of 5(R)- and 15 (R)-HETE to reduce fibrosis in mice,[35] and 17(R)-OHDHA was also found to be involved in resolution in humans.[230,231,245] We observed no effect with treatment of 11(R)-HETE across the three concentrations tested. This was encouraging as it is not considered an ASA-triggered lipid mediator, but the oxygenation product of cytochrome P450 enzymes acting upon arachidonic acid.[6]

A limitation in this study is that the effects of ASA were assessed on a single cell type. The chronic inflammatory micro-environment implicated in scarring after filtration surgery involves the interaction of many different cell types, such as immune cells, which cannot be completely recreated with the exogenous inflammatory cytokine application.[70,71] Therefore, future research may explore the lipid mediator modulating effects of ASA within a fibroblast-immune cell co-culture system.

In conclusion, we have shown that ASA and its associated lipid mediators shift lipid mediator production from pro-inflammatory to pro-resolving, inhibit *in* vitro wound healing phenomena. Data support that these findings are likely due to a

reversal of TGFβ1-induced metabolic activity and protein expression in HTCFs with exposure to ASA/ASA-triggered lipid mediators. These findings expand our understanding of the mechanism of ASA as an anti-inflammatory and anti-scarring agent in TGFβ1-induced HTCFs and provide the foundation for future *in vivo* research, which may potentially explore the effects of topical ASA application for ophthalmic pathologies. With the well-established safety profile of ASA in human patients, translation of these findings may be rapid.

Chapter 5

5 Acetylation of COX2: An Immuno-resolving and Anti-Cicatrizing Ocular Intervention[3]

Building on the promising results of Chapter 5, this chapter sought to further modulate the production of LMs. Theoretically, after ASA acetylation, all enzymatic machinery downstream of PLA2 – acetyl-COX2 and the endogenous LOX enzymes – will act on PUFA precursors to generate RMs. This would suggest that agonizing the activity of PLA2 under these conditions would theoretically lead to increased PUFA precursor generation for downstream processing into RMs. In Chapter 6, to test this hypothesis, we used ASA and a second, more COX2 specific acetylating molecule to induce COX acetylation in the presence of two separate PLA2 agonists. The effects of these interventions on the *in vitro* wound healing phenomena of subconjunctival fibroblasts are presented.

[3] Parts of this chapter have been patented: **Armstrong, J.J.** and Hutnik, C.M.L. 2018. Compositions and methods for treating ocular inflammation and ocular scarring. U.S. Provisional Patent Application No. 62/677,284, filed May 2018. Provisional status until May 2019.

Submission for peer reviewed publication will follow patent approval.

5.1 Introduction

Inflammation driven myofibroblast transdifferentiation and activity is at the heart of much currently unanswered ocular morbidity. Glaucoma filtration surgery, corneal stromal injury, posterior capsule opacification after cataract surgery, proliferative vitreoretinopathy and the sub-macular scarring associated with age-related macular degeneration are some major contributors to ocular morbidity with myofibroblast transdifferentiation at the core of the pathology.[1] More broadly, estimates implicate fibroproliferative disease in 45% of morbidity and mortality at a systemic level in the developed world[246] – it is unsurprising that the eye mirrors the rest of the body in unanswered contributors to mortality. Recent changes in our understanding of inflammation and, more importantly, its endogenously controlled resolution necessitate a reimagining of treatments for inflammation driven scarring.

Over the past twenty years, our understanding of inflammation and its resolution has shifted. Long have the prostaglandin family of lipid mediators, derived from cyclooxygenase 2 (COX2), been tied with the cardinal signs of inflammation. More recently, however, we have come to understand the subsidence of these cardinal signs, or the resolution of inflammation, to be an active process that is endogenously controlled by the pro-resolving superfamily of lipid mediators.[16] These resolving mediators (RMs) are produced mainly from the actions of lipoxygenase (LOX) enzymes on the polyunsaturated fatty acid (PUFA) precursors arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).[10] The actions of RMs on inflammatory cells have

been well established (i.e. inhibiting tissue infiltration of polymorphonuclear (PNM) cells, promoting phagocytosis of cellular and wound debris, and decreasing the antibiotic requirements for bacterial clearance) and their cumulative effects are understood to decrease the duration and impact of inflammation-induced cellular activity.[28,247]

Fibroblasts have been considered by some to be early sentinels of the immune system, capable of both mounting and modulating inflammatory processes.[30] Further, the process of fibroproliferation itself could be considered an appropriate innate immune response to contain certain infectious organisms that can evade immune cell killing. If myofibroblasts were to be modelled as inflammation-activated fibroblasts, one would expect high levels of endogenous prostaglandin production to be observed within the myofibroblast phenotype. It would then follow that, if RM secretion was promoted and resolution triggered, myofibroblasts may abrogate their fibroproliferative activity. In fact, much recent work has demonstrated the anti-fibrotic properties of RMs when stimulated endogenously through overexpression of lipoxygenase enzymes,[35] or when applied exogenously as individual lipid mediators.[21,36,37,248]

Therapeutically, the endogenous stimulation of RMs can be accomplished through a specific *in situ* acetylation of COX2. Acetylation at specific residues induces a change in enzymatic function and repurposes COX2 from an endogenous producer of pro-inflammatory prostaglandins, to one that resembles a LOX enzyme in function and produces RMs. Recently, an enzyme endogenous to murine neurons was found to acetylate murine COX2 at Ser565. The authors

noted that acetylation of COX2 by this enzyme impaired PG production, enabled production of RMs and induced resolution of dysfunctional microglial inflammation within the Alzheimer's disease model they employed.[249] These findings illustrate a naturally occurring example of COX2 repurposing. Acetylsalicylic acid (ASA) was long thought to inactivate COX2 through acetylation of Ser516,[250] however we now understand this modification to not only inhibit prostaglandin production, but to also enable RM generation through the acetylated enzyme.[10,224,251–253] This property of ASA has been measured in enzymatic assays,[224] cell-based systems[228] as well as in human subjects taking ASA in a randomized controlled trial.[226]

Prior to understanding the potential immuno-resolving impact of generating RMs from acetylated COX2, several COX2 specific acetylators were developed as potential inhibitors of prostaglandin production.[225] However, little is known regarding the potential of more specific COX2 acetylators to trigger the production of LMs akin to ASA. As COX1 function is abrogated completely by acetylation, one would hypothesize that a more specific COX2 acetylator would lead to more acetyl groups being attached to COX2 enzymes relative to COX1 enzymes and therefore illicit greater RM production than a non-specific COX2 acetylator such as ASA. This would be especially true in the setting of high levels of inflammation induced COX2. From this we hypothesized that acetylating COX2 in a pro-inflammatory and wound healing microenvironment would lead to increased generation of RMs relative to prostaglandins and decreased *in vitro*

cell-mediated wound healing phenomena through the impairment of myofibroblast transdifferentiation and activity.

5.2 Materials and Methods

5.2.1 COX2 Acetylating Agents and PLA2 Agonists

To our knowledge, the most specific COX2 Ser516 acetylating agent identified to date is *o*-(acetoxyphenyl)hept-2-ynyl sulfide (APHS),[225,254] which was purchased from Santa-Cruz Biotechnology (Catalog #sc-200668). ASA was purchased from Sigma Aldrich (A5376). Both melittin (M4171) and gentamicin (G1391) were purchased from Sigma-Aldrich. Molecular structures with key moieties are displayed in Figure 5-1. Structures of COX1 and COX2 are shown in Figure 5-2.

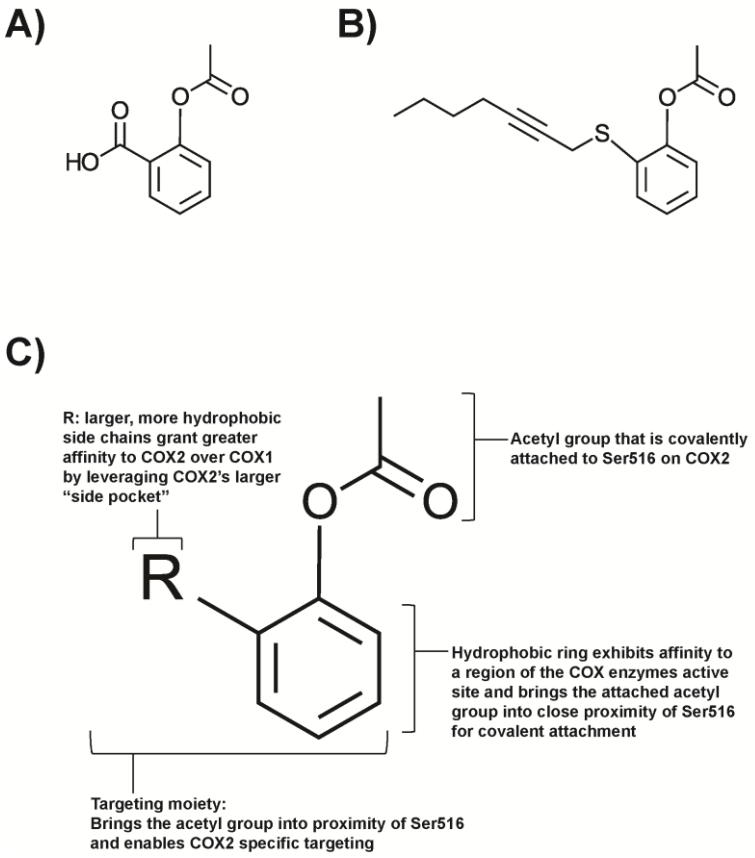


Figure 5-1 A) depicts the molecular structure of ASA. B) depicts the molecular structure of APHS. C) summarizes how the molecular moieties of ASA/APHS have been shown to interact with the active site of COX1/2.

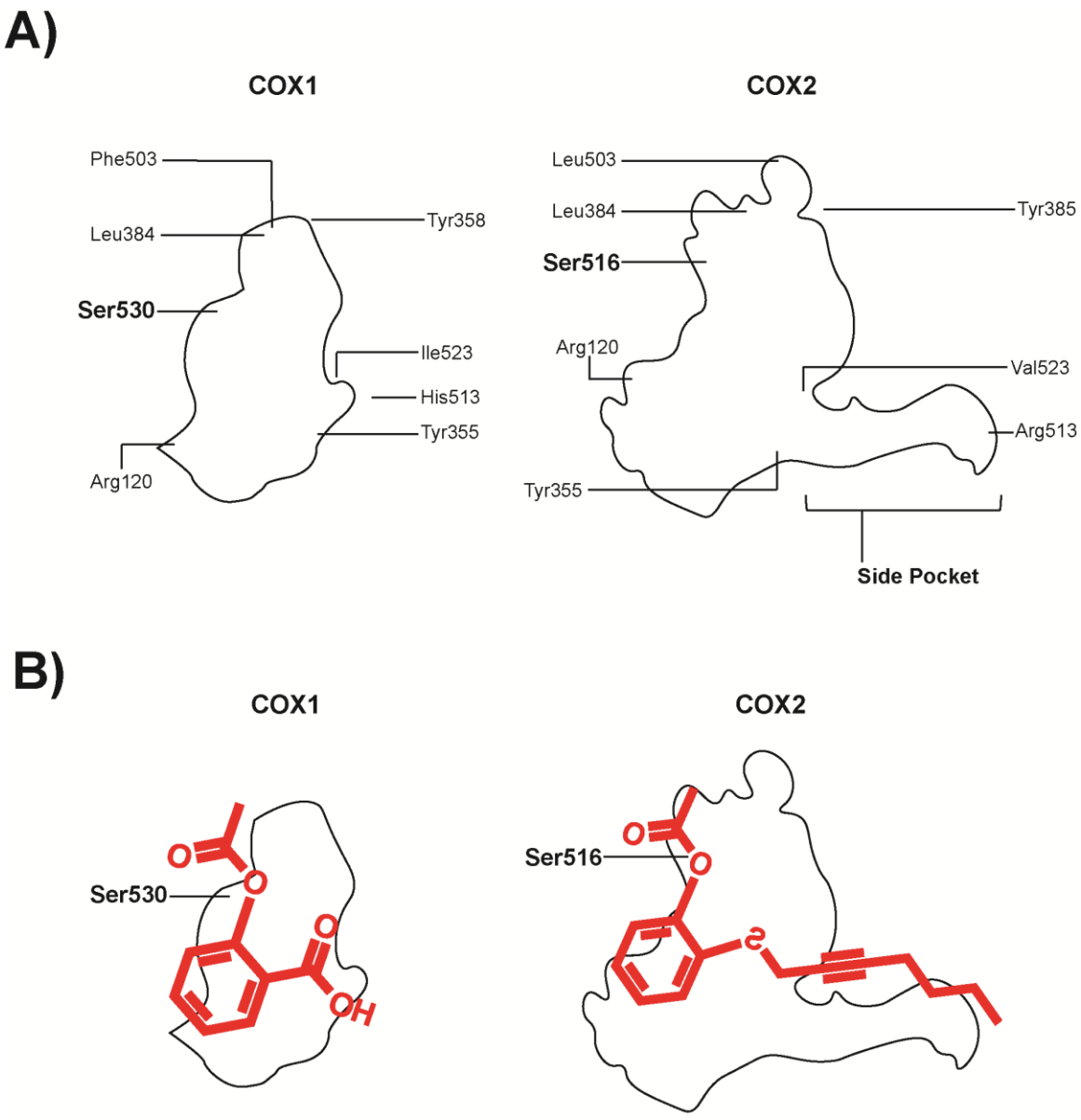


Figure 5-2: A) displays a schematic representation of COX1 and COX2. The location of Ser530 and Ser516 are indicated in bold (the residues acetylated by ASA and APHS on COX 1 and 2, respectively). Other key residues are indicated for schematic orientation. Important differences to note are the larger overall size of COX2 vs COX1 (72 kDA vs 68 kDa) and the hydrophobic side pocket on COX2 absent in COX1. B) displays the same schematic representations of COX1 and COX2, with ASA superimposed over the active site of COX1 and APHS superimposed over the active site of COX2. The longer, hydrophobic tail of APHS leverages the overall larger enzymatic structure and side pocket unique to COX2 in order to attain 60x greater affinity to COX2 compared to ASA. ASA has approximately 20x greater specificity to COX1 vs. COX2.

5.2.2 Inflammatory and Wound Healing Cytokines

Ocular tissues respond to injury or insult by eliciting the cellular programs defined grossly as inflammation and wound healing. Specific factors, found within the aqueous humor and surgical sites of ophthalmic patients, are thought to drive inflammatory and fibroproliferative responses observed in ocular tissues.[84–88] To model these *in vitro,* we used physiologically relevant mixtures (1ng/ml each) of inflammatory and wound healing cytokines IL-1β (SRP6169), TNFα (SRP2102), INFɣ (MSST0040) and TGFβ1 (T7039), all from Sigma-Aldrich.

5.2.3 Isolation and Culture of Tenon's Capsule Fibroblasts

This study followed the tenants of the Declaration of Helsinki and was approved by the office of Human Research Ethics at Western University (REB# 106783). Primary ocular fibroblast cell lines were derived from 2-4 mm^3 surgically resected segments of Tenon's capsule. After the acquisition of informed consent, tissue specimens were obtained from male and female glaucoma patients undergoing primary trabeculectomy at St. Josephs' Hospital, London, Canada. The samples were obtained by ophthalmic surgeons who removed segments of Tenon's capsule and placed them into primary culture growth media containing Dulbecco modified Eagle's minimal essential medium (DMEM), 10% Fetal Bovine Serum (FBS), 1% penicillin/streptomycin, and 1% amphotericin, all from Sigma-Aldrich (Oakville, Canada). After, specimens were placed in fibronectin (Sigma-Aldrich) coated 6-well culture plates, submerged in primary culture media, and subcultured upon confluency for further use. Cell cultures were used for

experimentation prior to passage five. **Table 3-1** provides a summary of donor characteristics for the ocular fibroblast cell lines used in experiments.

5.2.4 Stimulated Lipid Mediator of Inflammation / Resolution Secretion Assays

Relative quantification of secreted pro-inflammatory and pro-resolving lipid mediators was achieved using liquid chromatography tandem mass spectrometry (LC-MS/MS). Specifically, we focused our analyses on the COX2 derived lipid mediators, kPGF1a and PGE2; as well as the acetyl-COX2 derived mediators, 5-HETE, 15-HETE, 17-OHDHA and 18-HEPE; and their PUFA precursors, AA, DHA and EPA. Known quantities of deuterated internal standards of the analyzed the lipid mediators were added to each sample to enable relative quantification between samples (Item Numbers: #10007737, #320110, #314010, #11182, #315210, #319030, #390030, #10006410, #10006199, #334230, and #10008040, Cayman Chemical). This is a well-established method to determine the relative activity of lipid biosynthetic pathways.[233,234] In brief, the ratios of the integrated areas of the chromatographic peaks corresponding to each analyte and the integrated areas of the peaks corresponding to each analyte's internal standard (with known absolute quantity) are used to determine the relative quantity of each analyte in a given sample. After supernatant sample collection, 250ul of methanol containing 100pg of each deuterated internal standard was added. Samples were then vigorously vortexed and centrifuged at 10,000g for 10min before loading onto solid-phase extraction cartridges (Strata-X 33um

Polymeric Reversed Phase, 10mg, Phenomenex 8B-S100-AAK), that were previously activated with 2ml methanol and rinsed with 2ml water. Samples were diluted upon loading so that the final concentration of methanol was between 10 and 15% of total volume. After washing with 5ml water, extracts were eluted with 1ml methanol. Solvent was then evaporated under vacuum in a SpeedVac centrifuge, and the extract was resuspended in 100µl acetonitrile/water 60:40 (v/v). An aliquot of 20µl was injected into the LC-MS/MS system for analysis. A Sciex ExionLC Integrated System was used with a 0.3ml/min flow rate, initial mobile phase of 10% water / 0.1% formic acid followed by 100% acetonitrile / 0.1% formic acid on a Kinetex 2.6um C18 100 Å 100x2.1mm, Phenomax column (OOD-4462-AN). A Sciex Triple Quad 6500+ mass spectrometer with multiple reaction monitoring was used in negative ion mode. The chromatographic profile of the ion count for each *m/z* transition was monitored, and the area under the peaks (ion intensity vs elution time) was integrated using commercial software (MultiQuant, Sciex). Total cellular protein concentration was used for normalization of the supernatant samples.

5.2.5 Collagen Contraction Assays

The delayed release fibroblast populated collagen lattice model,[185] based on the model first proposed by Bell,[186] was used to assess the effects of experimental treatments on ocular fibroblast-mediated gel contraction. In brief, ocular fibroblasts were mixed within an extracellular matrix (ECM) mixture containing 80% type I collagen (1.8mg/ml; A1048301, Gibco), 16% neutralizing solution (equal parts Waymouth media (Sigma-Aldrich) and 0.275M NaOH) and 4%

vol./vol. ocular fibroblast conditioned media (concentrated to 25x to obtain a 1x final concentration within the 500µl construct volume) in order to achieve a final cell density of 2.5 x 10^5 cells/mL within the solution. Cell free collagen lattices were prepared identically, albeit without the inclusion of ocular fibroblasts, as negative control. The solution was pipetted gently to ensure homogenous distribution of ocular fibroblasts while avoiding the production of air bubbles, then 500µl were pipetted into each well of a 24-well tray. Collagen constructs were allowed to polymerize at 37°C and 5% CO_2 for 45 minutes before adding low serum culture media containing DMEM, 2% Fetal Bovine Serum (FBS), 1% penicillin/streptomycin.

After incubation at 37°C and 5% CO_2 for 72 hours, a sterile spatula was used to detach each collagen construct from the edges of the culture well. Plates were then immediately scanned on a flatbed laser scanner (Scanjet 8200, Hewlett-Packard) to record baseline area, and then periodically for the duration of the experiment. The surface area of each collagen construct was measured using ImageJ[187] and standardized against the baseline surface area measurement to express changes in area as a percentage of original surface area.

5.2.6 Collagen Remodeling Assays

To assess collagen remodeling unconfounded by cell-mediated contraction, collagen constructs were left attached to culture wells for the duration of the experiment. At experimental conclusion (12 days incubation), collagen constructs were fixed in 4% paraformaldehyde for 3h. After fixation, they were dehydrated in

ethanol, embedded in paraffin blocks, sectioned (5μM) and mounted on glass microscope slides using standard methods. Sections were deparaffinized and hydrated using standard protocols. Sections were then stained with picrosirius red. Briefly, a solution of 0.1% Sirius red in saturated picric acid was applied for 60 min, followed by 2 x 0.5% acetic acid washes. Collagen birefringence, used to determine collagen fibrillar hue,[188] was assessed by circularly polarized light microscopy of picrosirius red stained sections. Images were taken with an Abrio quantitative birefringence imaging system (Hinds Instruments) mounted on an Olympus BX-51 microscope. Specifically, a constant light intensity, a 45° angle to the polarizing filter and the same analyzer were used to facilitate comparisons between each sample.

When viewed under polarized light, the color of the collagen fibers stained with picrosirius red depends upon fiber thickness, spatial orientation and packing density; with the color changing from blue to yellow to orange to red as fiber maturity, thickness and density increase.[189,190] This method has been used previously in rabbit experimental filtration surgery to examine subconjunctival fibrosis, and blue(green)/yellow staining was associated with improved bleb function.[105] Using ImageJ, this property was leveraged to determine the relative proportions of different color fibers within the stained collagen constructs. This quantitative method has been previously described,[191] and is employed within the current experiment to assess changes in collagen architecture due to the remodelling activity of ocular fibroblasts. In short, relative color content of the images is obtained by separating the digital images into their hue, saturation and

value components. The hue component contains information on the color of each pixel within the image. Every pixel can have one of 256 possible colors. To identify the relative proportions of red, orange, yellow and blue pixels within a given image, a propriety script was written and run using the following hue definitions within ImageJ: red 2-9 and 230-256, orange 10-38, yellow 39-51 and green 52-128. The number of pixels within each hue range is calculated and expressed as a proportion of the total number of pixels.

5.2.7 Fluorescent Microscopy

Expression of the contractile protein alpha smooth muscle actin (α-SMA), the myofibroblastic phenotype marker,[255] by ocular fibroblasts within collagen constructs was assessed through immunohistochemistry. Deparaffinized and hydrated sections were permeabilized for 30 minutes with 1% Triton X-100 in PBS. After blocking of nonspecific sites with 1% BSA in PBS, sections were incubated for 40 minutes with Alexa Fluor 568–conjugated primary antibody against α-SMA (Abcam, CAT. NO: ab202295). Finally, slides were stained with 4,6-diamidino-2-phenylindole for 10 minutes. For each tissue section, the area positive for α-SMA staining was measured in ImageJ and then divided by the number of nuclei counted within that same frame, this number was then compared between treatment groups. Ten random frames were taken per tissue section, with five tissue sections imaged per patient cell line and treatment group.

Cellular proliferation was assessed through immunohistochemistry. Briefly, deparaffinized and hydrated sections were permeabilized for 30 minutes with 1%

Triton X-100 in PBS. After blocking of nonspecific sites with 1% BSA in PBS, sections were incubated for 40 minutes with Alexa Fluor 568–conjugated primary antibody against Ki-67 (Abcam, CAT. NO: ab197234) a marker of active cellular proliferation.[256] Slides were then stained with 4,6-diamidino-2-phenylindole for 10 minutes and imaged with a laser-scanning confocal microscope (A1R HD; Nikon Instruments Inc., Tokyo, Japan). Cells were counted by nuclei using ImageJ, and the proportion expressing Ki-67 was taken as an estimate of relative cellular proliferation between treatment groups. Ten random frames were taken per tissue section, with five tissue sections imaged per patient cell line and treatment group.

Collagen constructs were cast with equal cell density, therefore there should be equal variance in cell density between treatment groups after the experimental incubation period. This was assessed through fluorescent microscopy. Briefly, deparaffinized and hydrated sections were permeabilized for 30 minutes with 1% Triton X-100 (Sigma-Aldrich) in PBS (Sigma-Aldrich). Slides were then stained with 4,6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich) for 10 minutes and imaged with a laser-scanning confocal microscope (A1R HD; Nikon Instruments Inc., Tokyo, Japan). Relative cell density was determined by cell (nucleus) count standardized to area of collagen autofluorescence (in pixels) within each section and measured using ImageJ. Ten random frames were taken with the 40x objective per tissue section, with three tissue sections imaged per patient cell line and treatment group. The laser intensity settings were kept consistent between slides to facilitate consistent comparison between replicates.

5.2.8 LIVE/DEAD Cytotoxicity Assays

To assess the relative cellular viability between different experimental conditions, an *in situ* fluorescence-based LIVE/DEAD assay was used. This is a well reported method for estimating the cytotoxicity of an intervention. In brief, two florescent dyes, fluorescein diacetate and propidium iodide, are added to the culture media surrounding the collagen constructs. Fluorescein diacetate is converted into a blue fluorescent molecule by esterases within living cells. Propidium iodide (red) cannot pass through a viable cell's membrane, however it can penetrate disordered areas of dead cell membranes and then intercalates with the nuclear DNA. After a 5min incubation with the staining solution, collagen constructs were washed with PBS and immediately imaged on a laser-scanning confocal microscope (A1R HD; Nikon Instruments Inc., Tokyo, Japan). Ten random frames were taken with the 20x objective per tissue section, with three tissue sections imaged per patient cell line and treatment group. The laser intensity settings were kept consistent between samples to facilitate consistent comparison between replicates.

Using imageJ, blue signals were counted as living cells and totalled within every frame. The same was done for red signals, and these were counted as dead cells. The ratio of living (blue) to total (blue+red) cells was recorded.

5.2.9 Cell Culture and Western Blot

Ocular fibroblasts were grown in 6-well culture plates in DMEM with 10% FBS at 37°C and 5% CO_2 until 80-90% confluent. The cultures were then starved of serum for 24hrs, after which they were switched to experimental treatment media. For experiments involving inhibition of PPARɣ, we used the small molecule inhibitor GW9662 (Item Number: #70785, Cayman Chemical) at a concentration of 1uM.

After 48hrs incubation with the indicated experimental treatment, western blot was used to assess relative protein expression between the experimental treatment groups. Briefly, cells were lysed in lysis buffer (PhosphoSafe Extraction Reagent, Novagen) containing a protease inhibitor cocktail (P2714, Sigma-Aldrich) and the crude protein lysate (10µg) was resolved using a Novex WedgeWell 4-20% tris-glycine gel (Invitrogen). Using an iBlot Gel Transfer Device (IB1001, Invitrogen), the separated protein was transferred to a nitrocellulose membrane (IB301001, iBlot Transfer Stack, Invitrogen) which was then blocked with 5% (*w/v*) bovine serum albumin (Sigma- Aldrich) in Tris buffered saline (TBST) for 1 hour at room temperature. The membranes were incubated overnight at 4°C with primary antibody diluted in TBST containing 5% BSA (w/vol). Primary antibodies used were as follows: collagen 1 (ab138492, Abcam), αSMA (ab5694, Abcam), MMP9 (ab38898, Abcam), PPARɣ (sc-7273, SantaCruz Biotechnology), SMAD2/3 (ab63672, Abcam), pSMAD2/3 (ab63399, Abcam) and GAPDH (sc-47724, Santa Cruz Biotechnology, Inc.). After incubation with primary antibodies, the blots were washed and hybridized with 1:3000 (*v/v*)

dilutions of goat anti-rabbit or anti-mouse IgG conjugated with horseradish peroxidase (Santa Cruz Biotechnology, Inc.). Visualization was accomplished by applying WesternBright Quantum chemiluminescent reagent (Advansta, Inc.), with GAPDH used as a protein loading control. Imaging and relative densiometric quantification was accomplished using a ChemiDoc MP System (Bio-Rad Laboratories, Inc.) connected to Image Lab (Version 6, Bio-Rad Laboratories, Inc.).

5.2.10 Statistical Analysis

For reporting purposes, “N” denotes the number of biological replicates within an experiment and “n” denotes the number of technical replicates performed of each biological replicate. Comparisons between two groups were accomplished with Student’s *t* test. When more than two groups were compared to each other at a single time point, a one-way analysis of variance was used, followed by Tukey’s *post hoc* test if necessary. In cases where more than two groups were compared to each other at multiple time points, a two-way analysis of variance was used, followed by Dunnett’s multiple comparisons *post hoc* test with a single pooled variance if necessary. All statistical analyses were performed using Prism 8 (Version 8.01, GraphPad Software Inc.) statistical software. Values for $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$ were considered significant.

5.3 Results and Discussion

5.3.1 Within inflammation induced fibroblasts, melittin increases the production of acetyl-COX2 products relative to COX2 products after ASA exposure

Relatively small amounts of acetyl-COX2 products have been measured *in vivo* after ASA-induced COX2 acetylation in humans.[226] This led us to hypothesize that the acetylated COX2 enzyme may be limited in its production of acetyl-COX2 products, potentially by the competitive inhibitory activity of the co-localized salicylate ion – the byproduct of ASA's de-acetylation. In an effort to displace the salicylate ion from the active site of acetyl-COX2, we employed an extremely potent phospholipase A2 agonist to increase the availability of PUFA precursors for downstream processing through COX2 or acetyl-COX2. Melittin, isolated from wasp venom, directly increases the activity of PLA2 and is responsible for the swelling associated with their sting. It was previously measured to profoundly increase the production of AA by PLA2 in cell based systems.[257,258]

To assess the impact of COX2 Ser516 acetylation on inflammation induced ocular fibroblasts, they were pre-incubated with inflammatory and wound healing cytokines (Inf. Cytokines: 1ng/ml each of IL-1β, TNFα, INFɣ and TGFβ1) for 12 hours before being treated with vehicle, ASA alone or ASA+Melittin (a potent phospholipase A2 agonist). Melittin significantly increased the relative abundance of precursor PUFA's compared to both vehicle and ASA only treated ocular fibroblasts, by 200 to 400 fold (Figure 5-3A-C). ASA alone impaired the secretion of PG products kPGF1a (Figure5-3D) and PGE2 (Figure5-3E), however no significant increases were observed in acetyl-COX2 derived products, 5-HETE (Figure5-3F), 15-HETE (Figure5-3G), 17-OHDHA (Figure5-3H) and 18-HEPE (Figure5-3I). When melittin was combined with ASA, significant increases were observed in all lipid mediators analyzed (Figure5-3D-I). While the relative PG secretion was increased by approximately 2 to 20-fold, the relative RM secretion was increased by 3 to 400-fold – depending on the mediator analyzed. This suggested that a small fraction of un-acetylated COX2 was still present at the concentration of ASA assayed, however, a much greater proportion of the acetylated enzyme was likely present which accounts for the dramatic relative difference observed in COX2 vs. acetyl-COX2 product secretion.

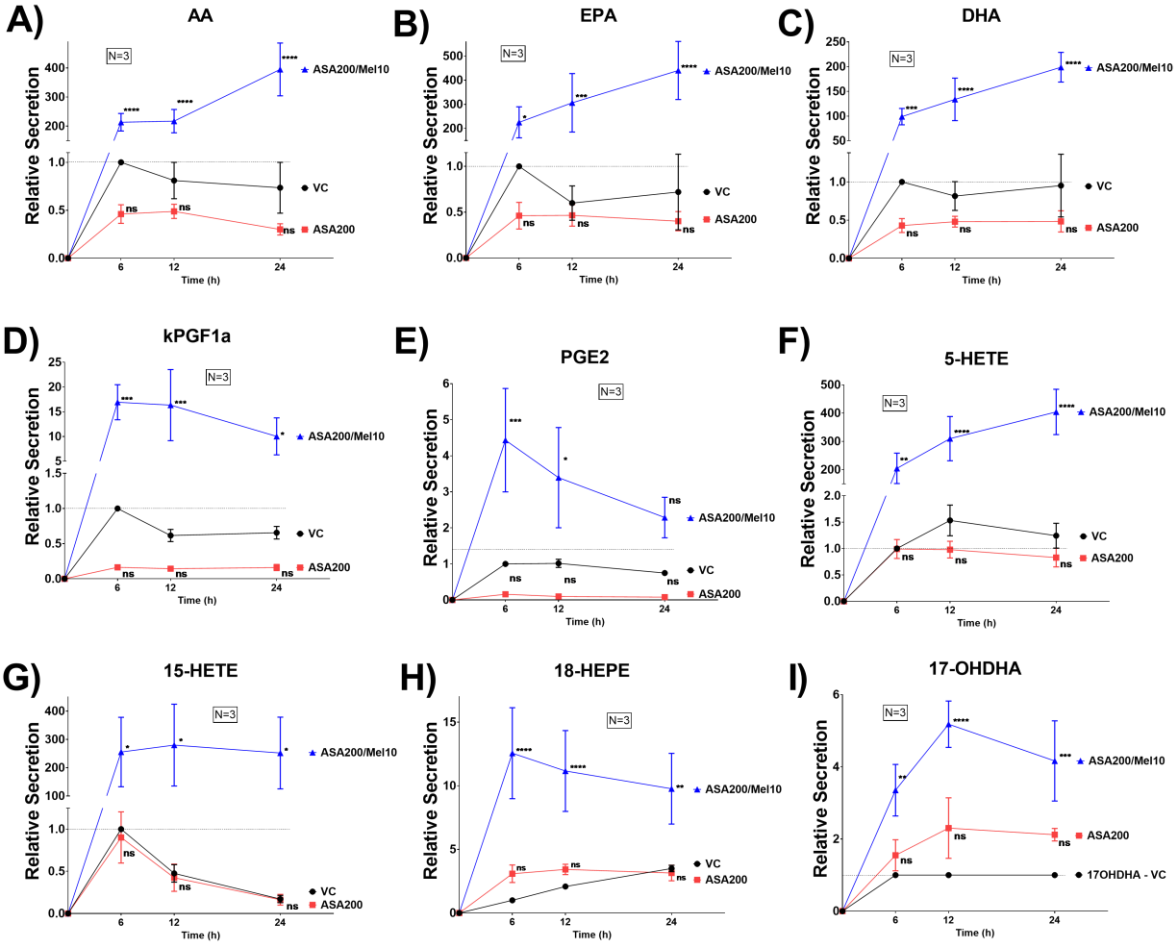


Figure 5-3 Relative inf. cytokine-induced LM secretion was determined by supernatant sampling and LC-MS/MS analysis at the indicated timepoints after exposure of inf. cytokine induced ocular fibroblasts to the indicated experimental treatment. A-C) PUFA precursors: AA, EPA and DHA. D-E) COX2 prostaglandin products: kPGF1a and PGE2. F-I) Acetyl-COX2 RM products: 5-HETE, 15-HETE, 18-HEPE and 17-OHDHA. A-I) Two-way ANOVA with Dunnett's *post hoc* test: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Figure 5-4 summarizes the relative biosynthetic activity of acetyl-COX2 vs. COX2 enzymes. The mean relative production of COX2 products (PGE2 and kPGF1a) was calculated and from this figure the mean relative production of acetyl-COX2 products (5-HETE, 15-HETE, 17-OHDHA and 18-HEPE) was subtracted. This way a value of zero would indicate equal relative biosynthetic activity between acetyl-COX2 and COX2 enzymes; positive values would indicate COX2 dominated biosynthesis of lipid mediators; and negative values would indicate acetyl-COX2 dominated lipid mediator biosynthesis. Under induced inflammatory conditions, ASA slightly reduces the proportion of COX2 derived products relative to acetyl-COX2 products, and that the addition of melittin causes a profound shift toward acetyl-COX2 dominated lipid mediator production - by several orders of magnitude (Figure5-4A).

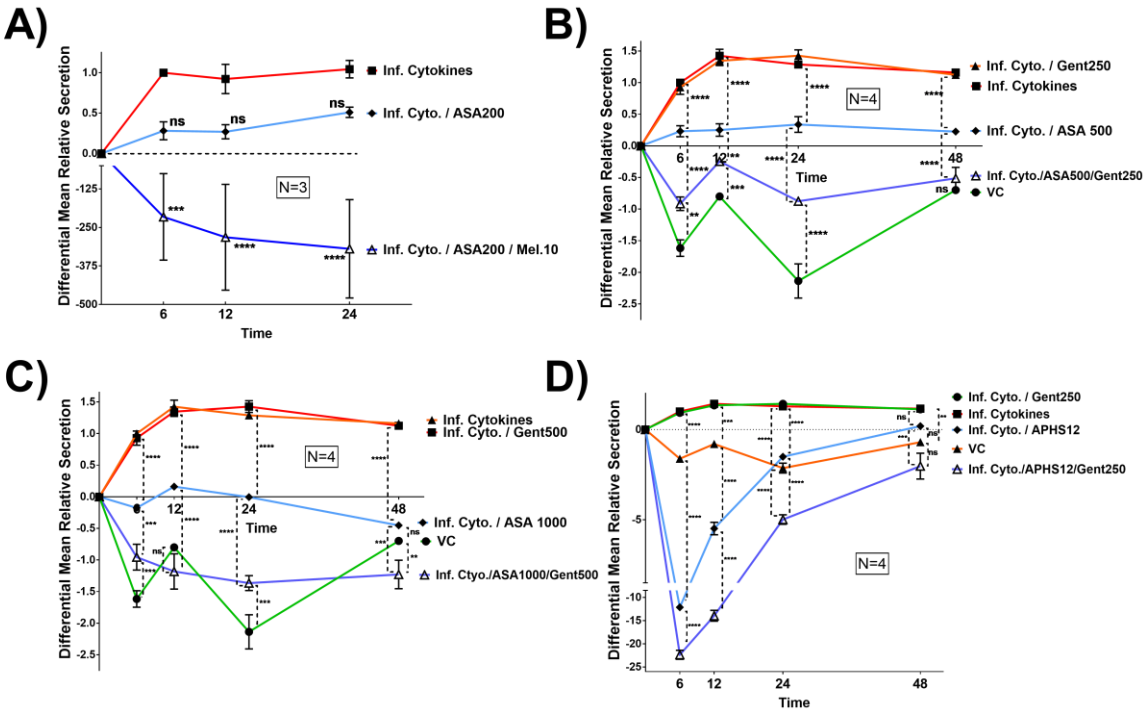


Figure 5-4 Lipid mediator secretion is shifted from a pro-inflammatory to a pro-resolving profile. Graphs display the differential mean relative secretion between pro-inflammatory mediators (PGE2 and kPGF1a) and pro-resolving mediators (5-HETE, 15-HETE, 18-HEPE and 17OH-DHA). A value of zero indicates equal relative biosynthetic activity between acetyl-COX2 and COX2; positive values indicate COX2 dominated biosynthesis of lipid mediators; and negative values indicate acetyl-COX2 dominated lipid mediator biosynthesis. Supernatant was sampled from cultured ocular fibroblasts after co-incubation with inf. cytokines and one of: A) ASA ± melittin (µg/ml, N=3), B and C) ASA ± gentamicin (µg/ml, N=4), or D) APHS ± gentamicin (µg/ml, N=4) at the indicated timepoints. The vehicle control was serum free DMEM and did not contain inf. cytokines. Differential mean relative secretion: ((PGE2 + kPGF1a) / 2) – ((5-HETE + 15-HETE + 18-HETE + 17-OHDHA)/4). Two-way ANOVA with Dunnett's post hoc: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

5.3.2 Less potent PLA2 agonist and more specific COX2 Ser516 acetylating agent exert similar modulatory effects on lipid mediator production

Previous authors have modelled lipid derived signaling molecules similar to neurotransmitters in that, theoretically, modulation of signaling activity should be preferred to its abrogation.[259] The concept of modulating the production of LMs to avoid the negative consequences of inflammation on patient tissues is not new. The competitive NSAID drugs partially block COX2, eliciting a dampening of PG production and therefore mitigating their impact. However, altering enzymatic activity in order to stimulate PUFA precursor production at the same time as redirecting the bulk of the downstream, now acetylated, COX2 enzymatic activity toward RM production is a novel approach, and has the potential to function as an immuno-resolvent intervention. Immuno-resolvents are theoretically preferable to immuno-suppressive modalities. Thus, we wished to determine if additional molecules with similar effects on COX2 and PLA2 could recreate the modulatory effects observed in the previous LM secretion experiment with ASA and melittin.

The second LM secretion experiment we conducted involved the gentler PLA2 agonist, gentamicin,[260] and the more potent COX2 Ser516 acetylating agent, APHS. APHS possesses the same COX2 Ser516 acetylating function as ASA, and, due to its larger lipophilic structure, it was shown to be approximately 20x more specificity for COX2 over COX1.[254] For reference, ASA has previously been

measured to have approximately 10 to 20 times greater specificity for COX1 over COX2.[261,262]

Gentamicin had a small stimulatory effect on the relative secretion of PUFA precursors AA, EPA and DHA when compared to the inf. cytokines induction group (Figure 5-5A-C). Both ASA and APHS were able to significantly reduce the secretion of PG products kPGF1a (Figure 5-5D-F) and PGE2 (Figure 5-5G-I). The addition of gentamicin to any experimental treatment group had no effect on PG synthesis (Figure 5-5D-I). These results confirmed the ability of both COX2 Ser516 acetylating agents to prevent PG synthesis within ocular fibroblasts.

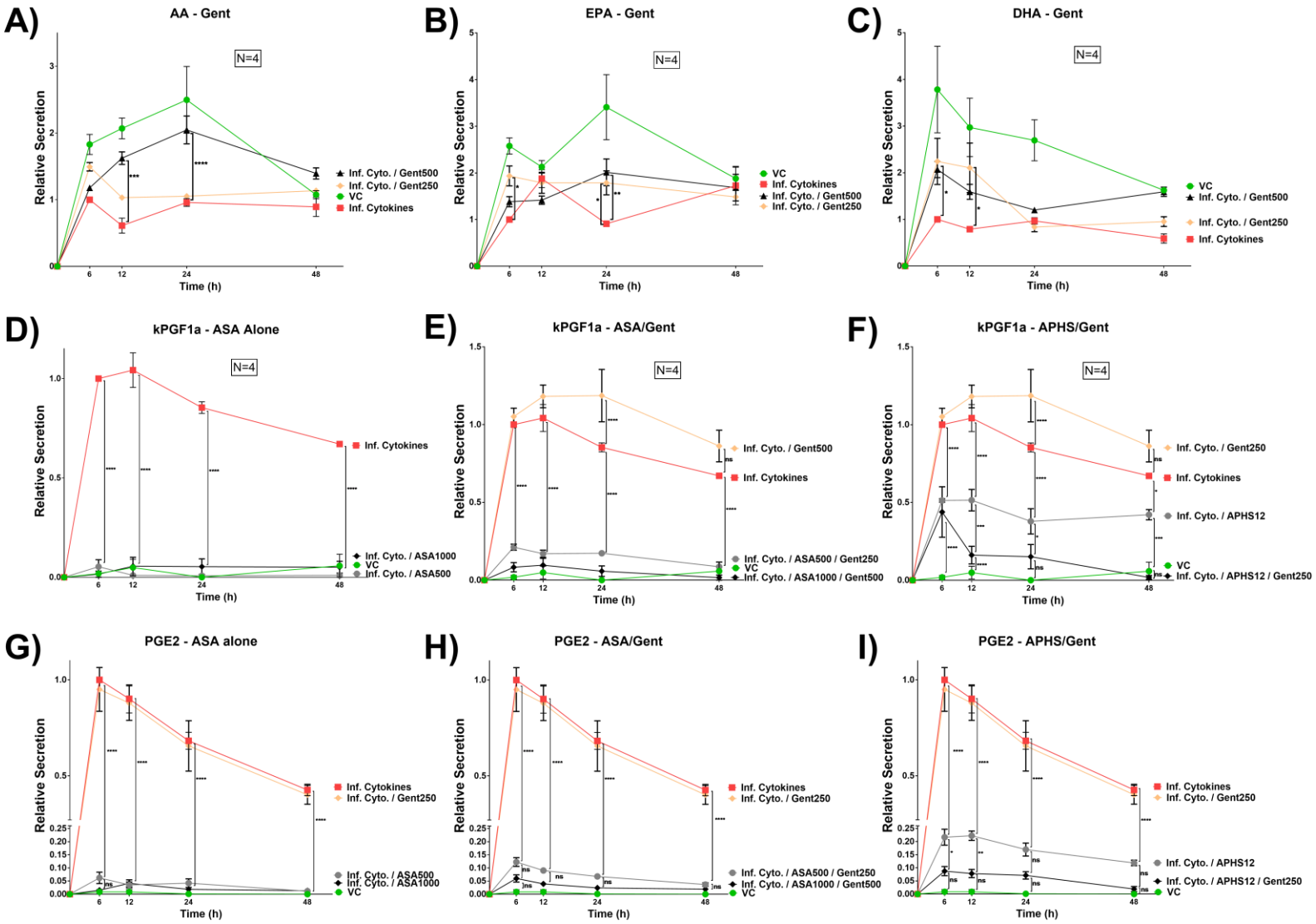

A)
AA - Gent
N=4
Relative Secretion
Time (h)
Inf. Cyto. / Gent500
Inf. Cyto. / Gent250
VC
Inf. Cytokines
B)
EPA - Gent
N=4
Relative Secretion
Time (h)
VC
Inf. Cytokines
Inf. Cyto. / Gent500
Inf. Cyto. / Gent250
C)
DHA - Gent
N=4
Relative Secretion
Time (h)
VC
Inf. Cyto. / Gent500
Inf. Cyto. / Gent250
Inf. Cytokines
D)
kPGF1a - ASA Alone
N=4
Relative Secretion
Time (h)
Inf. Cytokines
Inf. Cyto. / ASA1000
VC
Inf. Cyto. / ASA500
E)
kPGF1a - ASA/Gent
N=4
Relative Secretion
Time (h)
Inf. Cyto. / Gent500
Inf. Cytokines
Inf. Cyto. / ASA500 / Gent250
VC
Inf. Cyto. / ASA1000 / Gent500
F)
kPGF1a - APHS/Gent
N=4
Relative Secretion
Time (h)
Inf. Cyto. / Gent250
Inf. Cytokines
Inf. Cyto. / APHS12
VC
Inf. Cyto. / APHS12 / Gent250
G)
PGE2 - ASA alone
Relative Secretion
Time (h)
Inf. Cytokines
Inf. Cyto. / Gent250
Inf. Cyto. / ASA500
Inf. Cyto. / ASA1000
VC
H)
PGE2 - ASA/Gent
Relative Secretion
Time (h)
Inf. Cytokines
Inf. Cyto. / Gent250
Inf. Cyto. / ASA500 / Gent250
Inf. Cyto. / ASA1000 / Gent500
VC
I)
PGE2 - APHS/Gent
Relative Secretion
Time (h)
Inf. Cytokines
Inf. Cyto. / Gent250
Inf. Cyto. / APHS12
Inf. Cyto. / APHS12 / Gent250
VC

Figure 5-5 Relative inf. cytokine-induced LM secretion was determined by supernatant sampling and LC-MS/MS analysis at the indicated timepoints after exposure of inf. cytokine induced ocular fibroblasts to the indicated experimental treatment. A-C) PUFA precursors: AA, EPA and DHA. D-I) COX2 prostaglandin products: kPGF1a and PGE2. A-I) Two-way ANOVA with Dunnett's *post hoc* test: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

When examining the secretion of RMs, we noticed ASA had a small stimulatory effect on 5-HETE (Figure 5-6A), 15-HETE (Figure 5-6D) and 18-HEPE (Figure 5-6G) secretion in the presence of inf. cytokines, relative to inf. cytokines alone. However, the significant effects observed were transient and small. APHS alone had a profoundly significant stimulatory effect on the relative secretion of 5-HETE (Figure 5-6C), which peaked at 6hrs and returned to inf. cytokine treated only levels by 24hrs. APHS alone also had profound stimulatory effects on 15-HETE (Figure 5-6F) and 18-HEPE (Figure 5-6I) secretion under inflammatory conditions, relative to inf. cytokine treatment alone, and remained significant past 48hrs post-treatment. Finally, when ASA or APHS were combined with gentamicin, significant and lasting increases in 5-HETE (Figure 5-6B and C), 15-HETE (Figure 5-6E and D) and 18-HEPE (Figure 5-6H and I) in were observed in the presence of inf. cytokines, relative to either ASA or APHS alone in the presence of inf. cytokines. To our knowledge this is the first characterization of acetyl-COX2 products being triggered by APHS exposure under inflammatory conditions.

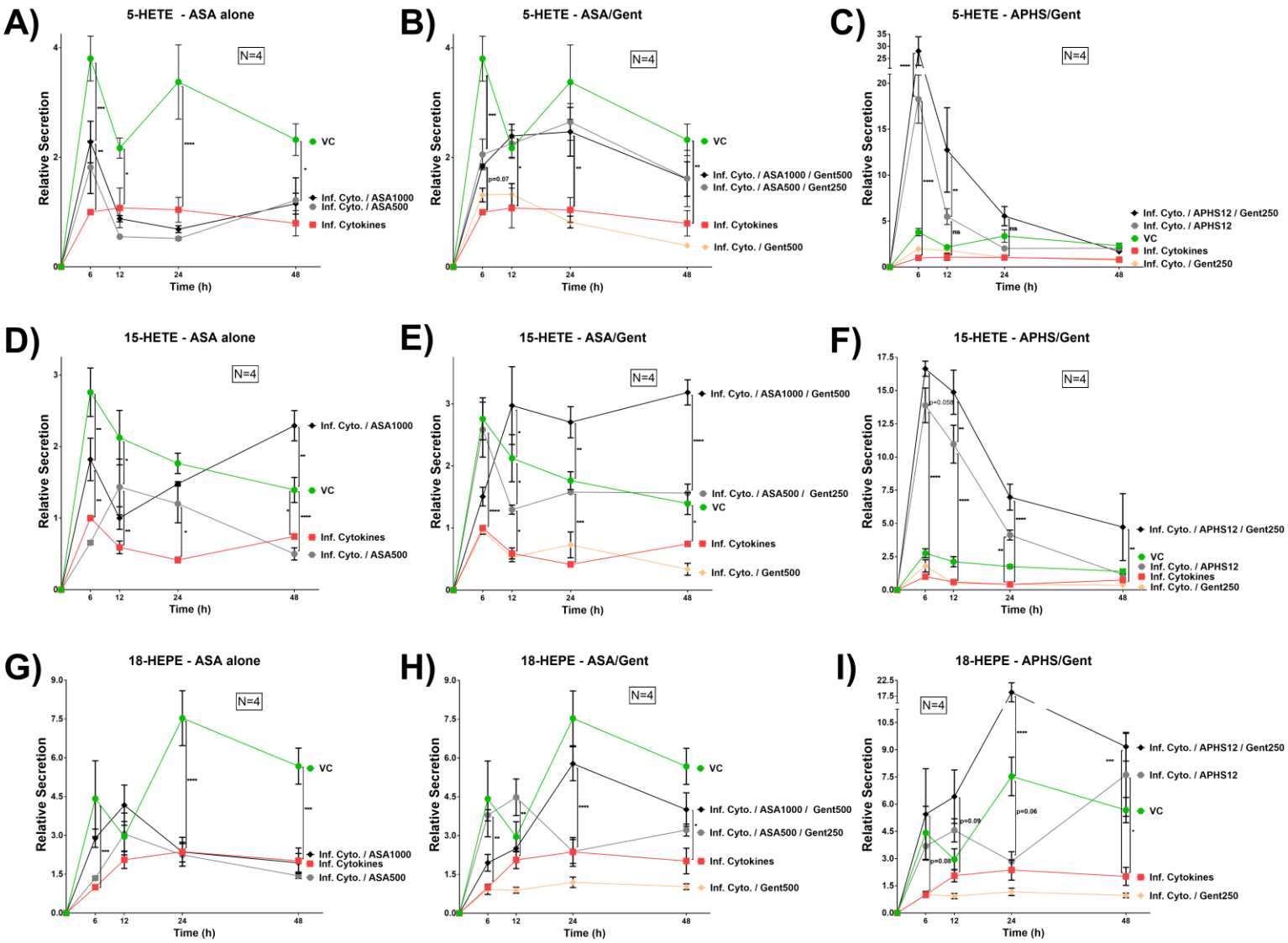

A)
5-HETE - ASA alone
N=4
Relative Secretion
Time (h)
VC
Inf. Cyto. / ASA1000
Inf. Cyto. / ASA500
Inf. Cytokines
B)
5-HETE - ASA/Gent
N=4
Relative Secretion
Time (h)
VC
Inf. Cyto. / ASA1000 / Gent500
Inf. Cyto. / ASA500 / Gent250
Inf. Cytokines
Inf. Cyto. / Gent500
C)
5-HETE - APHS/Gent
N=4
Relative Secretion
Time (h)
Inf. Cyto. / APHS12 / Gent250
Inf. Cyto. / APHS12
VC
Inf. Cytokines
Inf. Cyto. / Gent250
D)
15-HETE - ASA alone
N=4
Relative Secretion
Time (h)
Inf. Cyto. / ASA1000
VC
Inf. Cytokines
Inf. Cyto. / ASA500
E)
15-HETE - ASA/Gent
N=4
Relative Secretion
Time (h)
Inf. Cyto. / ASA1000 / Gent500
Inf. Cyto. / ASA500 / Gent250
VC
Inf. Cytokines
Inf. Cyto. / Gent500
F)
15-HETE - APHS/Gent
N=4
Relative Secretion
Time (h)
Inf. Cyto. / APHS12 / Gent250
VC
Inf. Cyto. / APHS12
Inf. Cytokines
Inf. Cyto. / Gent250
G)
18-HEPE - ASA alone
N=4
Relative Secretion
Time (h)
VC
Inf. Cyto. / ASA1000
Inf. Cytokines
Inf. Cyto. / ASA500
H)
18-HEPE - ASA/Gent
N=4
Relative Secretion
Time (h)
VC
Inf. Cyto. / ASA1000 / Gent500
Inf. Cyto. / ASA500 / Gent250
Inf. Cytokines
Inf. Cyto. / Gent500
I)
18-HEPE - APHS/Gent
N=4
Relative Secretion
Time (h)
Inf. Cyto. / APHS12 / Gent250
Inf. Cyto. / APHS12
VC
Inf. Cytokines
Inf. Cyto. / Gent250

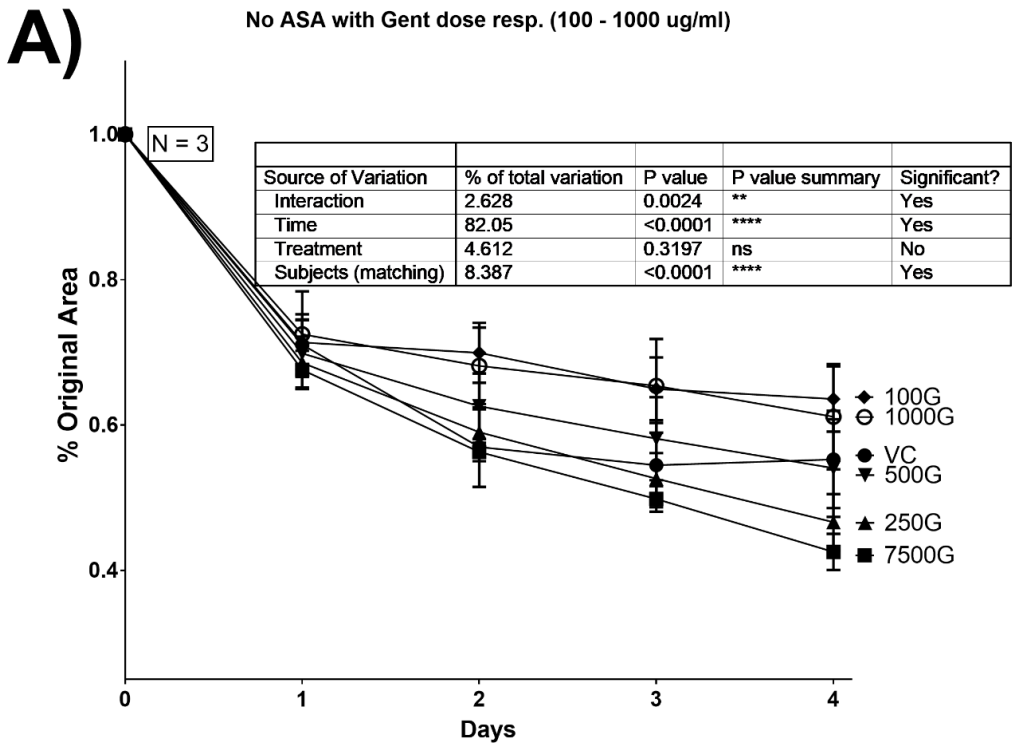
Figure 5-6: Relative inf. cytokine-induced LM secretion was determined by supernatant sampling and LC-MS/MS analysis at the indicated timepoints after exposure of inf. cytokine induced ocular fibroblasts to the indicated experimental treatment. A-C) PUFA precursors: AA, EPA and DHA. D-I) Acetyl-COX2 RM products: 5-HETE, 15-HETE and 18-HEPE. A-I) Two-way ANOVA with Dunnett's *post hoc* test: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Figure 5-4B, C and D illustrate the summary effects of ASA, APHS and gentamicin on the relative secretion of COX2 vs acetyl-COX2 products. ASA and APHS alone were both able to disrupt COX2 activity and induce a significant and dose dependent shift toward acetyl-COX2 dominated LM production. When gentamicin was combined with ASA or APHS, significantly greater acetyl-COX2 activity was observed relative to ASA or APHS alone in the presence of inf. cytokines. These results support ASA and APHS having similar modulatory effects on the production of pro-inflammatory and pro-resolving lipid mediators. Results also demonstrate that both melittin and gentamicin can augment the pro-resolving effects of COX2 acetylating molecules.

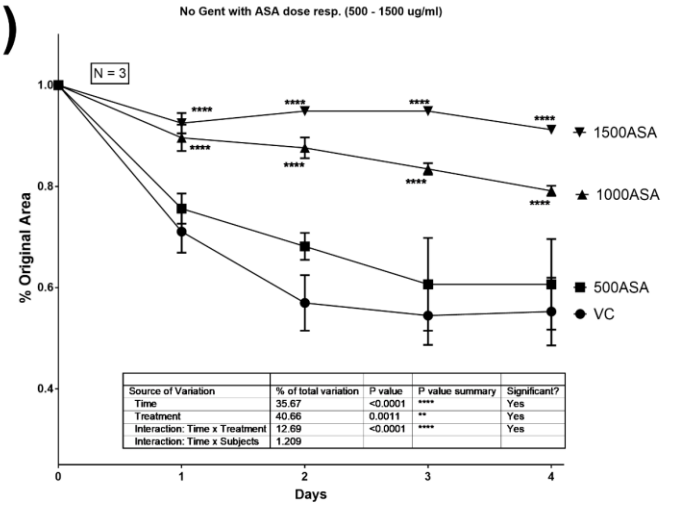
5.3.3 COX2 acetylation inhibits in vitro wound healing, PLA2 agonist increases effect

Cell-mediated extracellular matrix contraction is a hallmark of *in vivo* wound healing. We assessed the effects of lipid mediator modulation using ASA and gentamicin on cell-mediated collagen contraction. A collagen contraction assay with a double dose response for each drug was used to determine the optimal concentrations for inhibition of cell-mediated contraction. Gentamicin did not have a significant effect on cell-mediated collagen contraction at any tested

concentration (Figure 5-7A). Alone, ASA significantly impaired cell-mediated collagen contraction in a dose dependent manner (Figure 5-7B). Gentamicin was not able to increase the effect of low dose ASA on cell-mediated collagen contraction (Figure 5-7C); however, significantly greater inhibitory effects on cell-mediated contraction were seen when gentamicin was combined with higher doses of ASA (Figure 5-7D and E).

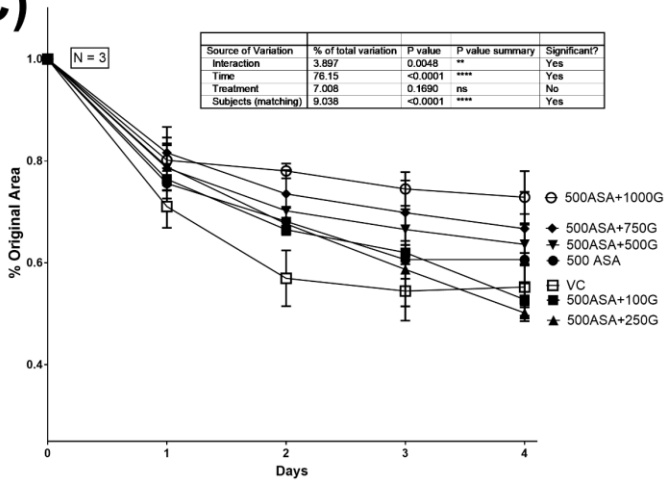


B)



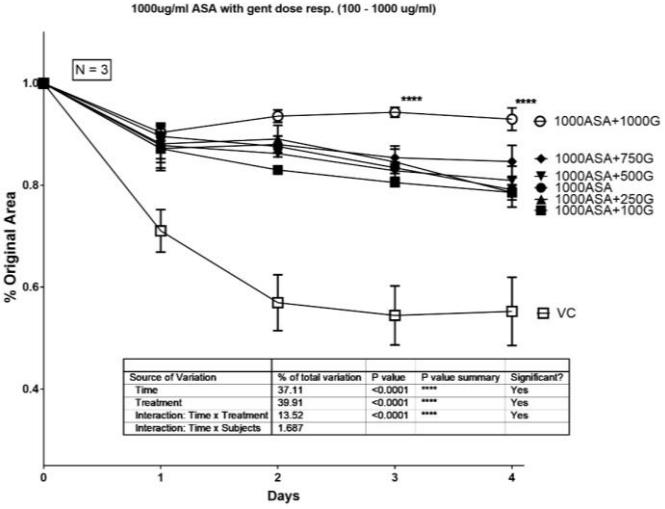
Source of Variation	% of total variation	P value	P value summary	Significant?
Time	35.67	<0.0001	****	Yes
Treatment	40.66	0.0011	**	Yes
Interaction: Time x Treatment	12.69	<0.0001	****	Yes
Interaction: Time x Subjects	1.209			

C)



Source of Variation	% of total variation	P value	P value summary	Significant?
Interaction	3.897	0.0048	**	Yes
Time	76.15	<0.0001	****	Yes
Treatment	7.008	0.1690	ns	No
Subjects (matching)	9.038	<0.0001	****	Yes

D)



Source of Variation	% of total variation	P value	P value summary	Significant?
Time	37.11	<0.0001	****	Yes
Treatment	39.91	<0.0001	****	Yes
Interaction: Time x Treatment	13.52	<0.0001	****	Yes
Interaction: Time x Subjects	1.687			

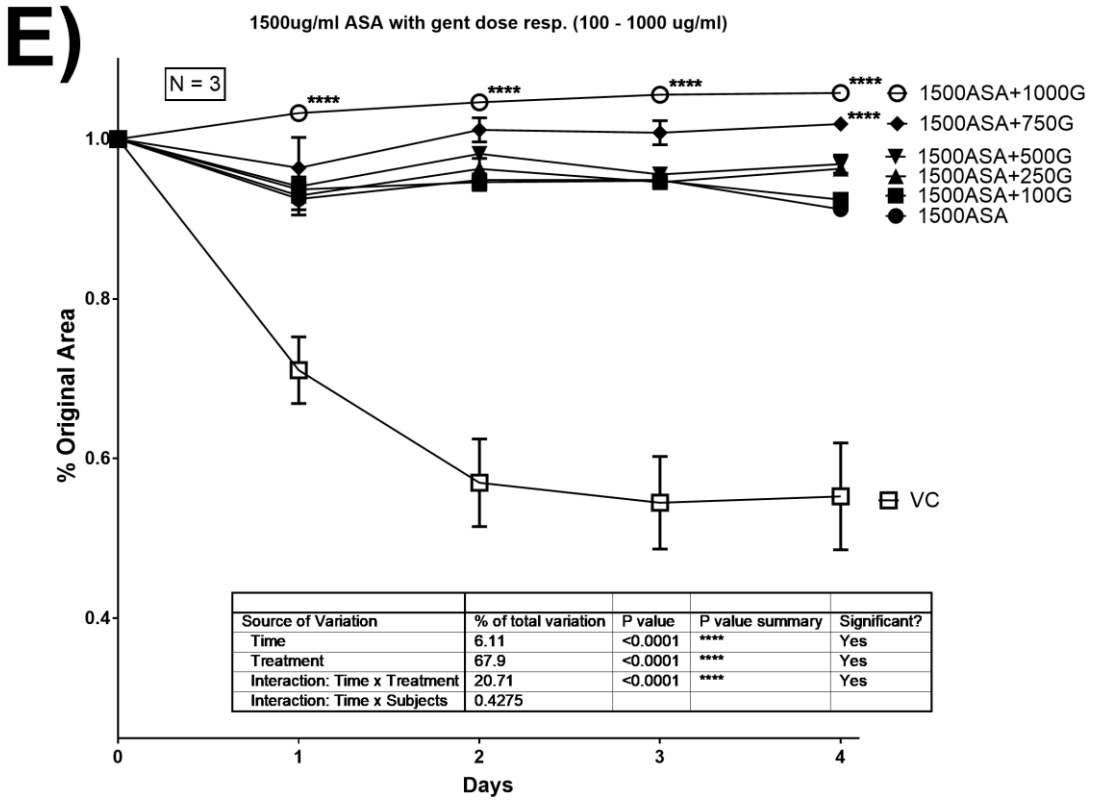
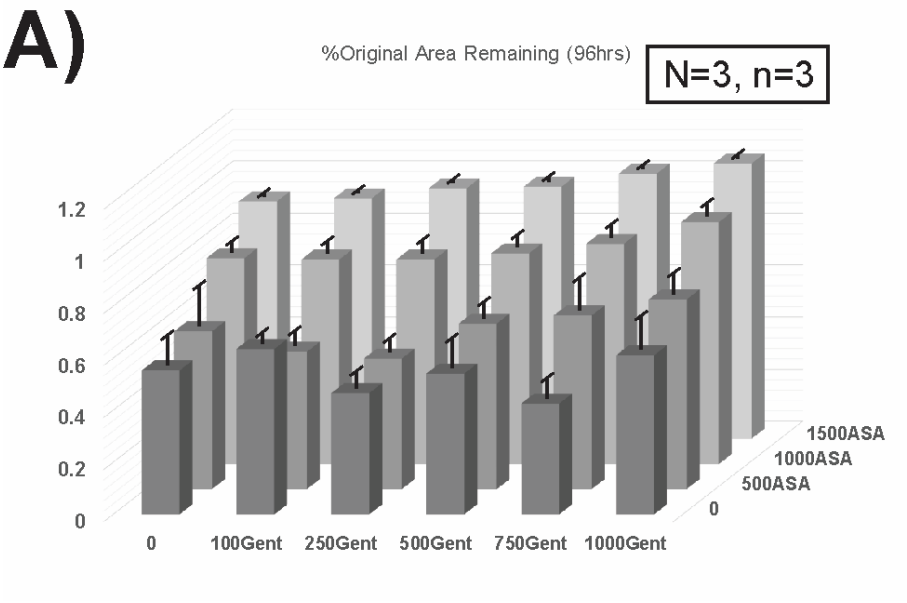


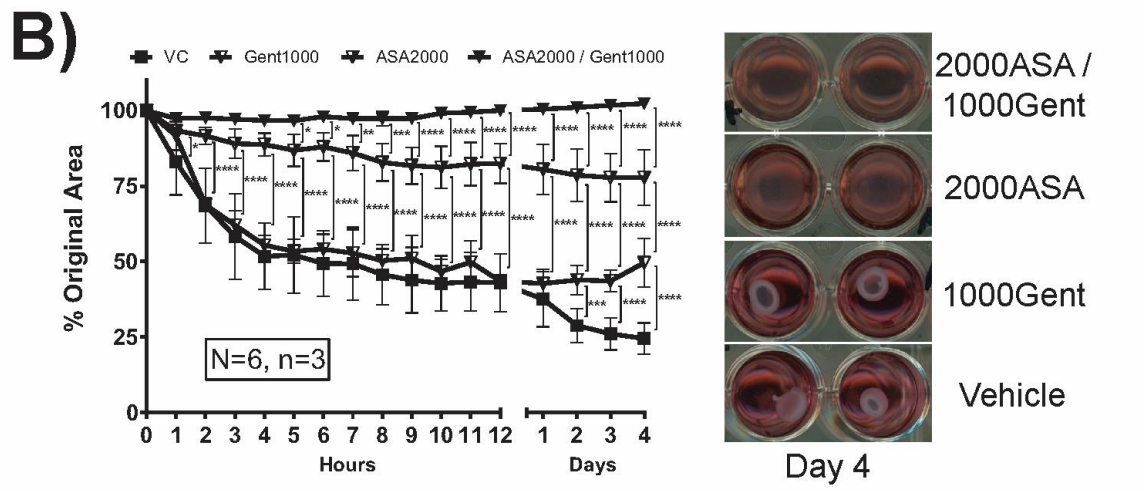
Figure 5-7 Effects of ASA and gentamicin on ocular-fibroblast mediated collagen contraction. A) Gentamicin alone had no significant effect relative to vehicle control on the area of collagen expressed as a proportion of baseline area at any timepoint. B) ASA alone had a dose dependent inhibitory effect. C) The effects of 500 µg/ml ASA were not significant, and not potentiated significantly by gentamicin, although a trend was evident. D) The effects of 1000 µg/ml ASA were significantly inhibitory, and gentamicin significantly increased the observed inhibition. E) The effects of 1500 µg/ml ASA were significantly inhibitory, and gentamicin significantly increased the inhibition observe. A-E) Two-way ANOVA with Dunnett's *post hoc* test: N=3, n=2; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

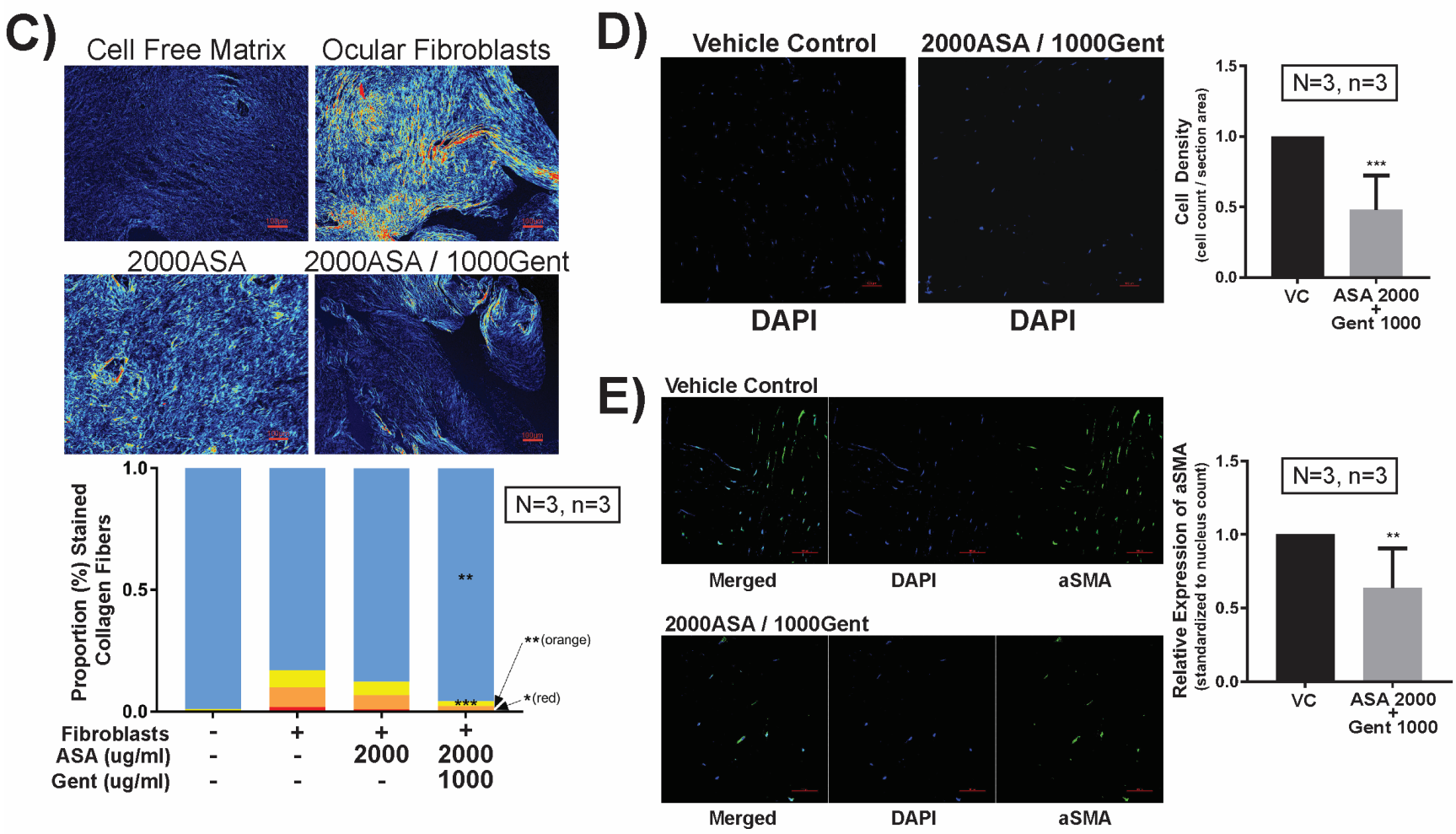
Two-drug dose-optimization is illustrated in Figure 5-8A. Complete inhibition of cell-mediated collagen contraction was achieved with 2000 μg/ml of ASA and 1000μg/ml gentamicin (Figure 5-8B). Cell-mediated collagen remodeling was also assessed under the indicated treatment conditions (Figure 5-8C). Cell free collagen matrices stained a uniform blue with picrosirius red staining, indicating a homogeneous and loosely packed collagen fibril arrangement. When ocular fibroblasts were cultured within the collagen matrices, areas of red, orange and yellow staining became apparent, which are regions of high collagen fibril density and are inferred to indicate the local occurrence of cell-mediated collagen remodeling. ASA alone was able to decrease the relative proportion of red, orange and yellow stained collagen fibrils within matrices containing ocular fibroblasts, implying an impairment of cell-mediated matrix remodeling. When gentamicin was combined with ASA, an even greater inhibition of cell-mediated matrix remodeling was observed relative to both ASA alone and vehicle control.

Matrices and the contained cells were fixed and stained immunohistochemically to evaluate cellularity (Figure 5-8D) as well as the expression of myofibroblastic and proliferative biomarkers, αSMA (Figure 5-8E) and Ki-67 (Figure 5-8F) respectively. Combined, ASA and gentamicin treatment had an inhibitory effect on cellularity, as measured by the number of nuclei per unit area of collagen section. Combined, ASA and gentamicin were able to significantly reduce the amount of αSMA staining per nuclei relative to vehicle control. Finally, the combination treatment was able to significantly decrease the proportion of cells expressing Ki-67 within the collagen matrix relative to vehicle control. These data

suggest that the LM modulating composition, ASA and gentamicin, causes an impairment of the wound healing related functions and biomarkers of ocular fibroblasts.






C)
Cell Free Matrix
Ocular Fibroblasts
2000ASA
2000ASA / 1000Gent
Proportion (%) Stained Collagen Fibers
1.0
0.5
0.0
N=3, n=3
**
***
**(orange)
*(red)
Fibroblasts - + + +
ASA (ug/ml) - - 2000 2000
Gent (ug/ml) - - - 1000
D)
Vehicle Control
2000ASA / 1000Gent
DAPI
DAPI
Cell Density
(cell count / section area)
1.5
1.0
0.5
0.0
N=3, n=3
***
VC
ASA 2000 + Gent 1000
E)
Vehicle Control
Merged
DAPI
aSMA
2000ASA / 1000Gent
Merged
DAPI
aSMA
Relative Expression of aSMA
(standardized to nucleus count)
1.5
1.0
0.5
0.0
N=3, n=3
**
VC
ASA 2000 + Gent 1000

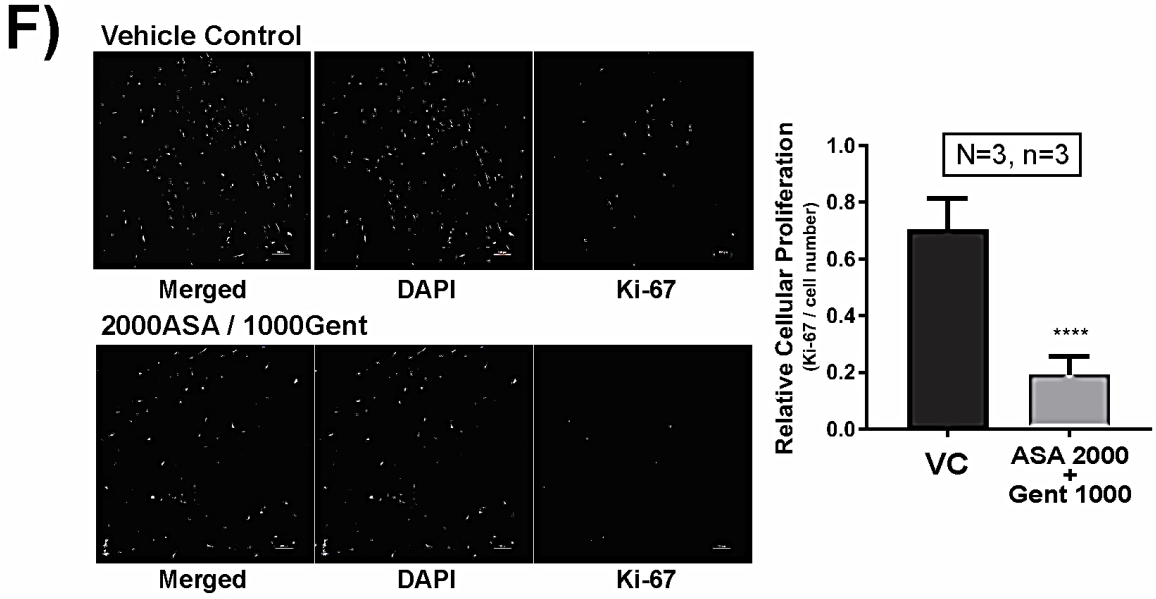
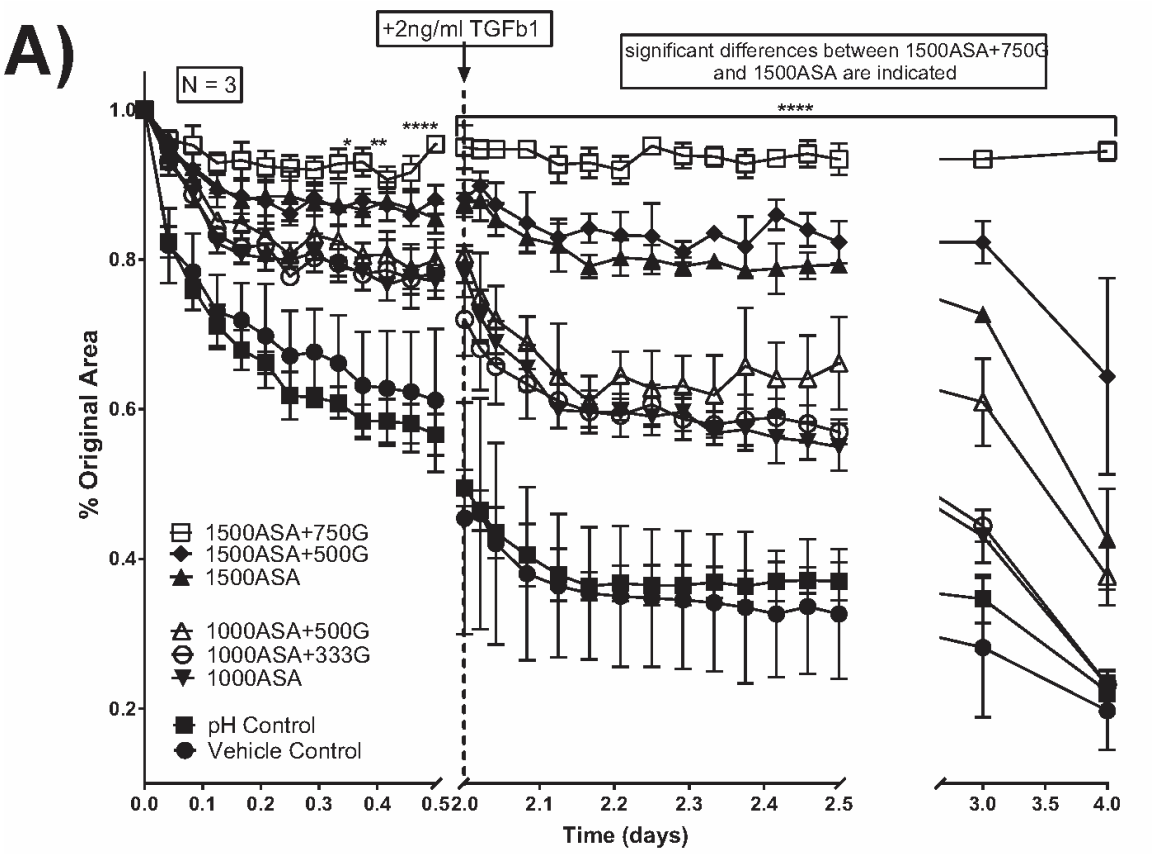
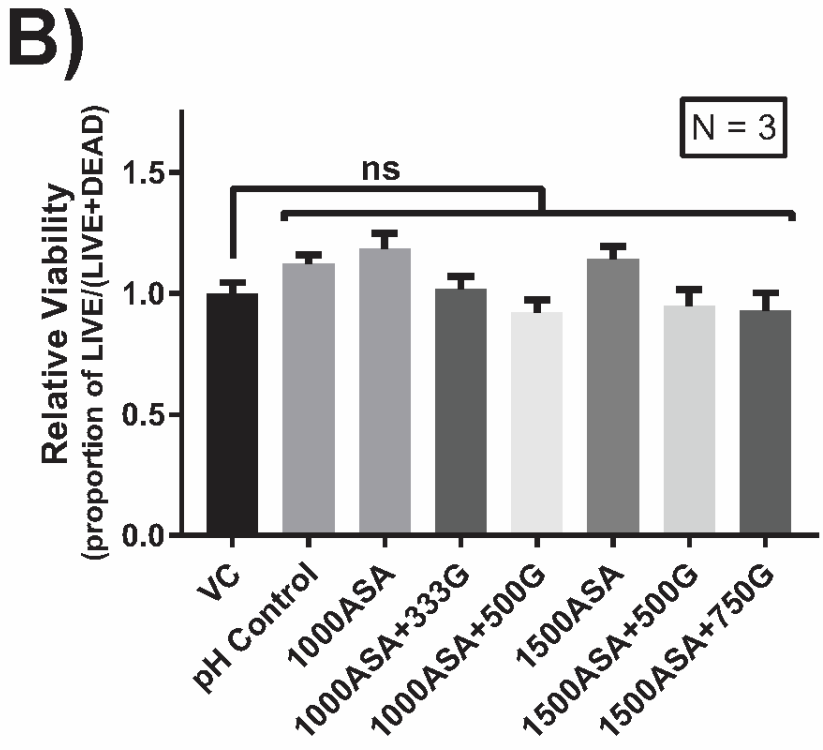


Figure 5-8 ASA ± gentamicin can modulate the unstimulated *in vitro* wound healing functions of ocular fibroblasts. A) Graph representing the effects of ASA and gentamicin (µg/ml) on the ocular fibroblast-mediated contraction of collagen matrices. Bars depict mean ± SD area (as a proportion of baseline area) of collagen remaining after 96hrs of contraction (N=3, n=3). B) Temporal effects of ASA ± gentamicin (µg/ml) on cell-mediated collagen contraction over a period of 96hrs. Two-way ANOVA with Dunnett's *post hoc* test (N=6, n=3; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001). C) The effects of ASA and gentamicin (ug/ml) on ocular fibroblast-mediated collagen remodelling after 12 days incubation. Picrosirius red stain: collagen fibril density increases as color changes from blue to yellow to orange to red. One-way ANOVA with Tukey's *post hoc* test: N=3, n=3, frames=10; *p<0.05, **p<0.01, ***p<0.001. D) After 12 days of culture, effects of ASA combined with gentamicin (µg/ml) on the number of fibroblasts per unit area of collagen matrix (N=3, n=3, frames=10. E) ASA and gentamicin (µg/ml) reduced the amount of area positive for αSMA per nuclei within the collagen matrix (N=3, n=3, frames=10), and F) the proportion of nuclei that stained positive for the proliferative biomarker, Ki-67, was also reduced (N=3, n=3, frames=10). D-F) Student's *t* test: **p<0.01, ***p<0.001, ****p<0.0001.

5.3.4 COX2 acetylation w/PLA2 agonist can overcome TGFβ1 induced collagen contraction

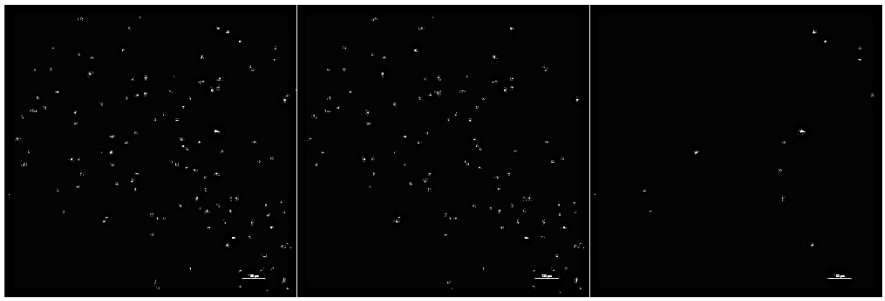
To assess how robust the inhibition of cell-mediated collagen contraction observed with COX2 acetylation was, we added 2ng/ml TGFβ1 to the culture media of actively contracting collagen constructs at the experiment's midpoint (Figure 5-9A). During the two days prior to TGFβ1 stimulation, a dose dependent inhibition of contraction was observed for both concentrations of ASA assessed. Further, gentamicin was able to significantly augment these effects. After TGFβ1 stimulation, contraction was accelerated greatly in the control groups, however less so in the ASA treated groups. In fact, after TGFβ1 stimulation, the dose dependent, ASA-induced impairment of contraction became more apparent and, the effect continued to be significantly augmented by gentamicin. Cellular viability was assessed within the collagen constructs by incubating them in FDA and PI to label living and dead cells, respectively, and then imaging on a confocal laser scanning microscope (Figure 5-9B and C). When the ratio of living cells to total cells was compared across treatment groups, there were no significant differences observed between any of the groups assessed. This suggests the observed treatment effects stem from a modulation of cellular activity as opposed to the by-product of a cytotoxic effect.


A)
+2ng/ml TGFb1
significant differences between 1500ASA+750G and 1500ASA are indicated
N = 3
****
*
**
****
% Original Area
1.0
0.8
0.6
0.4
0.2
1500ASA+750G
1500ASA+500G
1500ASA
1000ASA+500G
1000ASA+333G
1000ASA
pH Control
Vehicle Control
0.0 0.1 0.2 0.3 0.4 0.5
2.0 2.1 2.2 2.3 2.4 2.5
3.0 3.5 4.0
Time (days)


B)
N = 3
ns
Relative Viability
(proportion of LIVE/(LIVE+DEAD)
1.5
1.0
0.5
0.0
VC
pH Control
1000ASA
1000ASA+333G
1000ASA+500G
1500ASA
1500ASA+500G
1500ASA+750G

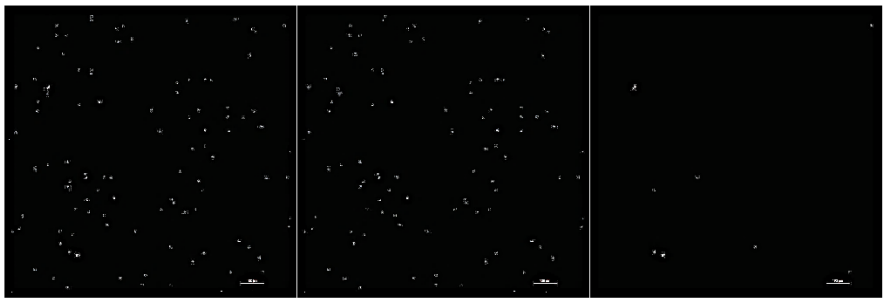
C)

Vehicle



Merged LIVE DEAD

1000ASA



Merged LIVE DEAD

1000ASA / 333Gent

Merged LIVE DEAD

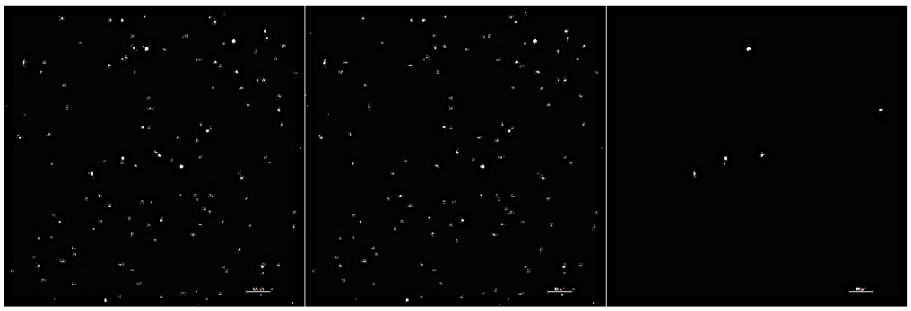
1000ASA / 500Gent

Merged LIVE DEAD

pH Control

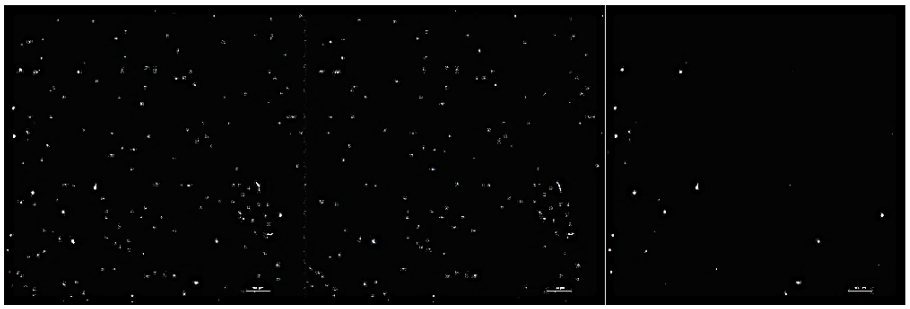
Merged LIVE DEAD

1500ASA



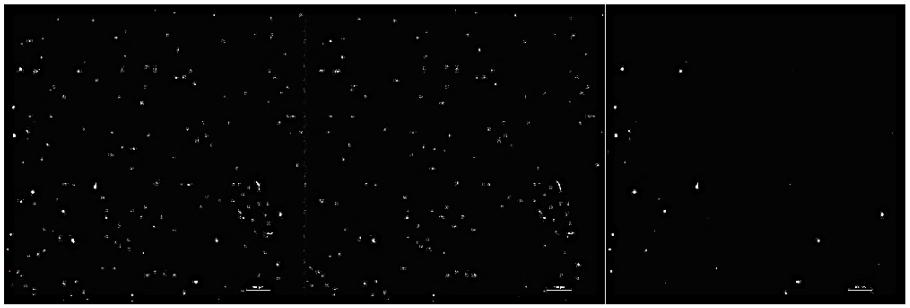
Merged LIVE DEAD

1500ASA / 500Gent



Merged LIVE DEAD

1500ASA / 750Gent



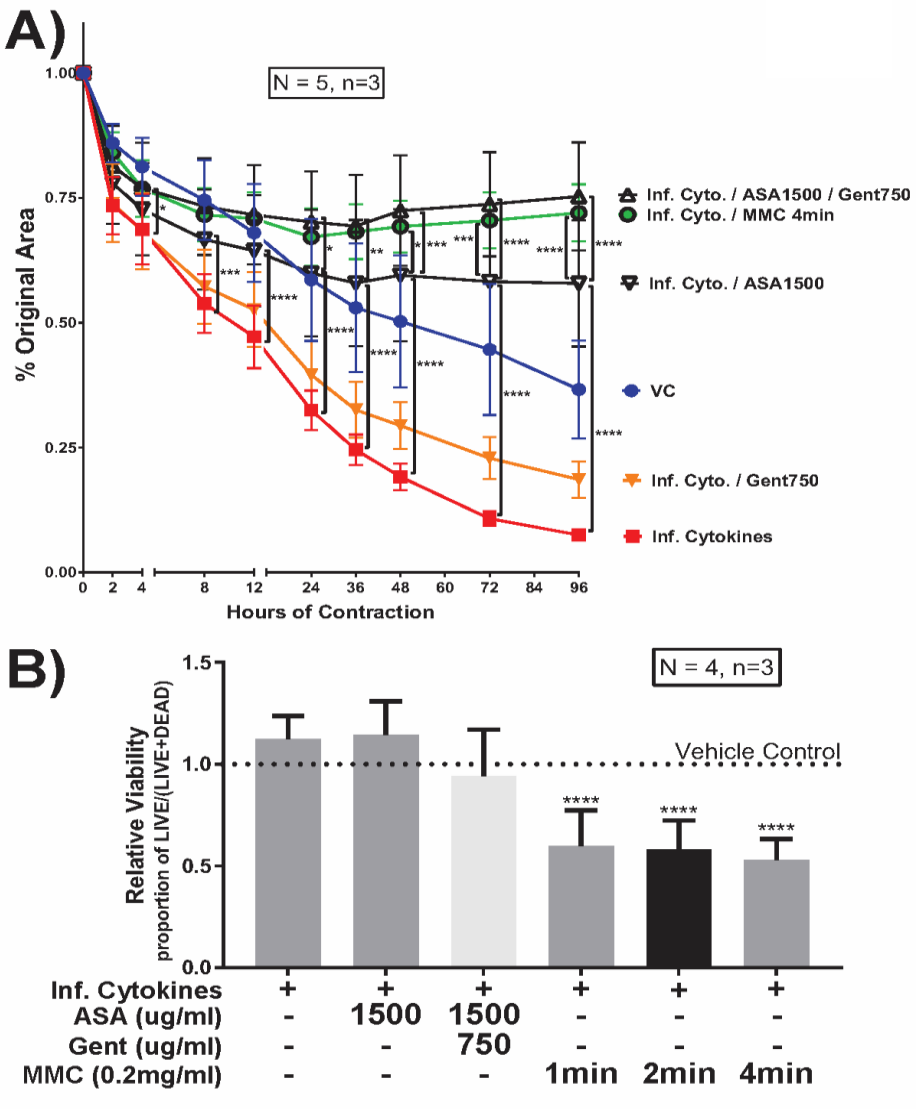
Merged LIVE DEAD

Figure 5-9 The effects of ASA and gentamicin can overcome TGFb1-induced cell-mediated collagen contraction without suppressing cellular viability. A) Un-induced contraction was assessed for the first 48hrs with the indicated experimental treatments, after 48hrs of experimental conditions, 2ng/ml was added to the culture media to assess the lasting ability of experimental treatments to impede TGFb1-induced contraction. No further experimental treatments were added at this point. Results of two-way repeated measures ANOVA with Dunnett's *post hoc* test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 for 1500ASA+750G vs. 1500ASA. B) Relative cellular viability was compared at the conclusion of the contraction assay by *in situ* LIVE/DEAD staining. One-way ANOVA showed no significant differences between groups (N=3, n=3, frames=10). C) Representative LIVE/DEAD fluorescent micrographs for the indicated treatment groups.

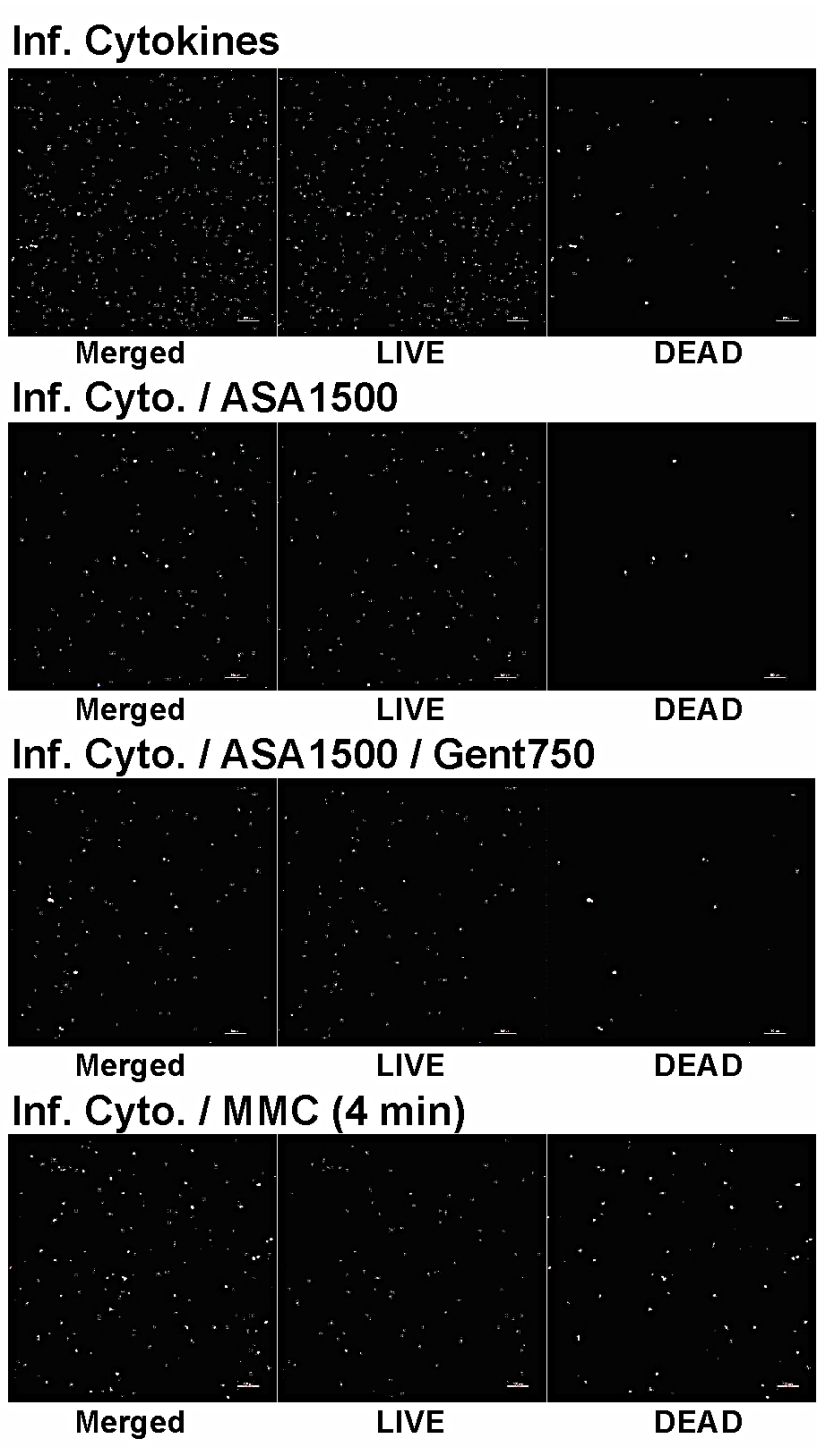
5.3.5 Inhibition of Inf. Cytokine-induced wound healing with COX2 acetylation and PLA2 agonist is comparable to that of mitomycin C *in vitro*

To model the ocular scarring *in vitro*, we assessed cell-mediated collagen contraction in the presence of inf. cytokines and compared the inhibitory actions of COX2 acetylation to that of a short duration exposure to MMC – the current clinical gold standard for reducing sub-conjunctival scarring (Figure 5-10A). Inf. cytokines significantly stimulated cell-mediated collagen contraction relative to vehicle control, supporting these growth factors' contribution to the pathological changes observed in the subconjunctiva after glaucoma surgery. On its own, ASA was able to significantly inhibit inf. cytokine-induced collagen contraction (Figure 5-10A). The effect size, however, was significantly smaller than that of a four-minute MMC application immediately prior to the initiation of contraction. When gentamicin was combined with ASA, the effect size increased significantly

and was statistically indistinguishable from that of a 4-minute exposure to MMC prior to contraction (Figure 5-10A and D). Cellular viability was assessed within the collagen constructs to assess the contribution of cytotoxicity to the observed effects on cell-mediated collagen contraction (Figure 5-10B and C). Relative to vehicle control, exposure to all tested durations of MMC exposure significantly decreased the proportion of living to total cells – confirming MMC's cytotoxic mechanism of action. No significant differences in cellular viability were observed relative to vehicle control with exposure to either ASA alone or in combination with gentamicin.



C)


Inf. Cytokines
Merged
LIVE
DEAD
Inf. Cyto. / ASA1500
Merged
LIVE
DEAD
Inf. Cyto. / ASA1500 / Gent750
Merged
LIVE
DEAD
Inf. Cyto. / MMC (4 min)
Merged
LIVE
DEAD

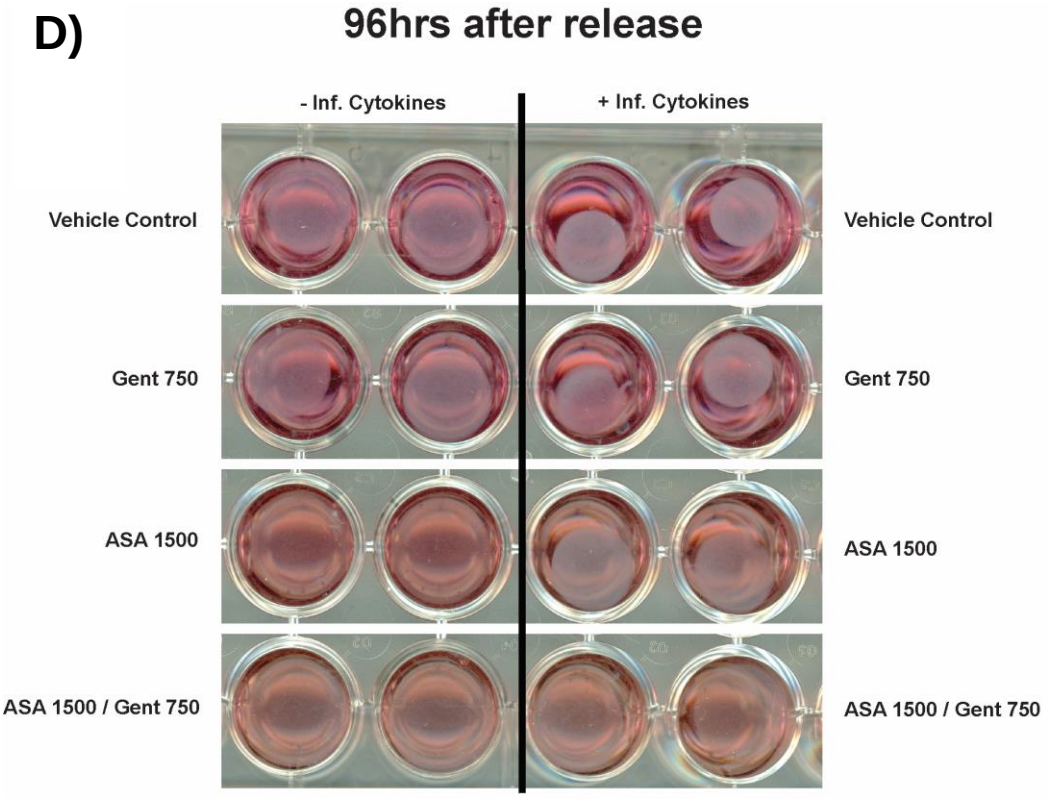
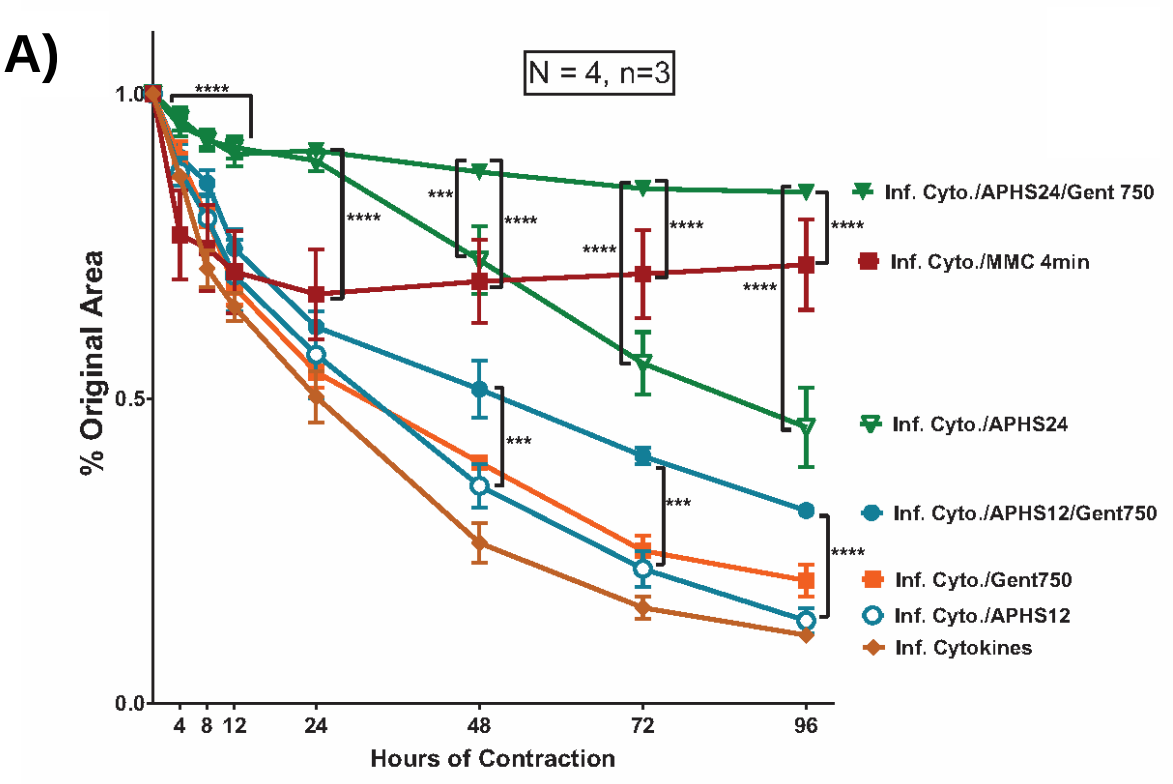


Figure 5-10 ASA ± gentamicin (µg/ml) can modulate inf. cytokine induced *in vitro* wound healing functions of ocular fibroblasts without increased cell death. A) Inf. cytokines potentiate ocular fibroblast-mediated collagen contraction relative to vehicle control. Proportion of baseline area remaining at the indicated timepoints is reported for the indicated experimental groups (N=5, n=3). B) After 96hrs of contraction, relative cytotoxicity of exposures was assessed by LIVE/DEAD staining of collagen matrices and comparing the proportion of living (blue) to total cells (blue + red) within the collagen matrices (N=4, n=3, frames=10). C) Representative LIVE/DEAD images of the indicated treatment groups. D) Representative scans of collagen matrices exposed to the indicated treatments and allowed to contract for 96hrs. A) Two-way ANOVA with Dunnett's *post hoc* test: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. B) One-way ANOVA with Tukey's *post hoc* test: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

APHS impaired cell-mediated collagen contraction in a dose dependent manner, and the effect was significantly augmented by gentamicin at all tested dose (Figure 5-11A). At the highest dose, APHS alone was significantly more effective at impairing contraction than a 4-minute exposure to MMC, however the effect was transient and lost significance after 48hrs. The combination of APHS and gentamicin, at the highest dose, was significantly more effective than a four-minute exposure to MMC at impairing inf. cytokine-induced collagen contraction. Figure 5-11B displays representative scans of contracted collagen matrices clearly displaying the described effects.



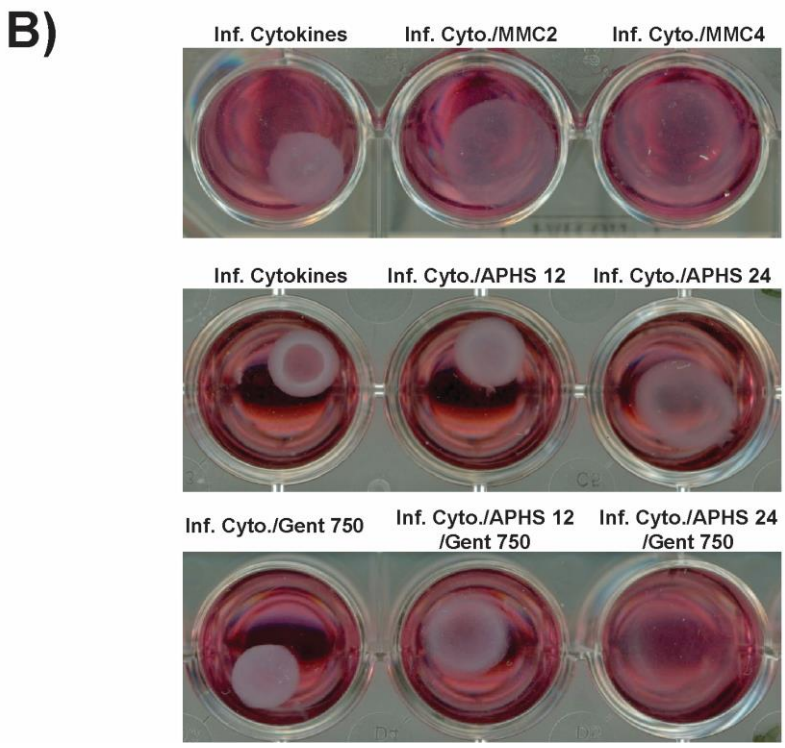


Figure 5-11 APHS, ASA ± gentamicin (µg/ml) can modulate inf. cytokine induced *in vitro* wound healing functions of ocular fibroblasts without increased cell death. A) effects of APHS ± gentamicin on the inf. cytokine-induce ocular fibroblast-mediated contraction of collagen matrices relative to a 4 minute exposure to MMC (N=4, n=3). B) Representative scans of collagen matrices exposed to the indicated treatments and allowed to contract for 96hrs. A) Two-way ANOVA with Dunnett's *post hoc* test: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. C) One-way ANOVA with Tukey's *post hoc* test: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Relative cellular viability was again compared between experimental treatments (Figure 5-12A and B). Relative to the inf. cytokine control, APHS alone or in combination with gentamicin did not have a significant effect on cellular viability. Together, these results suggest that the modulation of lipid mediators with COX2 Ser516 acetylating agents is associated with a modulation of fibroblast function. The effect of MMC appears to be mediated through cytotoxic means, whereas the effects of COX2 acetylating agents do not seem to be mediated through cytotoxic means.

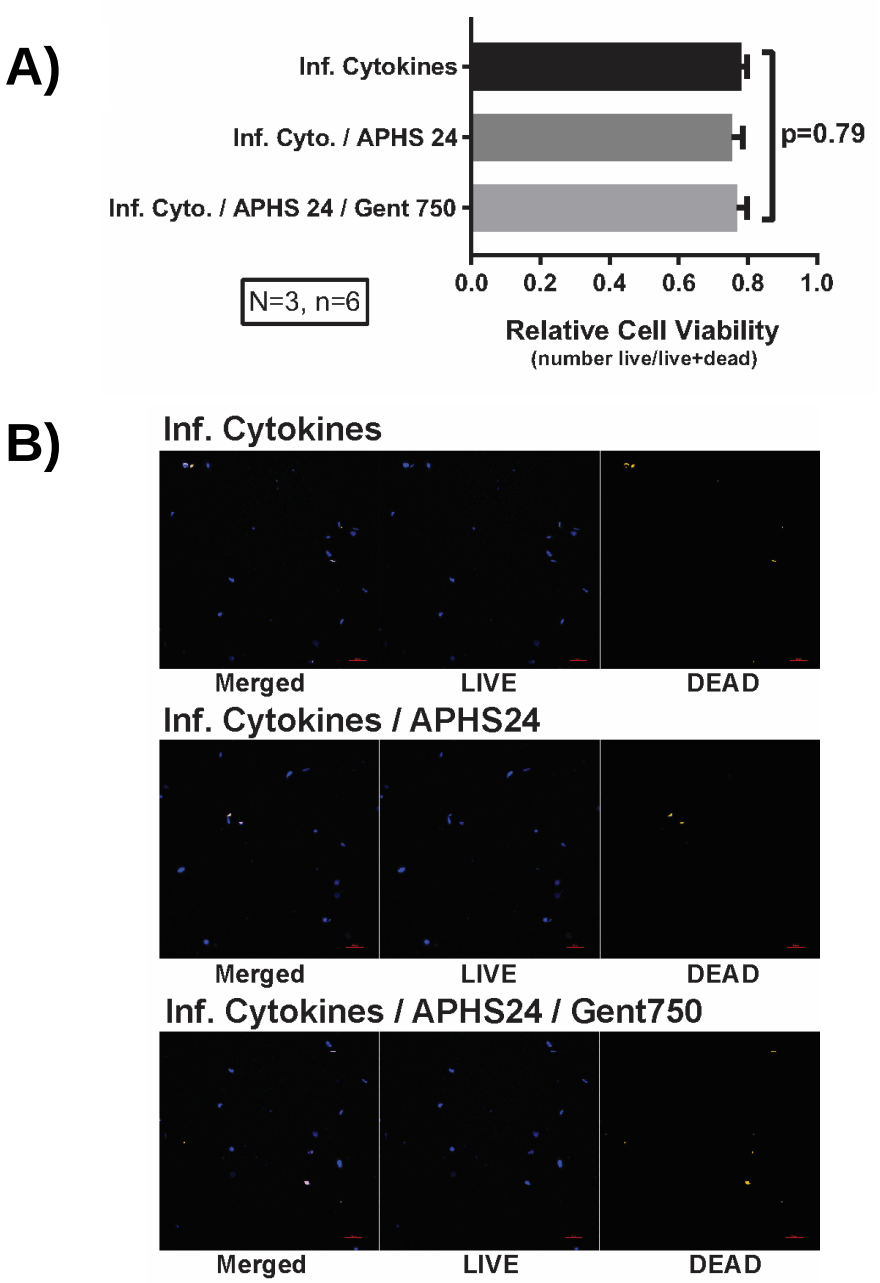


Figure 5-12 A) Effects of APHS ± gentamicin (µg/ml) on the cellular viability (N=3, n=6, frames=10). B) Representative LIVE/DEAD images of the indicated treatment groups. A) One-way ANOVA with Tukey's *post hoc* test: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

5.3.6 COX2 acetylation inhibits inf. cytokine-induced myofibroblast metabolic activity and pathognomonic protein expression

We wished to investigate the effects of COX2 acetylation on the fibroblast and myofibroblast phenotype as a means to better understand the mechanism responsible for modified function within collagen-based culture. First, we assessed the effect of ASA and APHS on the metabolic activity of TGFβ-induced myofibroblasts and control ocular fibroblasts (Figure 5-13A and B). Both COX2

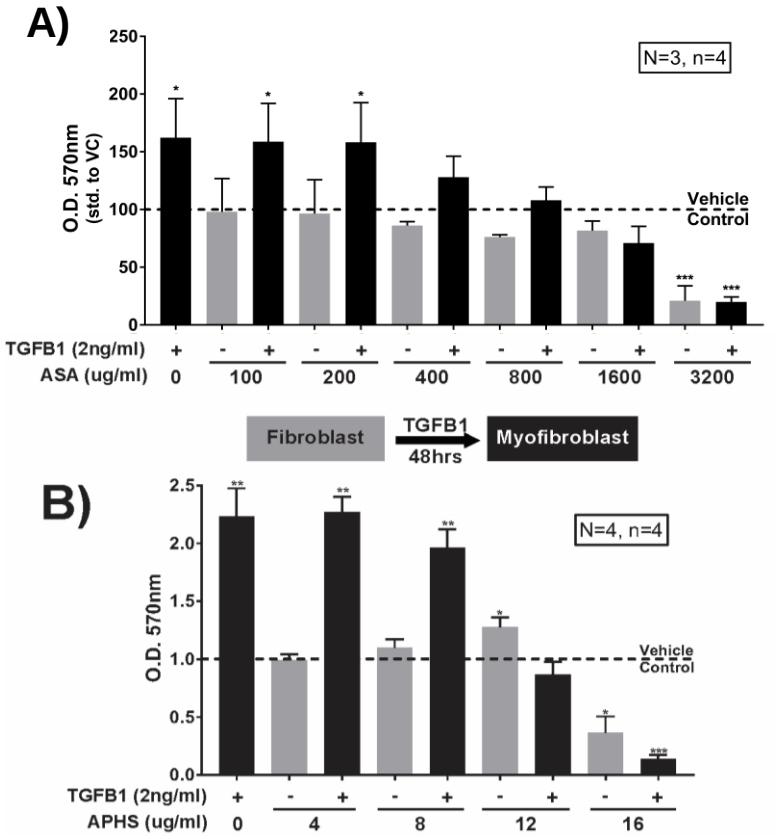


Figure 5-13 Effects of A) ASA, or B) APHS (µg/ml) on the TGFb1 (2ng/ml)-induced metabolic activity ocular fibroblasts. A-B) Results from MTT assays 48hrs after the indicated treatments, the optical density of the experimental groups was normalized to the vehicle control group. One-way ANOVA with Tukey's *post hoc* test: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

acetylating agents had a relatively small effect on the metabolic activity of the non-induced fibroblasts, until the extreme of the dose range tested. TGFβ1 had a significant stimulatory effect on fibroblast metabolic activity. When TGFβ1 was co-incubated with either COX2 acetylating agent, a significant reduction in metabolic activity was noted, with return to non-TGFβ stimulated levels in a dose dependent manner.

The protein alpha smooth muscle actin (αSMA) is pathognomonic of the myofibroblast phenotype and essential for contraction of the extracellular matrix to occur. Matrix metalloproteinase nine (MMP9) is essential for invasion and remodeling of the extracellular matrix. Using monolayer cell culture and western blot, we found that inf. cytokines induced differentiation of ocular fibroblasts into myofibroblasts, exhibiting increased expression of both αSMA and MMP9 relative to non-induced fibroblasts (Figure 5-14A and B). The inf. cytokines-induced expression of αSMA and MMP9 expression was significantly reversed ASA in a dose dependent manner (Figure 5-14A). APHS, at the concentration assessed (10μg/ml), has approximately equal COX2 acetylating power as 500μg/ml ASA ($1/60^{th}$ as many moles, with approx. 60x COX2 specificity). It was also able to significantly inhibit the observed inf. cytokine-induced expression of αSMA (Figure 5-14B). These results support the impairment of myofibroblast transdifferentiation by both COX2 acetylating agents.

The effects of adding the PLA2 agonist, gentamicin, was assayed using the same methodology (Figure 5-15A-C). Gentamicin alone had no significant effect on the inf. cytokine-induced expression of collagen 1 and αSMA. Both ASA and

APHS were able to impair the cytokine induced expression of collagen 1 and αSMA on their own. The effects of both COX2 acetylating agents on inf. cytokine-induced collagen 1 and αSMA expression were significantly potentiated by their co-incubation with gentamicin.

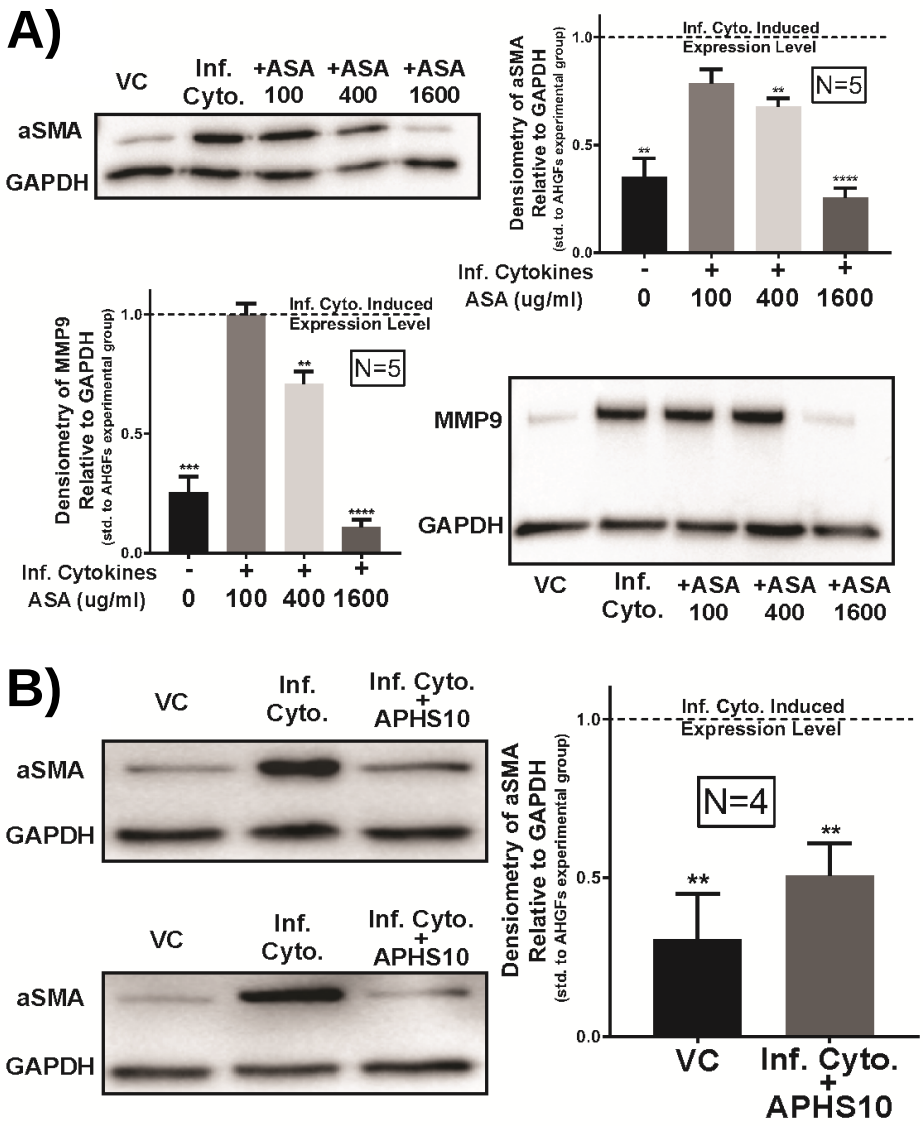
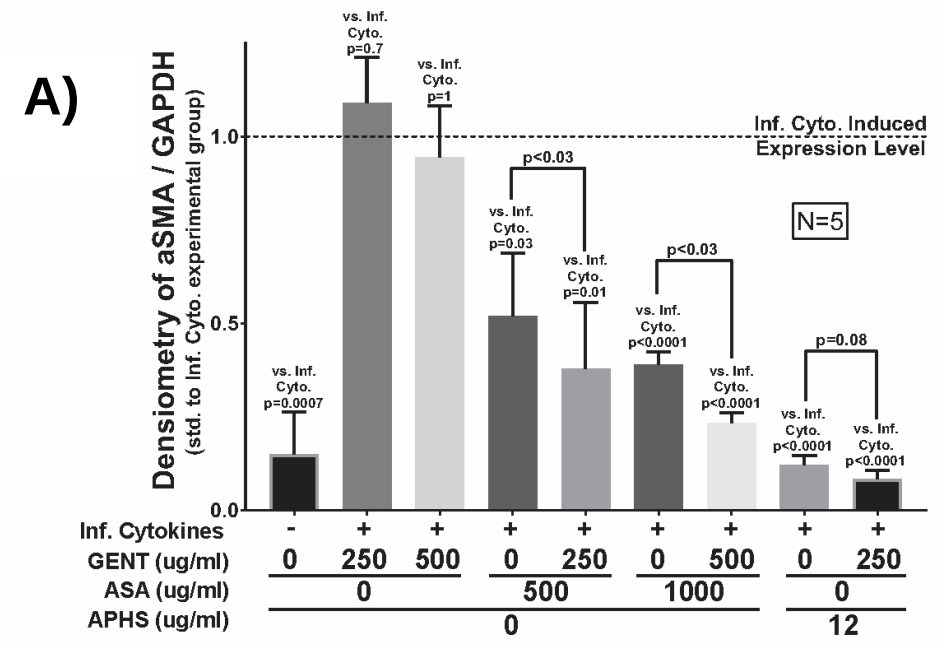
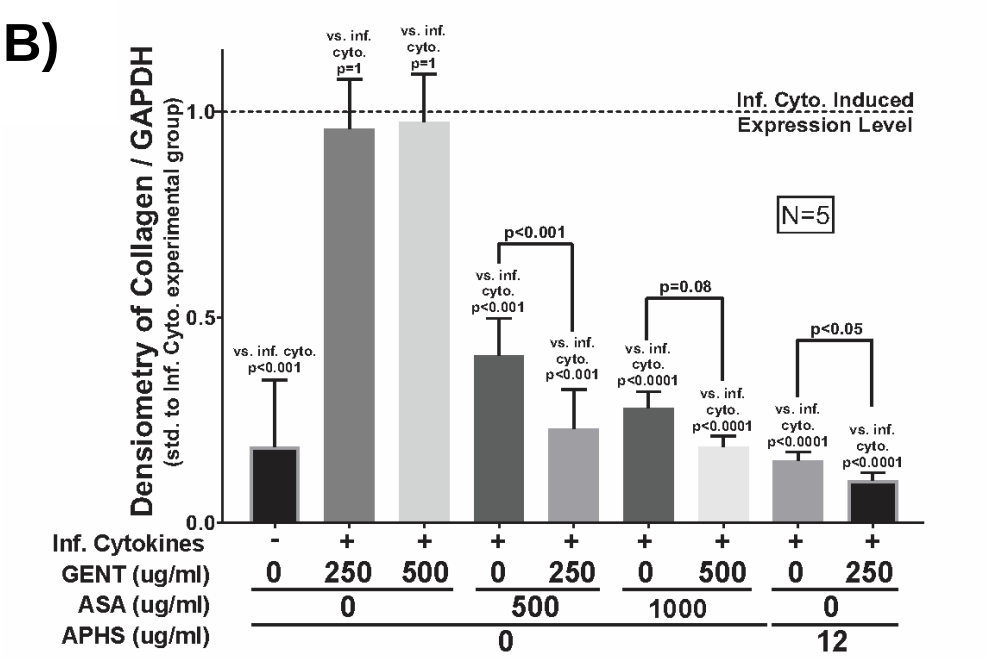


Figure 5-14 Assessment of inf. Cytokine induced αSMA and/or MMP9 expression by western blot of ocular fibroblast total protein lysate after 48hrs of culture with the indicated exposures (µg/ml). Results from a one-way ANOVA with Tukey's *post hoc* test are shown. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.


A)
Densiometry of aSMA / GAPDH
(std. to Inf. Cyto. experimental group)
Inf. Cyto. Induced
Expression Level
N=5
vs. Inf. Cyto. p=0.7
vs. Inf. Cyto. p=1
p<0.03
vs. Inf. Cyto. p=0.03
vs. Inf. Cyto. p=0.01
p<0.03
vs. Inf. Cyto. p<0.0001
vs. Inf. Cyto. p<0.0001
p=0.08
vs. Inf. Cyto. p<0.0001
vs. Inf. Cyto. p<0.0001
vs. Inf. Cyto. p=0.0007
1.0
0.5
0.0
Inf. Cytokines - + + + + + + + +
GENT (ug/ml) 0 250 500 0 250 0 500 0 250
ASA (ug/ml) 0 500 1000 0
APHS (ug/ml) 0 12


B)
Densiometry of Collagen / GAPDH
(std. to Inf. Cyto. experimental group)
Inf. Cyto. Induced
Expression Level
N=5
vs. inf. cyto. p=1
vs. inf. cyto. p=1
p<0.001
vs. inf. cyto. p<0.001
vs. inf. cyto. p<0.001
p=0.08
vs. inf. cyto. p<0.0001
vs. inf. cyto. p<0.0001
p<0.05
vs. inf. cyto. p<0.0001
vs. inf. cyto. p<0.0001
vs. inf. cyto. p<0.001
1.0
0.5
0.0
Inf. Cytokines - + + + + + + + +
GENT (ug/ml) 0 250 500 0 250 0 500 0 250
ASA (ug/ml) 0 500 1000 0
APHS (ug/ml) 0 12

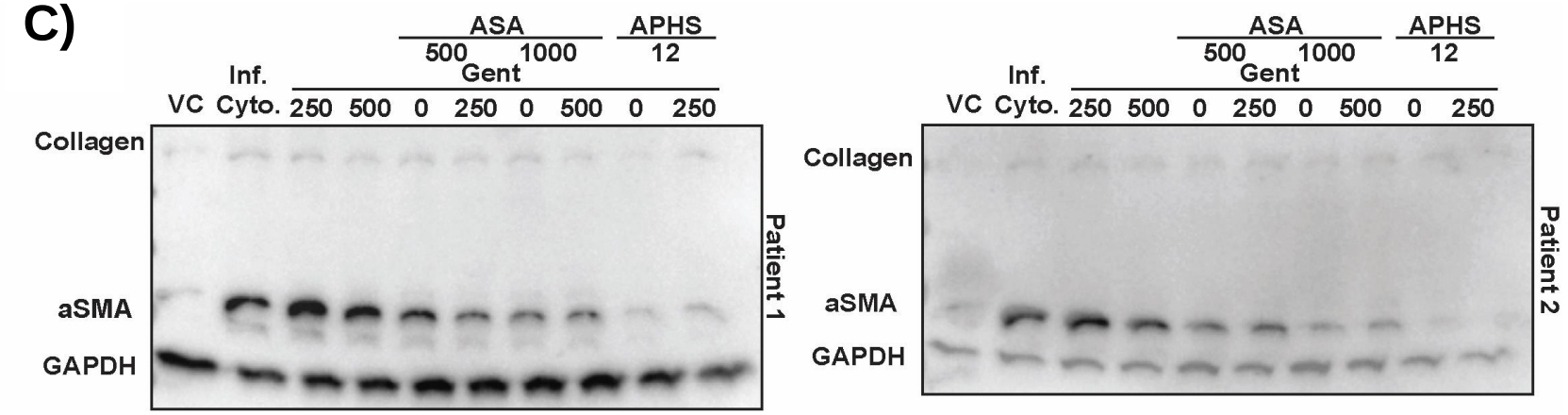


Figure 5-15 Effects of APHS, ASA ± gentamicin (µg/ml) on the cytokine-induced protein expression of ocular fibroblasts. A-B) Expression of αSMA and collagen 1 by western blot of ocular fibroblast total protein lysate after 48hrs of culture with the indicated exposures. C) Representative western blots from two patient replicates. A-B) One-way ANOVA with Tukey's *post hoc* multiple comparisons test: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

5.3.7 COX2 acetylation results in differential regulation of transcription factors PPARɣ and SMAD2/3

Previous work has demonstrated the ability of ASA to suppress MMP9 expression in mouse celiac macrophages.[239] These authors' conclusions implicated activation of PPARɣ as mechanistic rationale for the ASA-induced changes in protein expression. Conceptually, these findings are supported by our findings that the production of endogenous PPARɣ ligands, such as 15-HETE,[263] can be generated by the acetylated COX2 enzyme. Another study reported the effects of troglitazone, a potent synthetic PAPRy agonist, on collagen synthesis in human hypertrophic scar fibroblasts.[264] These authors found that agonizing PPARɣ led to impaired collagen expression and demonstrated this to occur through induction of miR-145, which itself causes inhibition of SMAD3. We hypothesized that the same intracellular mechanisms were responsible for the effects observed within ocular fibroblasts after exposure to ASA and APHS. We expected to see differential regulation of PPARɣ and SMAD2/3 after COX2 acetylation (Figure 5-16).

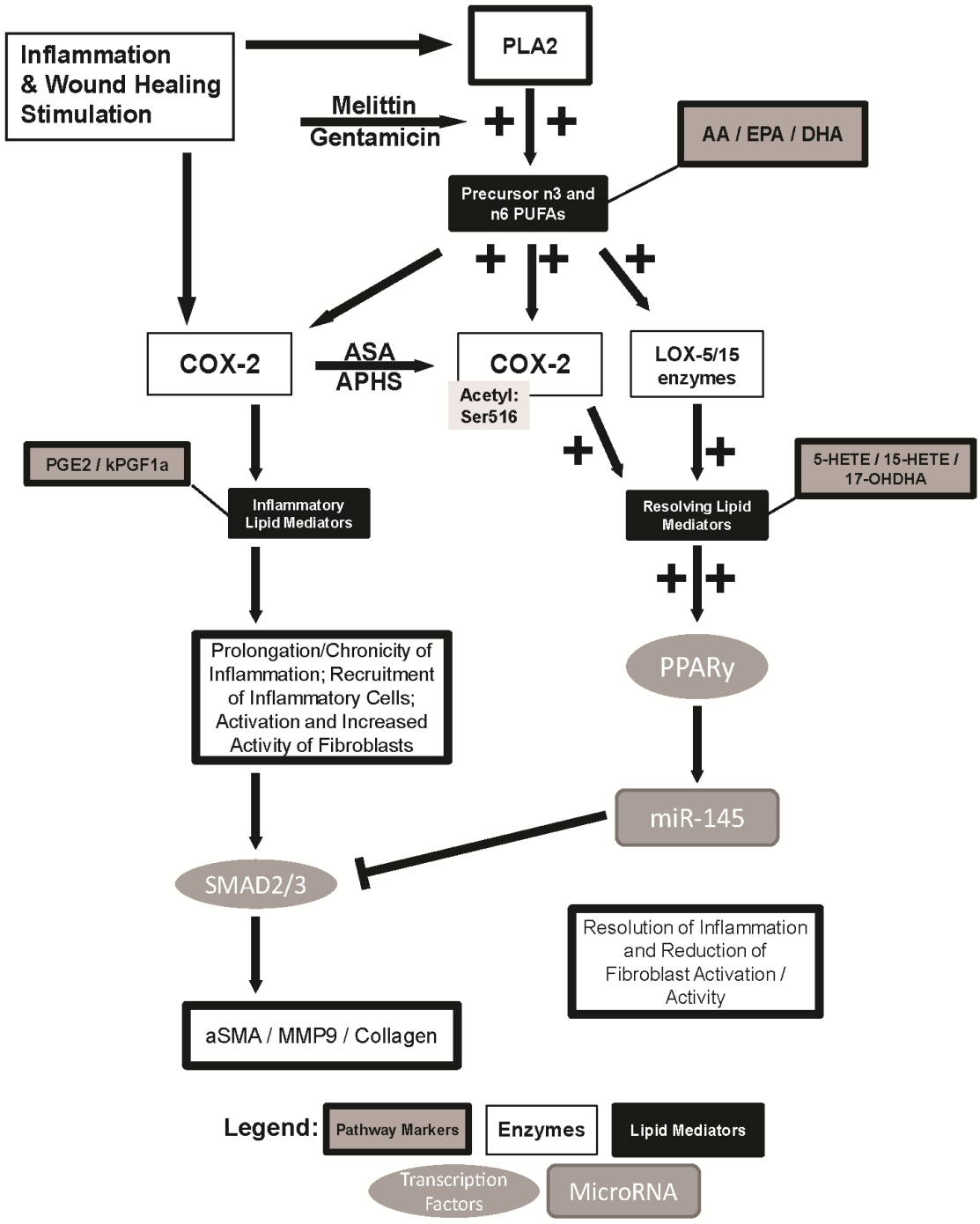


Figure 5-16 Schematic representation of the hypothesized mechanism leveraged for the modulation of lipid mediator production.

We first investigated the ability of ASA and APHS to influence PPARɣ after inf. cytokine-stimulation. Inf. cytokines significantly inhibited PPARɣ expression relative to vehicle control (Figure 5-17A and B). ASA was able to rescue inf. cytokine-induced PPARɣ expression in a dose dependent manner, exceeding the relative expression levels of the vehicle control group (Figure 5-17A). The effects of APHS alone on PPARɣ expression were inhibitory relative to vehicle control, however when APHS was co-incubated with inf. cytokine-induced fibroblasts, a significant increase in PPARɣ expression level was observed relative to both inf. cytokine control and vehicle control groups (Figure 5-17B). This finding in particular supports the requirement for COX2's induction before the therapeutic effects of its acetylation can be observed.

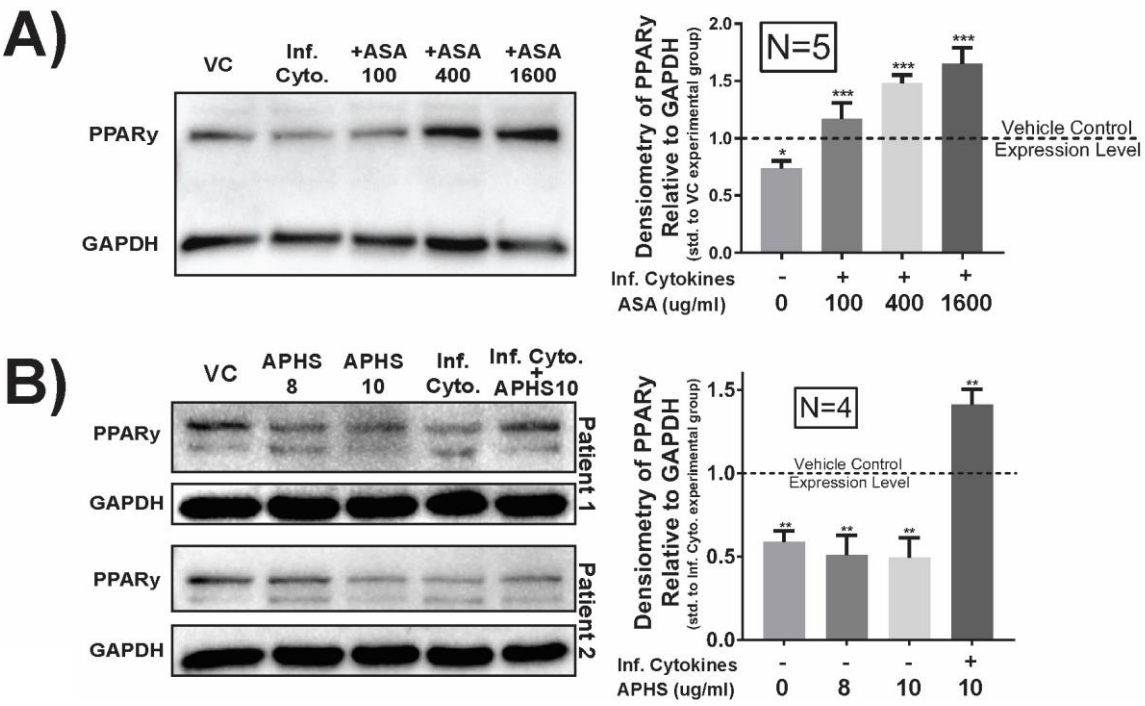


Figure 5-17 Effects of A) ASA and B) APHS (µg/ml) on the transcription factors PPARy. Expression of PPARy relative to GAPDH was assessed by western blot of ocular fibroblast total protein lysate after 48 hours of culture with the indicated exposures (N=5). A-B) One-way ANOVA with Tukey's *post hoc* multiple comparisons test: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

To investigate the occurrence of a corresponding SMAD2/3 downregulation, we analyzed the relative expression of both phosphorylated SMAD2/3 and total SMAD2/3 (Figure 5-18). Inf. cytokines induced high levels of SMAD2/3 expression and phosphorylation relative to vehicle control. Gentamicin had no effect on the inf. cytokine-induced phosphorylation or expression of SMAD2/3. ASA and APHS were able to significantly repress both the inf. cytokine-induced phosphorylation and expression of SMAD2/3, and gentamicin was able to significantly augment the effects of both COX2 acetylating agents. The ratio of

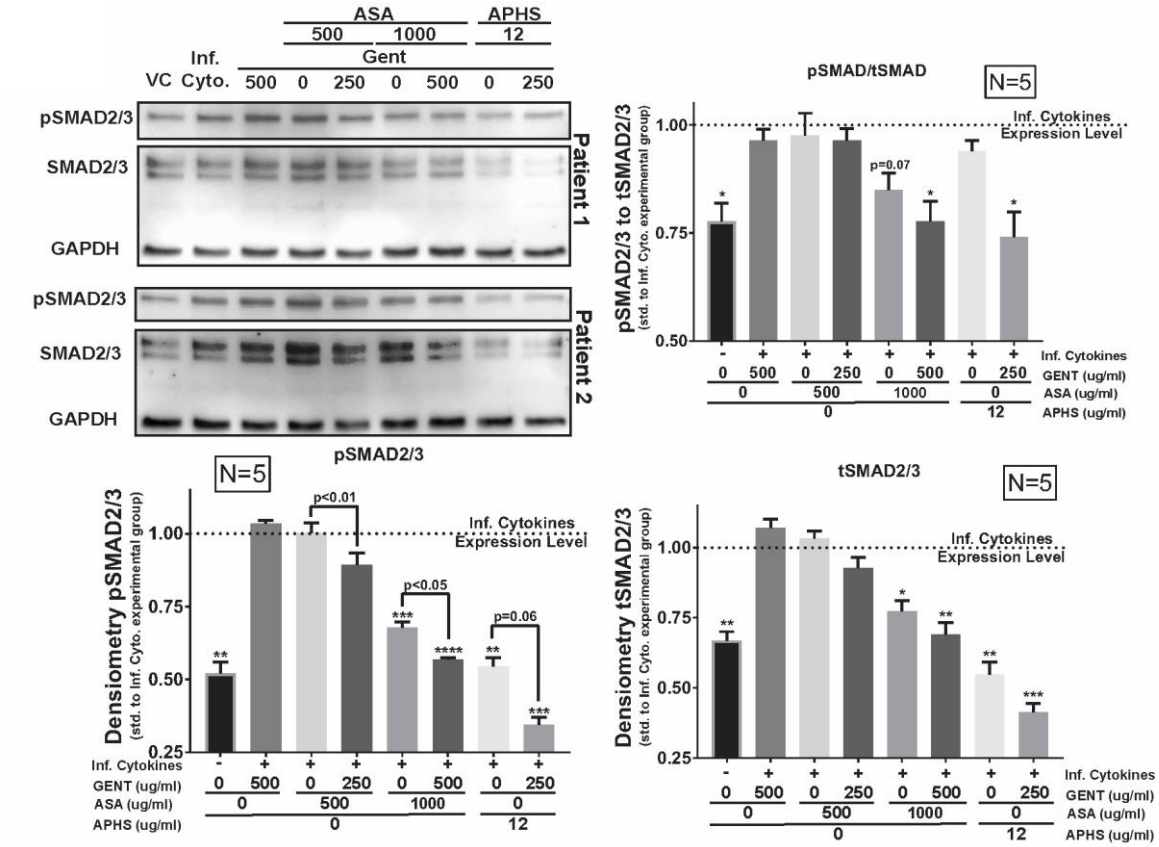


Figure 5-18 APHS, ASA ± gentamicin's (µg/ml) effects on the transcription factor SMAD2/3 total expression and phosphorylation state. Expression of phosphorylated SMAD2/3, total SMAD2/3 and the ratio of phosphorylated to total SMAD2/3 relative to GAPDH after 48hrs of culture with the indicated exposures (N=5). One-way ANOVA with Tukey's *post hoc* multiple comparisons test: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

activated (phosphorylated) to total SMAD2/3 was returned to vehicle control expression levels from inf. cytokine-induced levels with the application of 1000µg/ml ASA and 500µg/ml gentamicin or 12µg/ml APHS and 250µg/ml gentamicin.

To confirm PPARɣ's involvement in the effects observed, we co-incubated the COX2 acetylating agents with a PPARɣ inhibitor, GW9662 (1 µM). When either ASA or APHS were co-incubated with GW9662, no significant inhibition of inf. cytokine-induced αSMA expression within ocular fibroblasts was noted (Figure 5-19A and B). These findings support PPARɣ's role in mediating the effects of COX2 acetylating agents on myofibroblast transdifferentiation.

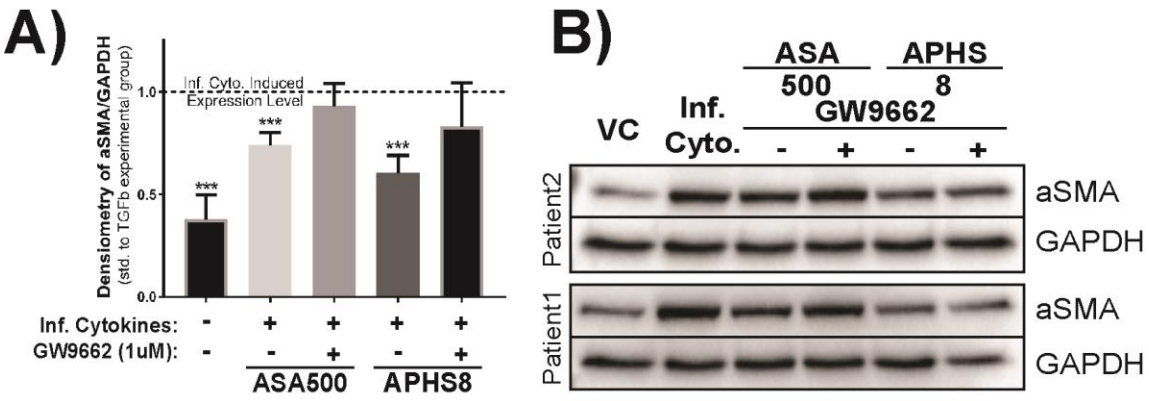


Figure 5-19 PPARγ inhibition (GW9662, 1 µM) attenuates the effects seen with COX2 Ser516 acetylating agents. A) Densiometric analysis of multiple (N=4) western blots. One-way ANOVA with Tukey's *post hoc* test: ***p<0.001. B) Representative western blot of total protein lysate from ocular fibroblasts 48hrs after the indicated exposures. ASA (µg/ml); APHS (µg/ml).

5.4 Conclusions

The presented evidence suggests that, within inflammation activated ocular fibroblasts, the modulation COX2 and PLA2 enzymatic function and activity can illicit a shift in pro-inflammatory and pro-resolving lipid mediator production that greatly favors the generation of pro-resolving mediators. Under these conditions, differential regulation of PPARɣ and SMAD2/3 were observed within ocular fibroblasts and corresponded with inhibition of inflammation-induced myofibroblast transdifferentiation. Together, these results suggest that the resolution of inflammatory and fibroproliferative signaling can be triggered within ocular fibroblasts by acetylation of COX2 at Ser516 and this effect appears to be augmented by stimulation of PLA2 activity.

Inflammation induced myofibroblast transdifferentiation and activity remains one of the largest unanswered contributors to ocular morbidity. To our knowledge, this is the first report of COX2 Ser516 acetylating agents being used to trigger endogenous mechanisms of resolution within ocular fibroblasts causing an impairment of myofibroblast transdifferentiation and function. Immuno-resolvent interventions are theorized to be associated with fewer off target effects, as they function to promote the ordered return to homeostasis after inflammatory insult. Immuno-suppressive interventions, on the other hand, attempt to prevent the initial inflammation-induced deviation from homeostasis and hinder the endogenous resolution of inflammation. A subtle difference, but one which implies that immuno-resolvent interventions attempt to engage synergistically

with an endogenous process, whereas immuno-suppressive interventions attempt to counter an endogenously occurring process. The ready availability of safety data pertaining to ASA's human use should provide a rapid path to assessing its efficacy in human ophthalmological pathologies. Based on the presented data, APHS has the potential to exhibit even greater efficacy, and animal studies assessing its ophthalmological safety and efficacy are warranted.

Chapter 6

6 Summary and Future Directions

6.1 Overall Findings and Implications

Excessive myofibroblast transdifferentiation and activity within delicate ocular structures is a major impediment to successful ophthalmological outcomes across the discipline. This thesis focused on the critical wound healing that occurs within the post-operative subconjunctival tissues of glaucoma surgery patients, however wound healing is a fundamental process, and lessons from the subconjunctiva could easily be translated to the other non-nervous tissues of the eye.

This thesis began with a survey of risk factors for glaucoma surgery revision. We used this large retrospective database study as a springboard for subsequent *in vitro* investigation. The subsequently generated data was enough to successfully fund a clinical trial that is scheduled to begin patient recruitment concurrent with this thesis's evaluation. Looking ahead and based on what was learned about RMs impact on wound healing, we subsequently designed a novel immuno-resolvent intervention that demonstrates significant efficacy *in vitro* at impairing cytokine-driven subconjunctival wound healing phenomena.

The implications of this body of work are far reaching. Immediate benefit to patients may be gained should NSAIDs outperform corticosteroids in the planned

randomized controlled trial – as many NSAID eye drops are currently approved and could easily be switched to as current standard of care. Long term benefits may be derived from a deeper understanding of the interactions between inflammatory mediator production, resolving mediator production and the development of scarring. Understanding the effects of COX2 Ser516 acetylation within animal models of progressive fibrotic diseases is warranted – especially considering the recent discovery of an endogenous COX2 acetylating protein that contributes to the resolution of neuroinflammation in mice.[249] Strategies that capitalize on the body's endogenous resolving mechanisms to avoid the dangers associated with inflammation are likely to enjoy more success than those which act against endogenous systems. Such immuno-resolvent modalities should gain a prominent position in the future fight against chronic inflammatory or dysregulated inflammatory diseases.

6.2 Limitations

The study which inspired all subsequent investigations was retrospective in nature, thus we could only assess exposures to drugs that are available to the public. This resulted in a potential blind spot; the inability to investigate the effects of novel wound modulating agents. Fortunately, there were two anti-inflammatory drugs within the database records that exhibited significantly different associations with surgical success, and I was subsequently inspired by their differing mechanisms of action. However, this initial study set the direction for future work, and this limitation undoubtedly restricted the theoretical framework

upon which Chapters 3 to 7 are based. It was fortunate that NSAIDs and corticosteroids lead me to investigate the interactions of LMs with wound healing, as this is a novel area of research and supported much subsequent investigation.

Although we make a case for fibroblasts themselves being immune cells – especially in the inflammatory and wound healing microenvironment – the presented studies do not incorporate more classical examples of immune cells in our models. The interaction of macrophages and other inflammatory cells with fibroblasts is critical to the wound healing process[34] and future studies should incorporate co-cultures of the two cell types to more accurately recreate the *in vivo* microenvironment. However, one can take comfort in the fact that the interventions discussed within this thesis have a very high likelihood of mitigating the impact of inflammatory cells in a similar manner – as almost all immune cells express PLA2 and COX2. In fact the overall effects of these interventions may be even more pronounced in inflammatory cells – as they have been shown to be extremely sensitive to RM signals and subsequently function to locally amplify their presence *in vivo* – potentially initiating a feedforward pro-resolution signal from which any local fibroblasts would also benefit.[18,265,266]

6.3 Future Directions

In Chapter 6 we explored the intracellular signaling cascade mediating the effects of COX2 Ser516 acetylators. We demonstrated involvement of SMAD2/3 and PPARɣ in the transduced effects. Previous work has implicated miR-145 as mediating PPARɣ-induced interference with SMAD2/3 signaling within human

hypertrophic scar fibroblasts.[264] Investigating the potential role of this microRNA in turning off myofibroblast associated gene sets in response to PPARɣ activation should yield new knowledge and strategies to impair myofibroblast transdifferentiation and activity.

Results presented warrant further investigation in animal models of ophthalmic disease. There are several validated animal model systems in which these interventions could be assessed for their anti-fibrotic properties. Corneal stromal injury,[267] posterior capsule opacification after cataract surgery,[268] and ocular neovascularization[269] all have validated animal models and are natural translations for the presented interventions.

Given the results in Chapter 6, APHS should be explored further as an immuno-resolvent agent. Subsequent animal safety and toxicity studies, should they prove promising, could open whole new avenues of research into more specific COX2 acetylating molecules and a new frontier for immuno-resolvent interventions.

References

1. Shu, D. Y. & Lovicu, F. J. Myofibroblast transdifferentiation: The dark force in ocular wound healing and fibrosis. *Prog. Retin. Eye Res.* (2017). doi:10.1016/j.preteyeres.2017.08.001

2. Skuta, G. & Parrish, R. Wound healing in glaucoma fltering surgery. *Surv. Ophthalmol.* **32**, (1987).

3. Yamanaka, O., Kitano-Izutani, A., Tomoyose, K. & Reinach, P. S. Pathobiology of wound healing after glaucoma filtration surgery. *BMC Ophthalmol.* **15 Suppl 1**, 157 (2015).

4. Zada, M., Pattamatta, U. & White, A. Modulation of Fibroblasts in Conjunctival Wound Healing. *Ophthalmology* **125**, 179–192 (2017).

5. Serhan, C. N. & Savill, J. Resolution of inflammation: the beginning programs the end. *Nat. Immunol.* **6**, 1191–1197 (2005).

6. Serhan, C. N. & Levy, B. D. Resolvins in inflammation : emergence of the pro-resolving superfamily of mediators. **128**, 2657–2669 (2018).

7. Coyne, C. B., Zeh, H. J., Lotze, M. T., Tang, D. & Kang, R. PAMPs and DAMPs: signal 0s that spur autophagy and immunity. *Immunol. Rev.* **249**, 158–75 (2012).

8. Rubartelli, A. & Lotze, M. T. Inside, outside, upside down: damage-associated molecular-pattern molecules (DAMPs) and redox. *Trends Immunol.* **28**, 429–436 (2007).

9. Mack, M. Inflammation and fibrosis. *Matrix Biology* (2018). doi:10.1016/j.matbio.2017.11.010

10. Serhan, C. N., Arita, M., Hong, S. & Gotlinger, K. Resolvins, docosatrienes, and neuroprotectins, novel omega-3-derived mediators, and their endogenous aspirin-triggered epimers. *Lipids* **39**, 1125–1132 (2004).

11. Smith, W. L. Prostanoid biosynthesis and mechanisms of action. *Am. J. Physiol.* **263**, F181–F191 (1992).

12. Leslie, C. C. Cytosolic phospholipase A $_2$: physiological function and role in disease. *J. Lipid Res.* **56**, 1386–1402 (2015).

13. Dhall, S. *et al.* Arachidonic acid-derived signaling lipids and functions in impaired healing. *Wound Repair Regen.* **23**, 644–656 (2015).

14. Oga, T. Prostaglandin F2α receptor signaling facilitates bleomycin-induced pulmonary fibrosis independently of transforming growth factor-β. *Nat. Med.* **15**, (2009).

15. Liu, Y., Ko, J., Yanai, R., Kimura, K. & Chikama, T. Induction by Latanoprost of Collagen Gel Contraction Mediated by Human Tenon Fibroblasts : Role of Intracellular Signaling Molecules. *Investig. Ophthalmol. Vis. Sci.* **2**, (2008).

16. Levy, B. D., Clish, C. B., Schmidt, B., Gronert, K. & Serhan, C. N. Lipid mediator class switching during acute inflammation: signals in resolution. *Nat. Immunol.* **2**, 612–619 (2001).

17. Serhan, C. N. Discovery of specialized pro-resolving mediators marks the dawn of resolution physiology and pharmacology. *Mol. Aspects Med.* (2017). doi:10.1016/j.mam.2017.03.001

18. Lopategi, A. *et al.* Frontline Science: Specialized proresolving lipid mediators inhibit the priming and activation of the macrophage NLRP3 inflammasome. *Journal of Leukocyte Biology* (2018). doi:10.1002/JLB.3HI0517-206RR

19. Serhan, C. N. Novel Pro-Resolving Lipid Mediators in Inflammation Are Leads for Resolution Physiology. *Nature* **510**, 92–101 (2014).

20. Serhan, C. N., Chiang, N. & Dalli, J. The resolution code of acute inflammation: Novel pro-resolving lipid mediators in resolution. *Semin. Immunol.* **27**, 200–215 (2015).

21. Tian, H., Lu, Y., Sherwood, A. M., Hongqian, D. & Hong, S. Resolvins E1 and D1 in choroid-retinal endothelial cells and leukocytes: Biosynthesis and mechanisms of anti-inflammatory actions. *Investig. Ophthalmol. Vis. Sci.* **50**, 3613–3620 (2009).

22. de la Rosa, X. *et al.* Identification and Complete Stereochemical Assignments of the New Resolvin Conjugates in Tissue Regeneration in Human Tissues that Stimulate Proresolving Phagocyte Functions and Tissue Regeneration. *Am. J. Pathol.* (2018). doi:10.1016/j.ajpath.2018.01.004

23. Powell, W. S. & Rokach, J. Biosynthesis, biological effects, and receptors of hydroxyeicosatetraenoic acids (HETEs) and oxoeicosatetraenoic acids (oxo-ETEs) derived from arachidonic acid. *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids* (2015). doi:10.1016/j.bbalip.2014.10.008

24. Marion-Letellier, R., Savoye, G. & Ghosh, S. Fatty acids, eicosanoids and PPAR gamma. *Eur. J. Pharmacol.* **785**, 44–49 (2016).

25. Hong, C. & Tontonoz, P. Coordination of inflammation and metabolism by PPAR and LXR nuclear receptors. *Curr. Opin. Genet. Dev.* **18**, 461–467 (2008).

26. Li, N., He, J., Schwartz, C. E., Gjorstrup, P. & Bazan, H. E. P. Resolvin E1 Improves Tear Production and Decreases Inflammation in a Dry Eye Mouse Model. *J. Ocul. Pharmacol. Ther.* **26**, 431–439 (2010).

27. Recchiuti, A. & Serhan, C. N. Pro-resolving lipid mediators (SPMs) and their actions in regulating miRNA in novel resolution circuits in inflammation. *Front. Immunol.* **3**, (2012).

28. Fullerton, J. N. & Gilroy, D. W. Resolution of inflammation: A new therapeutic

frontier. *Nat. Rev. Drug Discov.* **15**, 551–567 (2016).

29. Serhan, C. N. *et al.* Proresolving lipid mediators resolvin D1, resolvin D2, and maresin 1 are critical in modulating T cell responses. *Sci. Transl. Med.* **8**, 353ra111-353ra111 (2016).

30. Bautista-Hernández, L. A., Gómez-Olivares, J. L., Buentello-Volante, B. & Bautista-de Lucio, V. M. Fibroblasts: the unknown sentinels eliciting immune responses against microorganisms. *Eur. J. Microbiol. Immunol.* **7**, 151–157 (2017).

31. Yao, C. *et al.* Toll-like receptor family members in skin fibroblasts are functional and have a higher expression compared to skin keratinocytes. *Int. J. Mol. Med.* **35**, 1443–1450 (2015).

32. Kendall, R. T. & Feghali-Bostwick, C. A. Fibroblasts in fibrosis: Novel roles and mediators. *Frontiers in Pharmacology* (2014). doi:10.3389/fphar.2014.00123

33. Artlett, C. M. Inflammasomes in wound healing and fibrosis. *Journal of Pathology* (2013). doi:10.1002/path.4116

34. Borthwick, L. A., Wynn, T. A. & Fisher, A. J. Cytokine mediated tissue fibrosis. *Biochim. Biophys. Acta - Mol. Basis Dis.* **1832**, 1049–1060 (2013).

35. Krönke, G. *et al.* The 12/15-lipoxygenase pathway counteracts fibroblast activation and experimental fibrosis. *Ann. Rheum. Dis.* **71**, 1081–1087 (2012).

36. Qu, X. *et al.* Resolvins E1 and D1 inhibit interstitial fibrosis in the obstructed kidney via inhibition of local fibroblast proliferation. *J. Pathol.* **228**, 506–519 (2012).

37. Zheng, S. *et al.* ResolvinD1stimulates epithelial wound repair and inhibits TGF-β-induced EMT whilst reducing fibroproliferation and collagen production. *Lab. Investig.* **98**, 130–140 (2018).

38. Gonzalez, A. C. de O., Andrade, Z. de A., Costa, T. F. & Medrado, A. R. A. P. Wound Healing - A literature review. *Ann. Bras. Dermatology* **91**, 614–620 (2016).

39. Hinz, B. Myofibroblasts. *Exp. Eye Res.* **142**, 56–70 (2015).

40. Paul Ehrlich, H., Sun, B., Kainth, K. S. & Kromah, F. Elucidating the mechanism of wound contraction: Rapid versus sustained myosin ATPase activity in attached-delayed-released compared with free-floating fibroblast-populated collagen lattices. *Wound Repair Regen.* **14**, 625–632 (2006).

41. Peters, A. S., Brunner, G., Krieg, T. & Eckes, B. Cyclic mechanical strain induces TGFβ1-signalling in dermal fibroblasts embedded in a 3D collagen lattice. *Arch. Dermatol. Res.* **307**, 191–197 (2015).

42. Kessler, D. *et al.* Fibroblasts in Mechanically Stressed Collagen Lattices Assume a ‘Synthetic’ Phenotype. *J. Biol. Chem.* **276**, 36575–36585 (2001).

43. Hinz, B. The extracellular matrix and transforming growth factor-β1: Tale of a strained relationship. *Matrix Biol.* **47**, 54–65 (2015).

44. Tomasek, J. J., Gabbiani, G., Hinz, B., Chaponnier, C. & Brown, R. a. Myofibroblasts and mechano-regulation of connective tissue remodelling. *Nat. Rev. Mol. Cell Biol.* **3**, 349–63 (2002).

45. Karamanos, N. K., Theocharis, A. D., Neill, T. & Iozzo, R. V. Matrix modeling and remodeling: A biological interplay regulating tissue homeostasis and diseases. *Matrix Biol.* (2018). doi:10.1016/j.matbio.2018.08.007

46. Xue, M. & Jackson, C. J. Extracellular Matrix Reorganization During Wound Healing and Its Impact on Abnormal Scarring. *Adv. wound care* **4**, 119–136 (2015).

47. Tham, Y. C. *et al.* Global prevalence of glaucoma and projections of glaucoma burden through 2040: A systematic review and meta-analysis. *Ophthalmology* **121**, 2081–2090 (2014).

48. Fingeret, M. Glaucoma medications, glaucoma therapy, and the evolving paradigm. *J. Am. Optom. Assoc.* **69**, 115–21 (1998).

49. Kass, M. A., Gordon, M., Morley, R. E., Meltzer, D. W. & Goldberg, J. J. Compliance with topical timolol treatment. *Am. J. Ophthalmol.* **103**, 188–193 (1987).

50. Okeke, C. O. *et al.* Adherence with topical glaucoma medication monitored electronically: the Travatan Dosing Aid Study. *Ophthalmology* **116**, 191–199 (2009).

51. Robin, A. L., Novack, G. D., Covert, D. W., Crockett, R. S. & Marcic, T. S. Adherence in glaucoma: objective measurements of once-daily and adjunctive medication use. *Am. J. Ophthalmol.* **144**, 533–540 (2007).

52. Tsai, J. C. A comprehensive perspective on patient adherence to topical glaucoma therapy. *Ophthalmology* **116**, S30–S36 (2009).

53. Kholdebarin, R., Campbell, R. J., Jin, Y.-P., Buys, Y. M. & Group, C. C. S. Multicenter study of compliance and drop administration in glaucoma. *Can. J. Ophthalmol. Can. d'Ophtalmologie* **43**, 454–461 (2008).

54. Stone, J. L., Robin, A. L., Novack, G. D., Covert, D. W. & Cagle, G. D. An objective evaluation of eyedrop instillation in patients with glaucoma. *Arch. Ophthalmol.* **127**, 732–736 (2009).

55. Baudouin, C. *et al.* The Ocular Surface of Glaucoma Patients Treated over the Long Term Expresses Inflammatory Markers Related to Both T-Helper 1 and T-Helper 2 Pathways. *Ophthalmology* **115**, 109–115 (2008).

56. Ammar, D. A., Noecker, R. J. & Kahook, M. Y. Effects of benzalkonium chloride-and polyquad-preserved combination glaucoma medications on cultured human ocular surface cells. *Adv. Ther.* **28**, 501–510 (2011).

57. Everitt, D. E. & Avorn, J. Systemic effects of medications used to treat glaucoma. *Ann. Intern. Med.* **112**, 120–125 (1990).

58. Müller, M. E., van der Velde, N., Krulder, J. W. & Van Der Cammen, T. J. Syncope and falls due to timolol eye drops. *BMJ* **332**, 960–961 (2006).

59. Gardiner, B. S., Smith, D. W., Coote, M. & Crowston, J. G. Computational modeling of fluid flow and intra-ocular pressure following glaucoma surgery. *PLoS One* **5**, (2010).

60. Johnstone, M. A. The aqueous outflow system as a mechanical pump - Evidence from examination of tissue and aqueous movement in human and non-human primates. *J. Glaucoma* **13**, 421–438 (2004).

61. Seibold, L. K., Sherwood, M. B. & Kahook, M. Y. Wound Modulation After Filtration Surgery. *Surv. Ophthalmol.* **57**, 530–550 (2012).

62. Yamanaka, O., Kitano-izutani, A., Tomoyose, K. & Reinach, P. S. Pathobiology of wound healing after glaucoma filtration surgery. *BMC Ophthalmol.* **15**, (2015).

63. Shin, J. Y. *et al.* The morphometric analysis of filtering bleb using anterior segment optical coherence tomography: pilot study. *J. Korean Ophthalmol. Soc.* **51**, 234–240 (2010).

64. Mietz, H., Raschka, B. & Krieglstein, G. K. Risk factors for failures of trabeculectomies performed without antimetabolites. *Br. J. Ophthalmol.* **83**, 814–821 (1999).

65. Araujo, S. V., Spaeth, G. L., Roth, S. M. & Starita, R. J. A Ten-year Follow-up on a Prospective, Randomized Trial of Postoperative Corticosteroids after Trabeculectomy. *Ophthalmology* **102**, 1753–1759 (1995).

66. Awai-Kasaoka, N. *et al.* Prognostic factors in trabeculectomy with mitomycin C having history of previous glaucoma surgery. *Jpn. J. Ophthalmol.* **57**, 514–519 (2013).

67. Armstrong, J. J., Welk, B. K., Reid, J. N. S., Kansal, V. & Hutnik, C. M. L. Secondary surgical intervention after primary glaucoma filtration surgery: an Ontario population-based study. *Can. J. Ophthalmol.* 1–11 (2018). doi:10.1016/j.jcjo.2018.04.004

68. Boimer, C. & Birt, C. M. Preservative exposure and surgical outcomes in glaucoma patients: The PESO study. *J. Glaucoma* **22**, 730–5 (2013).

69. Broadway, D. C., Bates, A. K., Lightman, S. L., Grierson, I. & Hitchings, R. A. The importance of cellular changes in the conjunctiva of patients with uveitic glaucoma undergoing trabeculectomy. *Eye (Lond).* **7 (Pt 4)**, 495–501 (1993).

70. Broadway, D. C., Grierson, I., O'Brien, C. J. & Hitchings, R. A. Adverse Effects of Topical Antiglaucoma Medication II: The Outcome of Filtration Surgery. *Arch. Ophthalmol.* **112**, 1446–1454 (1994).

71. Broadway, D. C., Grierson, I. & O'Brien, C. J. Adverse Effects of Topical Antiglaucoma Medication I. The Conjunctival Cell Profile. *Arch. Ophthalmol.* **112**, 1437–1445 (1994).

72. Broadway, D. C. & Chang, L. P. Trabeculectomy, risk factors for failure and the preoperative state of the conjunctiva. *J. Glaucoma* **10**, 237–249 (2001).

73. Awai-Kasaoka, N. *et al.* Impact of phacoemulsification on failure of trabeculectomy with mitomycin-C. *J. Cataract Refract. Surg.* **38**, 419–424 (2012).

74. Cantor, L. B. *et al.* Economic evaluation of medication, laser trabeculoplasty and filtering surgeries in treating patients with glaucoma in the US. *Curr. Med. Res. Opin.* **24**, 2905–2918 (2008).

75. Noecker, R. Effects of common ophthalmic preservatives on ocular health. *Adv. Ther.* **18**, 205–15 (2001).

76. Mietz, H., Niesen, U. & Krieglstein, G. K. The effect of preservatives and antiglaucomatous medication on the histopathology of the conjunctiva. *Graefe's Arch. Clin. Exp. Ophthalmol.* **232**, 561–565 (1994).

77. Lacey, J., Cate, H. & Broadway, D. C. Barriers to adherence with glaucoma medications: a qualitative research study. *Eye* **23**, 924–932 (2009).

78. Baudouin, C. *et al.* Prevalence and risk factors for ocular surface disease among patients treated over the long term for glaucoma or ocular hypertension. *Eur. J. Ophthalmol.* **23**, 47–54 (2012).

79. Ceballos, E. M., Beck, A. D. & Lynn, M. J. Trabeculectomy with antiproliferative agents in uveitic glaucoma. *J. Glaucoma* **11**, 189–196 (2002).

80. Freedman, J. & Iserovich, P. Pro-inflammatory cytokines in glaucomatous aqueous and encysted Molteno implant blebs and their relationship to pressure. *Investig. Ophthalmol. Vis. Sci.* **54**, 4851–4855 (2013).

81. Kawai, M. *et al.* Elevated levels of monocyte chemoattractant protein-1 in the aqueous humor after phacoemulsification. *Invest. Ophthalmol. Vis. Sci.* **53**, 7951–60 (2012).

82. Inoue, T. *et al.* Simultaneous increases in multiple proinflammatory cytokines in the aqueous humor in pseudophakic glaucomatous eyes. *J. Cataract Refract. Surg.* **38**, 1389–1397 (2012).

83. Gater, R. *et al.* Investigation of Conjunctival Fibrosis Response Using a 3D Glaucoma Tenon's Capsule + Conjunctival Model. *Invest. Ophthalmol. Vis. Sci.* (2019). doi:10.1167/iovs.18-25335

84. Epstein, E. Fibrosing Response To Aqueous: Its Relation to Glaucoma. *Br. J. Ophthalmol.* **43**, 641–647 (1959).

85. Sharma, R. K., Rogojina, A. T. & Chalam, K. V. Multiplex immunoassay analysis of biomarkers in clinically accessible quantities of human aqueous humor. *Mol.*

Vis. **15**, 60–69 (2009).

86. Tripathi, R. C., Li, J., Chan, W. F. & Tripathi, B. J. Aqueous humor in glaucomatous eyes contains an increased level of TGF-beta 2. *Experimental eye research* **59**, 723–727 (1994).

87. Chua, J. *et al.* Expression profile of inflammatory cytokines in aqueous from glaucomatous eyes. *Mol. Vis.* **18**, 431–8 (2012).

88. Kuchtey, J., Rezaei, K. A., Jaru-Ampornpan, P., Sternberg, P. & Kuchtey, R. W. Multiplex cytokine analysis reveals elevated concentration of interleukin-8 in glaucomatous aqueous humor. *Investig. Ophthalmol. Vis. Sci.* **51**, 6441–6447 (2010).

89. Tailor, R., Batra, R. & Mohamed, S. A national survey of glaucoma specialists on the preoperative (trabeculectomy) management of the ocular surface. in *Seminars in ophthalmology* **31**, 519–525 (Taylor & Francis, 2016).

90. Yuen, D. *et al.* Corticosteroids versus NSAIDs on intraocular pressure and the hypertensive phase after Ahmed glaucoma valve surgery. *J. Glaucoma* **20**, 439–444 (2011).

91. Siriwardena, D., Edmunds, B., Wormald, R. P. L. & Khaw, P. T. National survey of antimetabolite use in glaucoma surgery in the United Kingdom. *Br. J. Ophthalmol.* **88**, 873–6 (2004).

92. Khaw, P. T. Advances in glaucoma surgery: evolution of antimetabolite adjunctive therapy. *J. Glaucoma* **10**, S81–S84 (2001).

93. Dhingra, S. & Khaw, P. T. The Moorfields Safer Surgery System. *Middle East Afr. J. Ophthalmol.* **16**, 112–115 (2009).

94. Cordeiro, M. F., Constable, P. H., Alexander, R. A., Bhattacharya, S. S. & Khaw, P. T. Effect of varying the mitomycin-C treatment area in glaucoma filtration surgery in the rabbit. *Investig. Ophthalmol. Vis. Sci.* **38**, 1639–1646 (1997).

95. Nguyen, K. D. & Lee, D. a. Effect of steroids and nonsteroidal antiinflammatory agents on human ocular fibroblast. *Invest. Ophthalmol. Vis. Sci.* **33**, 2693–2701 (1992).

96. Armaly, M. F. Inheritance of Dexamethasone Hypertension and Glaucoma. *Arch. Ophthalmol.* **77**, 747–751 (1967).

97. Chang, D. F., Tan, J. J. & Tripodis, Y. Risk factors for steroid response among cataract patients. *J. Cataract Refract. Surg.* **37**, 675–681 (2011).

98. Kronfeld, P. C. Functional Characteristics of Surgically Produced Outflow Channels: The 25th Edward Jackson Memorial Lecture. *Am. J. Ophthalmol.* **67**, 451–463 (1969).

99. Muecke, J. & Brian, G. Steroid-induced ocular hypertension in the presence of a functioning Molteno seton. *Clin. Experiment. Ophthalmol.* **23**, 67–68 (1995).

100. Seibold, L. K., Sherwood, M. B. & Kahook, M. Y. Wound Modulation After Filtration Surgery. *Surv. Ophthalmol.* **57**, 530–550 (2012).

101. Thomas, R. & Jay, J. L. Raised intraocular pressure with topical steroids after trabeculectomy. *Graefe's Arch. Clin. Exp. Ophthalmol.* **226**, 337–340 (1988).

102. Wilensky, J. T., Snyder, D. & Gieser, D. Steroid-induced ocular hypertension in patients with filtering blebs. *Ophthalmology* **87**, 240–244 (1980).

103. Zhan, G. L., Miranda, O. C. & Bito, L. Z. Steroid glaucoma: Corticosteroid-induced ocular hypertension in cats. *Exp. Eye Res.* **54**, 211–218 (1992).

104. Klein, R. *et al.* Fifteen-Year Cumulative Incidence of Age-Related Macular Degeneration The Beaver Dam Eye Study. *Ophthalmology* 253–262 (2007). doi:10.1016/j.ophtha.2006.10.040

105. How, A. *et al.* Combined treatment with bevacizumab and 5-Fluorouracil attenuates the postoperative scarring response after experimental glaucoma filtration surgery. *Investig. Ophthalmol. Vis. Sci.* **51**, 928–932 (2010).

106. Noor, N. A., Mustafa, S. & Artini, W. Glaucoma drainage device implantation with adjunctive intravitreal bevacizumab in neovascular glaucoma: 3-year experience. *Clin. Ophthalmol.* **11**, 1417–1422 (2017).

107. Van Bergen, T., Vandewalle, E., Moons, L. & Stalmans, I. Complementary effects of bevacizumab and MMC in the improvement of surgical outcome after glaucoma filtration surgery. *Acta Ophthalmol.* **93**, 667–678 (2015).

108. Cheng, J., Cheng, S., Wei, R. & Lu, G. Anti-vascular endothelial growth factor for control of wound healing in glaucoma surgery. *Cochrane Database Syst. Rev.* (2016).

109. Daniel, E. *et al.* Risk of Scar in the Comparison of Age-related Macular Degeneration Treatments Trials. *Ophthalmology* **121**, 656–666 (2014).

110. Daniel, E. *et al.* Development and Course of Scars in the Comparison of Age-Related Macular Degeneration Treatments Trials. *Ophthalmology* **125**, 1037–1046 (2018).

111. Tillmanns, J. *et al.* Fibroblast activation protein alpha expression identifies activated fibroblasts after myocardial infarction. *J. Mol. Cell. Cardiol.* **87**, 194–203 (2015).

112. Hinz, B., Celetta, G., Tomasek, J. J., Gabbiani, G. & Chaponnier, C. Alpha-Smooth Muscle Actin Expression Upregulates Fibroblast Contractile Activity. *Mol. Biol. Cell* **12**, 2730–2741 (2001).

113. Annes, J. P., Munger, J. S. & Rifkin, D. B. Making sense of latent TGFbeta activation. *J. Cell Sci.* **116**, 217–24 (2003).

114. Rahimi, R. A. & Leof, E. B. TGF-b signaling: A tale of two responses. *J. Cell. Biochem.* **102**, 593–608 (2007).

115. Picht, G. & Grehn, F. Development of the filtering bleb after trabeculectomy. Classification, histopathology, wound healing process. *Der Ophthalmol. Zeitschrift der Dtsch. Ophthalmol. Gesellschaft* **95**, W380-7 (1998).

116. Agnifili, L. *et al.* Histological findings of failed gold micro shunts in primary open-Angle glaucoma. *Graefe's Arch. Clin. Exp. Ophthalmol.* (2012). doi:10.1007/s00417-011-1778-6

117. Thieme, H., Choritz, L., Hofmann-Rummelt, C., Schloetzer-Schrehardt, U. & Kottler, U. B. Histopathologic findings in early encapsulated blebs of young patients treated with the ahmed glaucoma valve. *J. Glaucoma* **20**, 246–251 (2011).

118. Zhu, X. *et al.* Evaluation of chitosan/aptamer targeting TGF-β receptor II thermo-sensitive gel for scarring in rat glaucoma filtration surgery. *Investig. Ophthalmol. Vis. Sci.* **56**, 5465–5476 (2015).

119. Mead, A. L., Wong, T. T. L., Cordeiro, M. F., Anderson, I. K. & Khaw, P. T. Evaluation of anti-TGF-b2 antibody as a new postoperative anti-scarring agent in glaucoma surgery. *Investig. Ophthalmol. Vis. Sci.* **44**, 3394–3401 (2003).

120. Cordeiro, M. F., Gay, J. A. & Khaw, P. T. Human Anti-Transforming Growth Factor-b2 Antibody: A New Glaucoma Anti-Scarring Agent. *Ophthalmol. Vis. Sci.* **40**, 2225–2234 (1999).

121. CAT-152 0102 Trabeculectomy Study Group. A Phase III Study of Subconjunctival Human Anti-Transforming Growth Factor b2 Monoclonal Antibody (CAT-152) to Prevent Scarring after First-Time Trabeculectomy. *Ophthalmology* **114**, (2007).

122. Siriwardena, D. *et al.* Human antitransforming growth factor β2 monoclonal antibody—a new modulator of wound healing in trabeculectomy: a randomized placebo controlled clinical study. *Ophthalmology* **109**, 427–431 (2002).

123. Sapitro, J. *et al.* Suppression of transforming growth factor-β effects in rabbit subconjunctival fibroblasts by activin receptor-like kinase 5 inhibitor. *Mol. Vis.* **16**, 1880–92 (2010).

124. Yu-wai-man, C. & Khaw, P. T. Developing novel anti-fibrotic therapeutics to modulate post-surgical wound healing in glaucoma : big potential for small molecules. *Expert Rev. Ophthalmol.* **9899**, (2015).

125. Daniels, J. T. *et al.* Matrix metalloproteinase inhibition modulates fibroblast-mediated matrix contraction and collagen production in vitro. *Investig. Ophthalmol. Vis. Sci.* **44**, 1104–1110 (2003).

126. Wong, T. T. L., Mead, A. L. & Khaw, P. T. Prolonged antiscarring effects of ilomastat and MMC after experimental glaucoma filtration surgery. *Investig. Ophthalmol. Vis. Sci.* **46**, 2018–2022 (2005).

127. Mohamed-Ahmed, A. H. A. *et al.* An ilomastat-CD eye drop formulation to treat ocular scarring. *Investig. Ophthalmol. Vis. Sci.* **58**, 3425–3431 (2017).

128. Kitajima, S. *et al.* Overcoming resistance to dual innate immune and MEK inhibition downstream of KRAS. *Cancer Cell* **34**, 439–452 (2018).

129. De Saint, J. M. *et al.* Toxicity of preserved and unpreserved antiglaucoma topical drugs in an in vitro model of conjunctival cells. *Curr Eye Res* **20**, 85–94 (2000).

130. Gedde, S. J. *et al.* Treatment outcomes in the tube versus trabeculectomy (TVT) study after five years of follow-up. *Am. J. Ophthalmol.* **153**, 789-803.e2 (2012).

131. Cankaya, A. B. & Elgin, U. Comparison of the outcome of repeat trabeculectomy with adjunctive mitomycin C and initial trabeculectomy. *Korean J. Ophthalmol.* **25**, 401–8 (2011).

132. Jampel, H. D. *et al.* Perioperative complications of trabeculectomy in the Collaborative Initial Glaucoma Treatment Study (CIGTS). *Am. J. Ophthalmol.* **140**, 16–22 (2005).

133. Rosentreter, A., Schild, A. M., Jordan, J. F., Krieglstein, G. K. & Dietlein, T. S. A prospective randomised trial of trabeculectomy using mitomycin C vs an ologen implant in open angle glaucoma. *Eye* **24**, 1449–1457 (2010).

134. Khaw, P. *et al.* Factors Affecting the Outcome of Trabeculectomy. *Ophthalmology* **114**, 1831-1838.e4 (2007).

135. Mietz, H., Raschka, B. & Krieglstein, G. K. Risk factors for failures of trabeculectomies performed without antimetabolites. *Br. J. Ophthalmol.* **83**, 814–821 (1999).

136. Schwartz, A. L. *et al.* The advanced glaucoma intervention study (AGIS): 5. Encapsulated bleb after initial trabeculectomy. *Am. J. Ophthalmol.* **127**, 8–19 (1999).

137. Sugimoto, Y. *et al.* Intraocular Pressure Outcomes and Risk Factors for Failure in the Collaborative Bleb-Related Infection Incidence and Treatment Study. *Ophthalmology* **122**, 2223–2233 (2015).

138. Edmunds, B., Bunce, C. V., Thompson, J. R., Salmon, J. F. & Wormald, R. P. Factors Associated with Success in First-Time Trabeculectomy for Patients at Low Risk of Failure with Chronic Open-Angle Glaucoma. *Ophthalmology* **111**, 97–103 (2004).

139. AL-Obeidan, S. A., Mousa, A., Naseem, A., Abu-Amero, K. K. & Osman, E. A. Efficacy and safety of non-penetrating deep sclerectomy surgery in saudi patients with uncontrolled open angle glaucoma. *Saudi Med. J.* **34**, 54–61 (2013).

140. Bovee, C. E. & Pasquale, L. R. Evolving surgical interventions in the treatment of glaucoma. in *Seminars in ophthalmology* **32**, 91–95 (Taylor & Francis, 2017).

141. Lockwood, A., Brocchini, S. & Khaw, P. T. New developments in the pharmacological modulation of wound healing after glaucoma filtration surgery. *Curr. Opin. Pharmacol.* **13**, 65–71 (2013).

142. Elm, E. von *et al.* Annals of Internal Medicine Academia and Clinic The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement : Guidelines for Reporting. *Ann. Intern. Med.* **147**, 573–578 (2014).

143. Bressler, B. *et al.* Rates of new or missed colorectal cancers after colonoscopy and their risk factors: a population-based analysis. *Gastroenterology* **132**, 96–102 (2007).

144. Williams, J. I. & Young, W. A summary of studies on the quality of health care administrative databases in Canada. *Patterns Heal. care Ontario ICES Pract. atlas. 2nd ed. Ottawa Can. Med. Assoc.* **339**, 45 (1996).

145. Levy, A. R., O'Brien, B. J., Sellors, C., Grootendorst, P. & Willison, D. Coding accuracy of administrative drug claims in the Ontario Drug Benefit database. *Can. J. Clin. Pharmacol. J. Can. Pharmacol. Clin.* **10**, 67–71 (2002).

146. Tu, K., Chen, Z. & Lipscombe, L. L. Prevalence and incidence of hypertension from 1995 to 2005: a population-based study. *Cmaj* **178**, 1429–1435 (2008).

147. Guttmann, A. *et al.* Validation of a health administrative data algorithm for assessing the epidemiology of diabetes in Canadian children. *Pediatr. Diabetes* **11**, 122–128 (2010).

148. Gershon, A. S. *et al.* Identifying patients with physician-diagnosed asthma in health administrative databases. *Can. Respir. J.* **16**, 183–188 (2009).

149. Juurlink, D. *et al.* Canadian institute for health information discharge abstract database: a validation study. *ICES Investig. report. Inst. Clin. Eval. Sci. Toronto* (2006).

150. Borugian, M. J., Spinelli, J. J., Mezei, G. & Wilkins, R. Childhood Leukemia and Socioeconomic Status in Canada. *Epidemiology* **16**, 526–531 (2005).

151. Deyo, R. & Ciol, M. A. Adapting a Clinical Comorbidity Index for Use With ICD-9-CM Administrative Databases. *J. Clin. Epidemiol.* **6196**, (1992).

152. Quan, H. *et al.* Data Coding Algorithms for Defining Comorbidities in ICD-9-CM and ICD-1 0 Administrative Data. *Med. Care* **43**, 1130–1139 (2005).

153. Tu, K. *et al.* Validity of administrative data for identifying patients who have had a stroke or transient ischemic attack using emrald as a reference standard. *Can. J. Cardiol.* **29**, 1388–1394 (2013).

154. McIsaac, D. I. *et al.* Identifying Obstructive Sleep Apnea in Administrative Data. *Anesthesiology* **123**, 253–263 (2015).

155. Borucka, J. Extensions of Cox Model for Non-Proportional Hazards Purpose. *Ekonometrika* **3**, 85–101 (2014).

156. Austin, P. C. & Fine, J. P. Practical recommendations for reporting Fine-Gray model analyses for competing risk data. *Stat. Med.* **36**, 4391–4400 (2017).

157. Landers, J., Martin, K., Sarkies, N., Bourne, R. & Watson, P. A twenty-year follow-up study of trabeculectomy: Risk factors and outcomes. *Ophthalmology* **119**, 694–702 (2012).

158. Gedde, S. J. *et al.* Treatment Outcomes in the Tube Versus Trabeculectomy Study After One Year of Follow-up. *Am. J. Ophthalmol.* (2007). doi:10.1016/j.ajo.2009.06.018

159. Mietz, H., Jacobi, P. C., Welsandt, G. & Krieglstein, G. K. Trabeculectomies in fellow eyes have an increased risk of Tenon's capsule cysts. *Ophthalmology* **109**, 992–997 (2002).

160. Iwasaki, K. *et al.* Comparing trabeculectomy outcomes between first and second operated eyes: A multicenter study. *PLoS One* **11**, 1–13 (2016).

161. Lochhead, J., Casson, R. J. & Salmon, J. F. Long term effect on intraocular pressure of phacotrabeculectomy compared to trabeculectomy. *Br. J. Ophthalmol.* **87**, 850–852 (2003).

162. Murthy, S. K., Damji, K. F., Pan, Y. & Hodge, W. G. Trabeculectomy and phacotrabeculectomy, with mitomycin-C, show similar two-year target IOP outcomes. *Can. J. Ophthalmol. Can. d'Ophtalmologie* **41**, 51–59 (2006).

163. Breusegem, C. *et al.* Preoperative nonsteroidal anti-inflammatory drug or steroid and outcomes after trabeculectomy: A randomized controlled trial. *Ophthalmology* **117**, 1324–1330 (2010).

164. Hottendorf, G. H. & Williams, P. D. Aminoglycoside Nephrotoxicity. *Toxicol. Pathol.* (1986).

165. Avent, M. L., Rogers, B. A., Cheng, A. C. & Paterson, D. L. Current use of aminoglycosides: Indications, pharmacokinetics and monitoring for toxicity. *Intern. Med. J.* **41**, 441–449 (2011).

166. Kupin, T. H., Juzych, M. S., Shin, D. H., Khatana, A. K. & Olivier, M. M. G. Adjunctive mitomycin C in primary trabeculectomy in phakic eyes. *Am. J. Ophthalmol.* **119**, 30–39 (1995).

167. Masoumpour, M. B., Nowroozzadeh, M. H. & Razeghinejad, M. R. Suppl 1: M5: Current and Future Techniques in Wound Healing Modulation after Glaucoma Filtering Surgeries. *Open Ophthalmol. J.* **10**, 68 (2016).

168. Panarelli, J. F., Nayak, N. V & Sidoti, P. A. Postoperative management of trabeculectomy and glaucoma drainage implant surgery. *Curr. Opin. Ophthalmol.* **27**, 170–176 (2016).

169. Fuller, J. R., Bevin, T. H., Molteno, A. C. B., Vote, B. J. T. & Herbison, P. Anti-inflammatory fibrosis suppression in threatened trabeculectomy bleb failure produces good long term control of intraocular pressure without risk of sight threatening complications. *Br. J. Ophthalmol.* **86**, 1352–1355 (2002).

170. Rodriguez Una, I. *et al.* Perioperative pharmacological management in patients

with glaucoma. *Arch. Soc. Esp. Oftalmol.* **90**, 274–284 (2015).

171. Kent, A. R. *et al.* The efficacy and safety of diclofenac 0.1% versus prednisolone acetate 1% following trabeculectomy with adjunctive mitomycin-C. *Ophthalmic Surgery, Lasers Imaging Retin.* **29**, 562–569 (1998).

172. Levkovitch-Verbin, H., Katz, G., Kalev-Landoi, M. & Goldenfeld, M. Postoperative Treatment With Topical Diclofenac Versus Topical Dexamethasone After Combined Phacotrabeculectomy With Mitomycin C. *J. Glaucoma* **22**, 1 (2011).

173. Scott, G. R. *et al.* Can topical ketorolac 0.5% improve the function of Ahmed glaucoma drainage devices? *Ophthalmic Surg. Lasers Imaging2* **42**, 190–195 (2011).

174. Almatlouh, A., Bach-Holm, D. & Kessel, L. Steroids and nonsteroidal anti-inflammatory drugs in the postoperative regime after trabeculectomy - which provides the better outcome? A systematic review and meta-analysis. *Acta Ophthalmol.* 1–12 (2018). doi:10.1111/aos.13919

175. Levkovitch-Verbin, H., Katz, G., Kalev-Landoi, M. & Goldenfeld, M. Postoperative Treatment With Topical Diclofenac Versus Topical Dexamethasone After Combined Phacotrabeculectomy With Mitomycin C. *J. Glaucoma* **22**, 177–182 (2013).

176. Nakano, T., Ohara, O., Teraoka, H. & Arita, H. Glucocorticoids suppress group II phospholipase A2production by blocking mRNA synthesis and post-transcriptional expression. *J. Biol. Chem.* **265**, 12745–12748 (1990).

177. Funk, C. D. Prostaglandins and Leukotrienes : Advances in Eicosanoid Biology. *Science (80-.).* **294**, (2001).

178. Ricciotti, Emanuela and FitzGerald, G. A. Prostaglandins and Inflammation. *Arter. Thromb Vasc Biol.* **31**, 986–1000 (2011).

179. Dennis, E. A. & Norris, P. C. Eicosanoid Storm in Infection and Inflammation. *Nat. Rev. Immunol.* **15**, 511–523 (2016).

180. Almeida, D. R. P. *et al.* Effect of prophylactic nonsteroidal antiinflammatory drugs on cystoid macular edema assessed using optical coherence tomography quantification of total macular volume after cataract surgery. *J. Cataract Refract. Surg.* **34**, 64–69 (2008).

181. Asano, S. *et al.* Reducing angiographic cystoid macular edema and blood-aqueous barrier disruption after small-incision phacoemulsification and foldable intraocular lens implantation. Multicenter prospective randomized comparison of topical diclofenac 0.1% and betamethason. *J. Cataract Refract. Surg.* **34**, 57–63 (2008).

182. Laurell, C. G. & Zetterström, C. Effects of dexamethasone, diclofenac, or placebo on the inflammatory response after cataract surgery. *Br. J. Ophthalmol.* **86**, 1380–1384 (2002).

183. Sun, R., Gimbel, H. V, Liu, S., Guo, D. & Hollenberg, M. D. Effect of diclofenac sodium and dexamethasone on cultured human Tenon's capsule fibroblasts. *Ophthalmic Surgery, Lasers Imaging Retin.* **30**, 382–388 (1999).

184. Baker, B. M. & Chen, C. S. Deconstructing the third dimension: how 3D culture microenvironments alter cellular cues. *J. Cell Sci.* **125**, 3015–24 (2012).

185. Dallon, J. C., Ph, D. & Ehrlich, H. P. A Review of Fibroblast Populated Collagen Lattices. 1–20 (2008).

186. Bell, E., Ehrlich, H. P., Buttle, D. J. & Nakatsuji, T. Living tissue formed in vitro and accepted as skin-equivalent tissue of full thickness. *Science (80-.).* **211**, 1052–1054 (1981).

187. Eliceiri, K., Schneider, C. A., Rasband, W. S. & Eliceiri, K. W. NIH Image to ImageJ : 25 years of image analysis HISTORICAL commentary NIH Image to ImageJ : 25 years of image analysis. *Nat. Methods* **9**, 671–675 (2012).

188. Schmitz, N., Laverty, S., Kraus, V. B. & Aigner, T. Basic methods in histopathology of joint tissues. *Osteoarthr. Cartil.* **18**, S113–S116 (2010).

189. Eberth, J. F. *et al.* Importance of pulsatility in hypertensive carotid artery growth and remodeling. *J. Hypertens.* **27**, 2010–2021 (2009).

190. Whittaker, P., Kloner, R. A., Boughner, D. R. & Pickering, J. G. Quantitative assessment of myocardial collagen with picrosirius red staining and circularly polarized light. *Basic Res. Cardiol.* **89**, 397–410 (1994).

191. Rich, L. & Whittaker, P. Collagen and Picrosirius Red Staining : a Polarized Light Assessment of Fibrillar Hue and Spatial Distribution. *Braz J Morphol Sci* **22**, 97–104 (2005).

192. Ophir, A. Encapsulated filtering bleb. A selective review--new deductions. *Eye (Lond).* **6 (Pt 4)**, 348–52 (1992).

193. Mietz, H., Arnold, G., Kirchhof, B., Diestelhorst, M. & Krieglstein, G. K. Histopathology of episcleral fibrosis after trabeculectomy with and without mitomycin C. *Graefes Arch. Clin. Exp. Ophthalmol.* **234**, 364–8 (1996).

194. Mahale, A. *et al.* Histopathologic and immunohistochemical features of capsular tissue around failed Ahmed glaucoma valves. 1–13 (2017).

195. Shinde, A. V., Humeres, C. & Frangogiannis, N. G. The role of a-smooth muscle actin in fibroblast-mediated matrix contraction and remodeling. *Biochim. Biophys. Acta - Mol. Basis Dis.* **1863**, 298–309 (2017).

196. Sakaki, H. *et al.* Interleukin-1beta induces matrix metalloproteinase-1 expression in cultured human gingival fibroblasts: role of cyclooxygenase-2 and prostaglandin E2. *Oral Dis.* **10**, 87–93 (2004).

197. Mapleson, J. L. & Buchwald, M. Effect of cycloheximide and dexamethasone phosphate on hyaluronic acid synthesis and secretion in cultured human skin

fibroblasts. *J. Cell. Physiol.* **109**, 215–222 (1981).

198. Smith, T. J. Dexamethasone regulation of glycosaminoglycan synthesis in cultured human skin fibroblasts. Similar effects of glucocorticoid and thyroid hormones. *J. Clin. Invest.* **74**, 2157–2163 (1984).

199. Varkey, M., Ding, J. & Tredget, E. E. Differential collagen-glycosaminoglycan matrix remodeling by superficial and deep dermal fibroblasts: Potential therapeutic targets for hypertrophic scar. *Biomaterials* **32**, 7581–7591 (2011).

200. Baudouin, C. *et al.* Efficacy of indomethacin 0.1% and fluorometholone 0.1% on conjunctival inflammation following chronic application of antiglaucomatous drugs. *Graefe's Arch. Clin. Exp. Ophthalmol.* **240**, 929–935 (2002).

201. Rhee, S. Fibroblasts in three dimensional matrices: cell migration and matrix remodeling. *Exp. Mol. Med.* **41**, 858–865 (2009).

202. Weinreb, R. N., Aung, T. & Medeiros, F. A. The pathophysiology and treatment of glaucoma: a review. *Jama* **311**, 1901–1911 (2014).

203. Tsai, J. C. Medication adherence in glaucoma: approaches for optimizing patient compliance. *Curr. Opin. Ophthalmol.* **17**, 190–195 (2006).

204. Huang, C. & Ogawa, R. Fibroproliferative Disorders and Their Mechanobiology. *Connect. Tissue Res.* **53**, 187–196 (2012).

205. Saika, S., Yamanaka, O., Okada, Y. & Sumioka, T. Modulation of Smad signaling by non-TGFβ components in myofibroblast generation during wound healing in corneal stroma. *Exp. Eye Res.* **142**, 40–48 (2016).

206. Takai, Y., Tanito, M. & Ohira, A. Multiplex cytokine analysis of aqueous humor in eyes with primary open-angle glaucoma, exfoliation glaucoma, and cataract. *Investig. Ophthalmol. Vis. Sci.* **53**, 241–247 (2012).

207. Cvenkel, B., Kopitar, A. N. & Ihan, A. Inflammatory molecules in aqueous humour and on ocular surface and glaucoma surgery outcome. *Mediators Inflamm.* **2010**, (2010).

208. Darby, I. A., Zakuan, N., Billet, F. & Desmoulière, A. The myofibroblast, a key cell in normal and pathological tissue repair. *Cell. Mol. Life Sci.* 1–13 (2015). doi:10.1007/s00018-015-2110-0

209. Grinnell, F. Fibroblasts, Myofibroblasts, and Wound Contraction. *J. Cell Biol.* **124**, 511–516 (1994).

210. Gabbiani, G. The myofibroblast in wound healing and fibrocontractive diseases. *J. Pathol.* **200**, 500–503 (2003).

211. Toh, Z. H., Lee, C. S. Y., Chew, A. C. Y. & Perera, S. Time Heals All Wounds: Obstacles in Glaucoma Surgery from an Asian Perspective. *Proc. Singapore Healthc.* **24**, 103–112 (2015).

212. Schlötzer-Schrehardt, U., Lommatzsch, J., Küchle, M., Konstas, A. G. P. & Naumann, G. O. H. Matrix metalloproteinases and their inhibitors in aqueous humor of patients with pseudoexfoliation syndrome/glaucoma and primary open-angle glaucoma. *Investig. Ophthalmol. Vis. Sci.* **44**, 1117–1125 (2003).

213. Chintala, S. K. *et al.* Matrix metalloproteinase gelatinase B (MMP-9) is associated with leaking glaucoma filtering blebs. *Exp. Eye Res.* **81**, 429–436 (2005).

214. Acera, A., Vecino, E. & Duran, J. A. Tear MMP-9 levels as a marker of ocular surface inflammation in conjunctivochalasis. *Invest. Ophthalmol. Vis. Sci.* **54**, 8285–8291 (2013).

215. Serhan, C. N., Krishnamoorthy, S., Recchiuti, A. & Chiang, N. Novel anti-inflammatory--pro-resolving mediators and their receptors. *Curr. Top. Med. Chem.* **11**, 629–47 (2011).

216. Uderhardt, S. & Krönke, G. 12/15-Lipoxygenase during the regulation of inflammation, immunity, and self-tolerance. *J. Mol. Med.* **90**, 1247–1256 (2012).

217. Al Habash, A., Aljasim, L. A., Owaidhah, O. & Edward, D. P. A review of the efficacy of mitomycin C in glaucoma filtration surgery. *Clin. Ophthalmol. (Auckland, NZ)* **9**, 1945 (2015).

218. Chang, L., Crowston, J. G., Cordeiro, M. F., Akbar, A. N. & Khaw, P. T. The role of the immune system in conjunctival wound healing after glaucoma surgery. *Surv. Ophthalmol.* **45**, 49–68 (2000).

219. Spitzer, M. S. *et al.* Sodium hyaluronate gels as a drug-release system for corticosteroids: Release kinetics and antiproliferative potential for glaucoma surgery. *Acta Ophthalmol.* (2008). doi:10.1111/j.1755-3768.2007.01149.x

220. Cheng, J.-W., Cai, J.-P., Li, Y. & Wei, R.-L. Intraoperative mitomycin C for nonpenetrating glaucoma surgery: a systematic review and meta-analysis. *J. Glaucoma* **20**, 322–326 (2011).

221. Jampel, H. D. *et al.* Risk factors for late-onset infection following glaucoma filtration surgery. *Arch. Ophthalmol.* **119**, 1001–8 (2001).

222. Cells, M. *et al.* Dexamethasone Stiffens Trabecular Meshwork, Trabecular Meshwork Cells, and Matrix. (2018). doi:10.1167/iovs.15-16739

223. Armstrong, J. J., Denstedt, J. T., Trelford, C. B., Li, E. A. & Hutnik, C. M. L. Differential effects of dexamethasone and indomethacin on Tenon' s capsule fibroblasts: Implications for glaucoma surgery. *Exp. Eye Res.* **182**, 65–73 (2019).

224. Rowlinson, S. W. *et al.* Spatial Requirements for 15- (R) -Hydroxy-5 Z , 8 Z , 11 Z , 13 E - eicosatetraenoic Acid Synthesis within the Cyclooxygenase Active Site of Murine COX-2. *J. Biol. Chem.* **275**, 6586–6591 (2000).

225. Kalgutkar, A. S., Kozak, K. R., Crews, B. C., Hochgesang, G. P. & Marnett, L. J. Covalent modification of cyclooxygenase-2 (COX-2) by 2-acetoxyphenyl alkyl sulfides, a new class of selective COX-2 inactivators. *J. Med. Chem.* **41**, 4800–

4818 (1998).

226. Chiang, N., Bermudez, E. A., Ridker, P. M., Hurwitz, S. & Serhan, C. N. Aspirin triggers antiinflammatory 15-epi-lipoxin A4 and inhibits thromboxane in a randomized human trial. *Proc. Natl. Acad. Sci.* **101**, 15178–15183 (2004).

227. Claria, J. & Serhan, C. N. Aspirin triggers previously undescribed bioactive eicosanoids by human endothelial cell-leukocyte interactions. *Proc. Natl. Acad. Sci.* **92**, 9475–9479 (1995).

228. Takano, B. T. *et al.* Aspirin-triggered 15-Epi-Lipoxin A 4 (LXA 4) and LXA 4 Stable Analogues Are Potent Inhibitors of Acute Inflammation: Evidence for Anti-inflammatory Receptors. *J Exp Med* **185**, 1693–704 (1997).

229. Endo, J. *et al.* 18-HEPE, an n-3 fatty acid metabolite released by macrophages, prevents pressure overload–induced maladaptive cardiac remodeling. *J. Exp. Med.* **211**, 1673–1687 (2014).

230. Planagumà, A. *et al.* Docosahexaenoic acid (DHA) blunts liver injury by conversion to protective lipid mediators: protectin D1 and 17 S -hydroxy-DHA . *FASEB J.* **20**, 2537–2539 (2006).

231. Lannan, K. L., Spinelli, S. L., Blumberg, N. & Phipps, R. P. Maresin 1 induces a novel pro-resolving phenotype in human platelets. *J. Thromb. Haemost.* **15**, 802–813 (2017).

232. Purschke, M. *et al.* Omega-6 docosapentaenoic acid-derived resolvins and 17-hydroxydocosahexaenoic acid modulate macrophage function and alleviate experimental colitis. *Inflamm. Res.* **61**, 967–976 (2012).

233. Le Faouder, P. *et al.* LC-MS/MS method for rapid and concomitant quantification of pro-inflammatory and pro-resolving polyunsaturated fatty acid metabolites. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **932**, 123–133 (2013).

234. Martin-venegas, R., Olga, J. & Jose, J. Liquid chromatography-tandem mass spectrometry analysis of eicosanoids and related compounds in cell models. **964**, 41–49 (2014).

235. Saika, S. TGFβ pathobiology in the eye. *Lab. Investig.* **86**, 106–115 (2006).

236. Wang, X. *et al.* Aspirin suppresses cardiac fibroblast proliferation and collagen formation through downregulation of angiotensin type 1 receptor transcription. *Toxicol. Appl. Pharmacol.* **259**, 346–354 (2012).

237. Al-Nimer, M. S. M., Hameed, H. G. & Mahmood, M. M. Antiproliferative effects of aspirin and diclofenac against the growth of cancer and fibroblast cells: In vitro comparative study. *Saudi Pharm. J.* **23**, 483–486 (2015).

238. Viñals, M. *et al.* Aspirin increases CD36, SR-BI, and ABCA1 expression in human THP-1 macrophages. *Cardiovasc. Res.* **66**, 141–149 (2005).

239. Yiqin, Y., Meilin, X., Jie, X. & Keping, Z. Aspirin inhibits MMP-2 and MMP-9

expression and activity through PPARα/γ and TIMP-1-mediated mechanisms in cultured mouse celiac macrophages. *Inflammation* (2009). doi:10.1007/s10753-009-9125-3

240. Lai, M. Y., Huang, J. A., Liang, Z. H., Jiang, H. X. & Tang, G. D. Mechanisms underlying aspirin-mediated growth inhibition and apoptosis induction of cyclooxygenase-2 negative colon cancer cell line SW480. *World J. Gastroenterol.* **14**, 4227–4233 (2008).

241. Jian, Z. *et al.* Aspirin induces Nrf2-mediated transcriptional activation of haem oxygenase-1 in protection of human melanocytes from H2O2-induced oxidative stress. *J. Cell. Mol. Med.* **20**, 1307–1318 (2016).

242. Liu, P. P. *et al.* Aspirin alleviates cardiac fibrosis in mice by inhibiting autophagy. *Acta Pharmacol. Sin.* **38**, 488–497 (2017).

243. Mehta, J. L., Chen, J., Yu, F. & Li, D. Y. Aspirin inhibits ox-LDL-mediated LOX-1 expression and metalloproteinase-1 in human coronary endothelial cells. *Cardiovasc. Res.* **64**, 243–249 (2004).

244. Bhatt, L. K. & Addepalli, V. Attenuation of diabetic retinopathy by enhanced inhibition of MMP-2 and MMP-9 using aspirin and minocycline in streptozotocin-diabetic rats. *Am. J. Transl. Res.* **2**, 181–189 (2010).

245. Claria, J., Nguyen, B. T., Madenci, A. L., Ozaki, C. K. & Serhan, C. N. Diversity of lipid mediators in human adipose tissue depots. *AJP Cell Physiol.* (2013). doi:10.1152/ajpcell.00351.2012

246. Wynn, T. A. Fibrotic Disease and the TH1/TH2 Paradigm. *Nat Rev Immunol* **4**, 583–594 (2004).

247. Wei, J. & Gronert, K. Eicosanoid and Specialized Proresolving Mediator Regulation of Lymphoid Cells. (2018). doi:10.1016/j.tibs.2018.10.007

248. Rius, B. *et al.* The specialized pro-resolving lipid mediator maresin 1 protects hepatocytes from lipotoxic and hypoxia-induced endoplasmic reticulum stress. *FASEB J.* fj.201700394R (2017). doi:10.1096/fj.201700394R

249. Lee, J. Y. *et al.* Neuronal SphK1 acetylates COX2 and contributes to pathogenesis in a model of Alzheimer's Disease. *Nat. Commun.* **9**, (2018).

250. Roth, G. J., Stanford, N. & Majerus, P. W. Acetylation of prostaglandin synthase by aspirin. *Proc. Natl. Acad. Sci. U. S. A.* **72**, 3073–6 (1975).

251. Serhan, C. N. Lipoxins and aspirin-triggered 15-epi-lipoxins. pro-resolving mediators in anti-inflammation and resolution. *Handb. Cell Signaling, 2/e* **2**, 1235–1242 (2010).

252. Birnbaum, Y. *et al.* Aspirin augments 15-epi-lipoxin A4production by lipopolysaccharide, but blocks the pioglitazone and atorvastatin induction of 15-epi-lipoxin A4in the rat heart. *Prostaglandins Other Lipid Mediat.* **83**, 89–98 (2007).

253. Von Der Weid, P. Y., Hollenberg, M. D., Fiorucci, S. & Wallace, J. L. Aspirin-triggered, cyclooxygenase-2-dependent lipoxin synthesis modulates vascular tone. *Circulation* **110**, 1320–1325 (2004).

254. Kalgutkar, A. S. *et al.* Aspirin-like Molecules that Covalently Inactivate Cyclooxygenase-2. *Science (80-.).* **280**, 1268–1270 (1998).

255. Hinz, B. Formation and function of the myofibroblast during tissue repair. *J. Invest. Dermatol.* **127**, 526–537 (2007).

256. Sun, X. & Kaufman, P. D. Ki-67: more than a proliferation marker. *Chromosoma* 1–12 (2018).

257. Pedersen, S. F., Poulsen, K. A. & Lambert, I. H. Roles of phospholipase A_2 isoforms in swelling- and melittin-induced arachidonic acid release and taurine efflux in NIH3T3 fibroblasts. *Am. J. Physiol. Physiol.* **291**, C1286–C1296 (2006).

258. Cajal, Y. & Jain, M. K. Synergism between mellitin and phospholipase A2 from bee venom: Apparent activation by intervesicle exchange of phospholipids. *Biochemistry* **36**, 3882–3893 (1997).

259. Schuster, V. L., Chi, Y. & Lu, R. *The Prostaglandin Transporter: Eicosanoid Reuptake, Control of Signaling, and Development of High-Affinity Inhibitors as Drug Candidates*. *Transactions of the American Clinical and Climatological Association* **126**, (2015).

260. Martinez-Salgado, C., Rodriguez-Barbero, A., Rodriguez-Puyol, D., Pérez de Lema, G. & Lopez-Novoa, J. M. Involvement of phospholipase A2 in gentamicin-induced rat mesangial cell activation. *Am. J. Physiol. Physiol.* **273**, F60–F66 (1997).

261. Vane, J. R. & Botting, R. M. Mechanism of action of anti-inflammatory drugs. *Scand. J. Rheumatol.* **25**, 9–21 (1996).

262. Meade, E. A., Smith, W. L. & DeWitt, D. L. Differential inhibition of prostaglandin endoperoxide synthase (cyclooxygenase) isozymes by aspirin and other non-steroidal anti-inflammatory drugs. *J. Biol. Chem.* **268**, 6610–6614 (1993).

263. Varga, T., Czimmerer, Z. & Nagy, L. PPARs are a unique set of fatty acid regulated transcription factors controlling both lipid metabolism and inflammation. *Biochimica et Biophysica Acta - Molecular Basis of Disease* **1812**, 1007–1022 (2011).

264. Zhu, H. Y. *et al.* Peroxisome proliferator-activated receptor-γ (PPAR-γ) agonist inhibits collagen synthesis in human hypertrophic scar fibroblasts by targeting Smad3 via miR-145. *Biochem. Biophys. Res. Commun.* (2015). doi:10.1016/j.bbrc.2015.02.061

265. Menon, R., Krzyszczyk, P. & Berthiaume, F. Pro-Resolution Potency of Resolvins D1, D2 and E1 on Neutrophil Migration and in Dermal Wound Healing. *Nano Life* **07**, 1750002 (2017).

266. Bannenberg, G. L. *et al.* Molecular Circuits of Resolution: Formation and Actions of Resolvins and Protectins. *J. Immunol.* **174**, 4345–4355 (2005).

267. Chen, W. S., Cao, Z., Leffler, H., Nilsson, U. J. & Panjwani, N. Galectin-3 inhibition by a small-molecule inhibitor reduces both pathological corneal neovascularization and fibrosis. *Investig. Ophthalmol. Vis. Sci.* **58**, 9–20 (2017).

268. Lois, N. *et al.* Effect of TGF-β2 and anti-TGF-β2 antibody in a new in vivo rodent model of posterior capsule opacification. *Investig. Ophthalmol. Vis. Sci.* **46**, 4260–4266 (2005).

269. Ishikawa, K., Kannan, R. & Hinton, D. R. Molecular mechanisms of subretinal fibrosis in age-related macular degeneration. *Exp. Eye Res.* **142**, 19–25 (2014).

270. Pleyer, U., Ursell, P. G. & Rama, P. Intraocular pressure effects of common topical steroids for post-cataract inflammation: are they all the same? *Ophthalmol. Ther.* **2**, 55–72 (2013).

271. Tajika, T., Miyake, K., Ogawa, T., Gow, J. A. & McNamara, T. R. Ocular Pharmacokinetics of a Single Dose of Bromfenac Sodium Ophthalmic Solution 0.1% in Human Aqueous Humor. *J. Ocul. Pharmacol. Ther.* **24**, 573–578 (2008).

272. Ahuja, M., Dhake, A. S., Sharma, S. K. & Majumdar, D. K. Topical Ocular Delivery of NSAIDs. *AAPS J.* **10**, 229–241 (2008).

273. Rubinstein, L. V, Gail, M. H. & Santner, T. J. Planning the duration of a comparative clinical trial with loss to follow-up and a period of continued observation. *J. Chronic Dis.* **34**, 469–479 (1981).

274. Shaarawy, T. M., Sherwood, M. B. & Grehn, F. *WGA Guidelines on Design and Reporting of Glaucoma Surgical Trials*. (2009).

Appendices

Appendix A: STROBE Statement—Checklist of items to be included in reports of cohort studies

	Item No.	Recommendation	Page No.
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	2
Objectives	3	State specific objectives, including any prespecified hypotheses	2
Methods			
Study design	4	Present key elements of study design early in the paper	3
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3-4
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	3-4
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4,5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4-5, Appendices A-F
Bias	9	Describe any efforts to address potential sources of bias	3-5
Study size	10	Explain how the study size was arrived at	Figure 1
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4-5
Statistical methods	12	(*a*) Describe all statistical methods, including	4-5

		those used to control for confounding	
		(*b*) Describe any methods used to examine subgroups and interactions	4-5
		(*c*) Explain how missing data were addressed	4-5
		(*d*) If applicable, explain how loss to follow-up was addressed	5, Figure 1
		(*e*) Describe any sensitivity analyses	4
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Figure 1
		(b) Give reasons for non-participation at each stage	Figure 1
		(c) Consider use of a flow diagram	Figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	6, 7, Table 1, S2
		(b) Indicate number of participants with missing data for each variable of interest	Table 2, Table S2
		(c) Summarise follow-up time (eg, average and total amount)	6
Outcome data	15*	Report numbers of outcome events or summary measures over time	6, Table 1
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Table 3
		(*b*) Report category boundaries when continuous variables were categorized	Table 2, Table S2
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	5,7
Discussion			
Key results	18	Summarise key results with reference to study objectives	8
Limitations	19	Discuss limitations of the study, taking into	8,9

		account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	8-10
Generalisability	21	Discuss the generalisability (external validity) of the study results	8-10
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	11

*Information given separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

Appendix B: Index Events and Outcome Definitions

Procedure	Algorithm
Solo-filtration surgery	Record of OHIP billing code E132, with no add-on codes, without a prior record of E132 or E214 over the previous 10 years, in addition to any one of the following CCI procedural codes: 1.CJ.52.LA, 1CJ.52.LA-SJ, 1.CJ.52.LA-QB and 1.CJ.52.WJ
Filtration surgery +cataract extraction	Record of OHIP billing code E214, with no add-on codes, without a prior record of E132 or E214 over the previous 10 years, in addition to any one of the following CCI procedural codes: 1.CJ.52.LA, 1CJ.52.LA-SJ, 1.CJ.52.LA-QB and 1.CJ.52.WJ
Filtration surgery +IDD	Record of OHIP billing code E132, with the E136 add-on code, without a prior record of E132 or E214 over the previous 10 years, in addition to any one of the following CCI procedural codes: 1.CJ.52.LA, 1CJ.52.LA-SJ, 1.CJ.52.LA-QB and 1.CJ.52.WJ
Filtration Surgery +cataract extraction +IDD	Record of OHIP billing code E214, with the E136 add-on code, without a prior record of E132 or E214 over the previous 10 years, in addition to any one of the following CCI procedural codes: 1.CJ.52.LA, 1CJ.52.LA-SJ, 1.CJ.52.LA-QB and 1.CJ.52.WJ
Revision Filtration Surgery	Within the follow-up period, a record of OHIP billing code E983 or E984 and/or CCI intervention codes: 1.CJ.52.LA, 1CJ.52.LA-SJ, 1.CJ.52.LA-QB, and 1.CJ.52.WJ (accompanied by the intervention attribute “revision”).
Bleb Repair	Within the follow-up period, record of OHIP billing code E212 or E213 and/or CCI intervention codes 1.CS.80, 1.CS.72, 1.CS.84, 1.CS.87, 1.CS.52, 1.CC.80, 1.CD.80 revision, 1.CJ.54, 1.CJ.55, 1.CJ.80, 1.CJ.87, and 1.CD.52.LA revision.

Appendix C: Comorbidity Coding Algorithms

Comorbidity	Algorithm
Diabetes	At least two OHIP claims bearing a diagnosis of diabetes or one OHIP fee code claim, or one CIHI admission within two years. OHIP diagnosis code: 250 OHIP fee codes: K030, Q040, K045, K046, K029 CIHI ICD-9 code: 250 CIHI ICD-10 codes: E10, E11, E13, E14
Hypertension	One hospital admission with a hypertension diagnosis or an OHIP claim with a hypertension diagnosis followed within two years by either an OHIP claim or hospital admission with a hypertension diagnosis. OHIP diagnosis codes: 401, 402, 403, 404, or 405 CIHI ICD-9 codes: 401x, 402x, 403x, 404x, or 405x CIHI ICD-10 codes: I10, I11, I12, I13, or I15
Asthma	One hospital admission with an asthma diagnosis or two OHIP claims with asthma diagnosis within two years. CIHI ICD-9: 493 CIHI ICD-10: J45, J46 OHIP diagnosis code: 493
Stroke	At least 2 OHIP claims within 1 year bearing any one of OHIP diagnostic codes (436, 432, 435) -or- if patient has any 1 CIHI-DAD ICD-10 code (I61 or I63 or I64 or H34.1 or G45 [ICD-9: 431, 433, 36231, 435]) within 1 year
Sleep Apnea	Any one OHIP billing code (J696, J896, J890, J690, J898, J899, J990, J897, J697, J889, J689) or any one CIHI-DAD ICD-10 code (G4730, G4730 [ICD-9: 32720, 78051, 78053, 78057) within 5 year lookback window

Appendix D: Ophthalmic Surgical History Variables

Variable	OHIP Definition	OHIP Code
Secondary Glaucoma Surgical Interventions		
Sclera	Sclerotomy, posterior	E127
	Removal of scleral implant	E161
	Scleral resection or buckling procedure (primary or secondary)	E152
Vitreous	Vitrectomy by infusion suction cutter technique	E148
	Vitrectomy by infusion suction cutter technique with transscleral retinal suturing	E938
	Preretinal membrane peeling or segmentation to include posterior vitrectomy and coagulation	E142
	Vitreous exchange	E936
Retina	Retinal and choroid re-attachment. Initial procedure	E151
	Retinal reattachment. Second time	E153
Conjunctival Disrupting		
Sclera	Sclerotomy, posterior	E127
	Removal of scleral implant	E161
	Scleral resection or buckling procedure (primary or secondary)	E152
Vitreous	Vitrectomy by infusion suction cutter technique	E148
	Vitrectomy by infusion suction cutter technique with transscleral retinal suturing	E938
	Preretinal membrane peeling or segmentation to include posterior vitrectomy and coagulation	E142
	Vitreous exchange	E936
Retina	Retinal and choroid re-attachment. Initial procedure	E151
	Retinal reattachment. Second time	E153
Conjunctiva	Excision of conjunctival lesion	E210
	Excision of conjunctival lesion w/mucous membrane graft	E948
	Excision of conjunctival lesion w/autogenous conjunctival transplant	E937
Ciliary	Ciliary body re-attachment	E135
Conjunctival Sparing		
Lens	Cataract extraction	E140
	Dislocated lens extraction or repositioning	E141
	Excision of secondary membrane with corneal section following cataract extraction	E143
	Fixation of intra-ocular lens and/or capsular tension device by suturing	E138

	Removal of intraocular lens	E144
	Repositioning dislocated intra-ocular lens	E145
	Insertion of secondary intra-ocular lens	E146
	Capsulotomy	E139
Cornea	Corneal Transplant, Penetrating	E121
	Corneal transplant, lamellar	E122
Iris	Laser iridotomy	E131
	Iridectomy	E130
Laser Angle Surgery	Laser Angle Surgery	E134
Photocoagulation	Photocoagulation, retina	E154
Retina	Cryopexy	E155
	Intravitreal injection for other than macular degeneration	E149

Appendix E: Glaucoma Eye Drops

Drug Class	Generic Name	Trade Name	Strength	DIN
Beta-blocker	LEVOBUNOLOL HCL	APO-LEVOBUNOLOL	0.5%	02241574
	LEVOBUNOLOL HCL	SANDOZ-LEVOBUNOLOL	0.5%	02241716
	LEVOBUNOLOL HCL	APO-LEVOBUNOLOL	0.25%	02241575
	LEVOBUNOLOL HCL	SANDOZ-LEVOBUNOLOL	0.25%	02241715
	TIMOLOL MALEATE	RATIO-TIMOLOL	0.5%	02240249
	BRIMONIDINE TARTRATE & TIMOLOL MALEATE	COMBIGAN	0.2/0.5%	02248347
	TIMOLOL MALEATE	TIMOLOL MALEATE-EX	0.25%	02242275
	TIMOLOL MALEATE	TIMOLOL MALEATE-EX	0.5%	02242276
	BRIMONIDINE TARTRATE & TIMOLOL MALEATE	COMBIGAN	0.2/0.5%	09857298
	BRINZOLAMIDE & TIMOLOL MALEATE	AZARGA	1%/0.5%	02331624
	TIMOLOL MALEATE	APO-TIMOP GEL	0.5%	02290812
	TIMOLOL MALEATE	APO-TIMOP	0.25%	00812277
	TIMOLOL MALEATE	APO-TIMOP	0.5%	00812285
	TIMOLOL MALEATE	APO-TIMOP	0.25%	00898082
	TIMOLOL MALEATE	APO-TIMOP	0.5%	00898090
	TIMOLOL MALEATE	TIMOPTIC-XE	0.5%	02171889
	TIMOLOL MALEATE	APO-TIMOP GEL	0.25%	02315858
	TIMOLOL MALEATE	TIMOPTIC OCUDOSE	0.5%	66123988
	TIMOLOL MALEATE	APO-TIMOP	0.25%	66898090
	LEVOBUNOLOL HCL	BETAGAN	0.25%	00801291
	LEVOBUNOLOL HCL	BETAGAN	0.25%	00801305
	LEVOBUNOLOL HCL	BETAGAN	0.5%	00811734
	LEVOBUNOLOL HCL	BETAGAN	0.5%	00895733
	LEVOBUNOLOL HCL	BETAGAN	0.5%	00907596

LEVOBUNOLOL HCL	NOVO-LEVOBUNOLOL	0.25%	02197456
LEVOBUNOLOL HCL	NOVO-LEVOBUNOLOL	0.5%	02197464
LEVOBUNOLOL HCL	LEVOBUNOLOL HCL	0.25%	02236792
LEVOBUNOLOL HCL	LEVOBUNOLOL HCL	0.5%	02236793
TIMOLOL MALEATE	TIMOPTIC	0.5%	00889431
BETAXOLOL HCL	BETOPTIC	0.5%	00896373
TIMOLOL MALEATE	TIMOPTIC	0.25%	00896829
TIMOLOL MALEATE	TIMOPTIC	0.5%	00896837
TIMOLOL MALEATE	TIM-AK	0.5%	02010240
TIMOLOL MALEATE	TIM-AK	0.25%	02010259
TIMOLOL MALEATE	T-LO	0.25%	02202492
TIMOLOL MALEATE	T-LO	0.5%	02202506
TIMOLOL MALEATE	TIMOLOL	0.25%	02230028
TIMOLOL MALEATE	TIMOLOL	0.5%	02230029
BETAXOLOL HCL	SANDOZ-BETAXOLOL	0.5%	02235971
TIMOLOL MALEATE	DOM-TIMOLOL	0.25%	02238770
TIMOLOL MALEATE	DOM-TIMOLOL	0.5%	02238771
TIMOLOL MALEATE	SANDOZ-TIMOLOL	0.25%	02241731
TIMOLOL MALEATE	SANDOZ-TIMOLOL	0.5%	02241732
TIMOLOL MALEATE	TIMOPTIC	-	49451207
TIMOLOL MALEATE	TIMOPTIC	0.25%	66889423
LEVOBUNOLOL HCL	LEVOBUNOLOL HCL	5MG/ML	00637696
TIMOLOL MALEATE	MED-TIMOLOL	0.25%	02084317
TIMOLOL MALEATE	MED-TIMOLOL	0.5%	02084325
TIMOLOL MALEATE	TEVA-TIMOLOL	0.25%	02094916
TIMOLOL MALEATE	TEVA-TIMOLOL	0.5%	02094924
TIMOLOL MALEATE	NU-TIMOLOL	0.25%	02094932
TIMOLOL MALEATE	NU-TIMOLOL	0.5%	02094940

LEVOBUNOLOL	RATIO-LEVOBUNOLOL	0.5%	02131167
TIMOLOL MALEATE	TIMOLOL	0.25%	02147475
TIMOLOL MALEATE	TIMOLOL	0.5%	02147483
TIMOLOL MALEATE	STORZ TIMOL	0.25%	02176025
TIMOLOL MALEATE	STORZ TIMOL	0.5%	02176033
LEVOBUNOLOL HCL	GEN-BUNOLOL	0.25%	02200813
LEVOBUNOLOL HCL	GEN-BUNOLOL	0.5%	02200821
LEVOBUNOLOL HCL	OPHTHO-BUNOLOL	0.25%	02200848
LEVOBUNOLOL HCL	OPHTHO-BUNOLOL	0.5%	02200856
BETAXOLOL HCL	ALTI-BETAXOLOL	0.5%	02230620
LEVOBUNOLOL HCL	PMS-LEVOBUNOLOL	0.25%	02237990
TIMOLOL MALEATE	CROWN-TIM	0.25%	02239300
TIMOLOL MALEATE	CROWN-TIM	0.5%	02239301
BETAXOLOL HCL	NOVO-BETAXOLOL	0.5%	02240050
TIMOLOL MALEATE	RATIO-TIMOLOL	0.25%	02240248
LEVOBUNOLOL	RATIO-LEVOBUNOLOL	0.5%	20311670
TIMOLOL MALEATE	COMPOUND - TIMOLOL PRESERVATIVE FREE	-	22123295
DORZOLAMIDE & TIMOLOL	APO-DORZO/TIMOP	20MG/5MG/ML	02299615
DORZOLAMIDE & TIMOLOL	CO DORZOTIMOLOL	2/0.5%	02404389
DORZOLAMIDE & TIMOLOL	SANDOZ-DORZOL/TIMOL	1%	02344351
DORZOLAMIDE & TIMOLOL	DORZOLAMIDE-TIMOLOL	1%	02357801
BETAXOLOL HCL & PILOCARPINE HCL	BETOPTIC/PILO	0.25% & 1.75%	02238539
LATANOPROST & TIMOLOL	XALACOM	5MG/5MCG/ML	02246619
TIMOLOL MALEATE & TRAVOPROST	DUOTRAV PQ	0.004/0.5%	02278251
TIMOLOL MALEATE & TRAVOPROST	DUOTRAV PQ	0.004/0.5%	09857333
LATANOPROST & TIMOLOL	SDZ-LATANOP/TIMOLOL	5MG/5MCG/ML	02394685

	LATANOPROST & TIMOLOL	GD-LATANOPROST/TIMOLOL	5MG/5MCG/ML	02373068
	TIMOLOL MALEATE & TRAVOPROST	DUOTRAV PQ	0.004/0.5%	09857512
	TIMOLOL MALEATE & TRAVOPROST	DUOTRAV PQ	0.004/0.5%	09857513
	LATANOPROST & TIMOLOL	PMS-LATANOPROST-TIMOLOL	5MG/50MCG/ML	02404591
CAI	DORZOLAMIDE	SANDOZ-DORZOLAMIDE	2%	02316307
	BRINZOLAMIDE	SANDOZ BRINZOLAMIDE	20MG/ML	02365235
	ACETAZOLAMIDE SODIUM	DIAMOX	2%	00014672
	ACETAZOLAMIDE SODIUM	DIAMOX	20MG	02039249
	ACETAZOLAMIDE SODIUM	DIAMOX	2%/.5%	02238074
	DORZOLAMIDE	DORZOLAMIDE	2%	02364999
	ACETAZOLAMIDE	ACETAZOLAMIDE	2%	99101175
	DORZOLAMIDE & TIMOLOL	APO-DORZO/TIMOP	20MG/5MG/ML	02299615
	DORZOLAMIDE & TIMOLOL	CO DORZOTIMOLOL	2/0.5%	02404389
	DORZOLAMIDE & TIMOLOL	SANDOZ-DORZOL/TIMOL	1%	02344351
	DORZOLAMIDE & TIMOLOL	DORZOLAMIDE-TIMOLOL	1%	02357801
Miotic	PILOCARPINE HCL	MINIMS PILOCARPINE	4%	02148471
	DORZOLAMIDE HCL & TIMOLOL MALEATE	COSOPT	20MG/5MG/ML	02258692
	PILOCARPINE HCL	PILOSTAT	4%	00772364
	CARBACHOL	ISOPTO CARBACHOL	0.75%	00000647
	PILOCARPINE HBR	ADSORBO-CARPINE	2%	00278831
	PILOCARPINE HCL	ISOPTO CARPINE	3%	00000876
	CARBACHOL CHLORIDE	MIOSTAT	0.01%	00042544
	PILOCARPINE HCL	ISOPTO CARPINE	8%	00252530
	PILOCARPINE HCL	AKARPINE	1%	00627739
	PILOCARPINE HCL	AKARPINE	2%	00627747

PILOCARPINE HCL	AKARPINE	4%	00627755
PILOCARPINE HCL	ISOPTO CARPINE	1%	22123017
PILOCARPINE NITRATE	P.V. CARPINE	1%	00001171
PILOCARPINE NITRATE	P.V. CARPINE	2%	00001198
PILOCARPINE NITRATE	P.V. CARPINE	4%	00001228
PILOCARPINE HCL	MIOCARPINE	1%	00028967
PILOCARPINE HCL	MIOCARPINE	2%	00028975
PILOCARPINE HCL	MIOCARPINE	4%	00028991
PILOCARPINE HCL	MIOCARPINE	6%	00029009
PILOCARPINE HCL	MIOCARPINE	1%	00265098
PILOCARPINE HCL	MIOCARPINE	2%	00265101
PILOCARPINE HCL	MIOCARPINE	3%	00265128
PILOCARPINE NITRATE	PILOCARPINE NITRATE	1%	00269115
EPINEPHRINE BITARTRATE & PILOCARPINE HCL	E-PILO # 1	1%	00281670
EPINEPHRINE BITARTRATE & PILOCARPINE HCL	E-PILO # 2	2%	00281689
EPINEPHRINE BITARTRATE & PILOCARPINE HCL	E-PILO # 4	4%	00281700
PILOCARPINE HCL	MIOCARPINE	0.5%	00281751
PILOCARPINE HCL	MIOCARPINE	4%	00281778
PILOCARPINE HCL	MIOCARPINE	6%	00281786
ECOTHIOPATE IODIDE	PHOSPHOLINE IOD	3MG	00283304
CHYMOTRYPSIN	CATARASE	300U/VIAL	00526355
PILOCARPINE HCL	MIOCARPINE	6%	00526738
PILOCARPINE HCL	MIOCARPINE	0.5%	00527521
PILOCARPINE HCL	MIOCARPINE	1%	00527548
PILOCARPINE HCL	MIOCARPINE	2%	00527556

	PILOCARPINE HCL	MIOCARPINE	3%	00527564
	PILOCARPINE HCL	MIOCARPINE	4%	00527572
	PILOCARPINE HCL	SPERSACARPINE	3%	00725420
	PILOCARPINE HCL	PILOSTAT	2%	00772372
	PILOCARPINE HCL	PILOSTAT	1%	00772380
	ECOTHIOPATE IODIDE	PHOSPHOLINE IOD	3MG	00860859
	ACETYLCHOLINE HYDROXIDE CHLORIDE	MIOCHOL-E	10MG/ML	00889334
	PILOCARPINE HCL	PILOCARPINE	2%	01907638
	PILOCARPINE HCL	PILOCARPINE	1%	01907654
	PILOCARPINE HCL	PILOCARPINE	4%	01907662
	DAPIPRAZOLE HCL	REV-EYES	25MG	01970348
	ACETYLCHOLINE HYDROXIDE CHLORIDE	MIOCHOL-E	10MG/ML	02133326
	CARBACHOL	CARBASTAT	0.01%	02195585
	ECOTHIOPATE IODIDE	PHOSPHOLINE IOD	6.25MG	02238076
	DORZOLAMIDE & TIMOLOL	TEVA-DORZOTIMOL	20MG/5MG/ML	02320525
	PILOCARPINE HCL	COMPOUND - PILOCARPINE	0.25%	22123015
	PILOCARPINE HCL	COMPOUND - PILOCARPINE	4%	22123019
	PILOCARPINE HCL	COMPOUND - PILOCARPINE	-	22123294
	PILOCARPINE HCL & EPINEPHRINE BITARTRATE	E-PILO 2	2% & 1%	00028851
	BETAXOLOL HCL & PILOCARPINE HCL	BETOPTIC/PILO	0.25% & 1.75%	02238539
PGA	TRAVOPROST	TRAVATAN	0.004%	02244896
	BIMATOPROST	LUMIGAN	0.03%	02245860
	TRAVOPROST	TRAVATAN Z	0.004%	02318008
	TRAVOPROST	TRAVATAN Z	0.004%	09857332
	BIMATOPROST	LUMIGAN RC	0.01%	0232499

	BIMATOPROST	LUMIGAN RC	0.01%	7 0985736 8
	LATANOPROST	APO-LATANOPROST	50MCG/ML	0229652 7
	LATANOPROST	GD-LATANOPROST	50MCG	0237304 1
	BIMATOPROST	LUMIGAN RC	0.01%	0985739 8
	LATANOPROST	CO LATANOPROST	0.05MG/ML	0225478 6
	LATANOPROST	SANDOZ- LATANOPROST	50MCG/ML	0236733 5
	TRAVOPROST	TEVA-TRAVOPROST Z	0.004%	0241206 3
	TRAVOPROST	TEVA-TRAVOPROST Z	0.004%	0985750 4
	TRAVOPROST	SANDOZ TRAVOPROST	0.004%	0241316 7
	TRAVOPROST	APO-TRAVOPROST Z	0.004%	0241573 9
	LATANOPROST	PMS-LATANOPROST	50MCG/ML	0231712 5
	BIMATOPROST	LUMIGAN	0.03%	0099014 6
	BIMATOPROST	LUMIGAN	0.03%	9224586 0
	LATANOPROST	LATANOPROST	50MCG/ML	0237550 8
	LATANOPROST & TIMOLOL	XALACOM	5MG/5MCG/ML	0224661 9
	TIMOLOL MALEATE & TRAVOPROST	DUOTRAV PQ	0.004/0.5%	0227825 1
	TIMOLOL MALEATE & TRAVOPROST	DUOTRAV PQ	0.004/0.5%	0985733 3
	LATANOPROST & TIMOLOL	SDZ- LATANOP/TIMOLOL	5MG/5MCG/ML	0239468 5
	LATANOPROST & TIMOLOL	GD- LATANOPROST/TIMOL OL	5MG/5MCG/ML	0237306 8
	TIMOLOL MALEATE & TRAVOPROST	DUOTRAV PQ	0.004/0.5%	0985751 2
	TIMOLOL MALEATE & TRAVOPROST	DUOTRAV PQ	0.004/0.5%	0985751 3
	LATANOPROST & TIMOLOL	PMS-LATANOPROST- TIMOLOL	5MG/50MCG/M L	0240459 1
Sympathomimeti cs	BRIMONIDINE TARTRATE	RATIO-BRIMONIDINE	0.2%	0224302 6
	BRIMONIDINE TARTRATE	ALPHAGAN P	0.15%	0224815 1
	BRIMONIDINE	PMS-BRIMONIDINE	0.2%	0224628

TARTRATE			4
BRIMONIDINE TARTRATE	APO-BRIMONIDINE	0.2%	0226007 7
BRIMONIDINE	SANDOZ-BRIMONIDINE	0.2%	0230542 9
BRIMONIDINE TARTRATE	APO-BRIMONIDINE P	0.15%	0230133 4
BRIMONIDINE TARTRATE	ALPHAGAN	0.5%	0223687 7
BRIMONIDINE TARTRATE	DOM-BRIMONIDINE	0.2%	0224628 5
BRIMONIDINE TARTRATE	BRIMONIDINE	0.2%	0224945 6
BRIMONIDINE TARTRATE	BRIMONIDINE TARTRATE	0.2%	0224594 2
KETOTIFEN FUMARATE	KETOTIFEN	0.25MG/ML	0240087 1
EPINEPHRYL BORATE	EPINAL	1%	0000073 6
EPINEPHRINE BITARTRATE & PILOCARPINE HCL	E-PILO # 2	2%	0003592 0
EPINEPHRINE BITARTRATE & PILOCARPINE HCL	E-PILO # 4	4%	0003594 7
EPINEPHRINE BITARTRATE & PILOCARPINE HCL	E-PILO # 3	3%	0028169 7
EPINEPHRINE BITARTRATE & PILOCARPINE HCL	E-PILO # 6	6%	0028185 9
EPINEPHRYL BORATE	EPPY	0.5%	0032364 0
EPINEPHRYL BORATE	EPPY	1%	0032365 9
EPINEPHRINE BITARTRATE & PILOCARPINE HCL	E-PILO # 1	1%	0052636 3
EPINEPHRINE BITARTRATE & PILOCARPINE HCL	E-PILO # 2	2%	0052637 1
EPINEPHRINE BITARTRATE & PILOCARPINE HCL	E-PILO # 3	3%	0052639 8
EPINEPHRINE BITARTRATE & PILOCARPINE HCL	E-PILO # 4	4%	0052640 1
EPINEPHRINE	E-PILO # 6	6%	0052642

	BITARTRATE & PILOCARPINE HCL			8
	BRIMONIDINE TARTRATE	BRIMONIDINE OPHTHALMIC	0.2%	0224628 3
	BRIMONIDINE TARTRATE	APX-BRIMONIDINE P	0.15%	0235073 4
	PILOCARPINE HCL & EPINEPHRINE BITARTRATE	E-PILO 2	2% & 1%	0002885 1

Appendix F: Other Eye Drop Medications

Drug Class	Generic Name	Trade Name	Strength	DIN
Antibiotic	FUSIDIC ACID	FUCITHALMIC W/O PRES	1%	02243861
	GRAMICIDIN & POLYMYXIN B SULFATE	POLYSPORIN	10MU	02239156
	BACITRACIN ZINC & POLYMYXIN B SULFATE	POLYSPORIN	10MU	02239157
	FUSIDIC ACID	FUCITHALMIC W/ PRES	1%	02243862
	CHLORAMPHENICOL	CHLOROPTIC	1%	00001341
	BACITRACIN ZINC & POLYMYXIN B SULFATE	POLYSPORIN	10MU	00004847
	CHLORTETRACYCLINE HCL	AUREOMYCIN	1%	00015075
	TETRACYCLINE HCL	ACHROMYCIN	1%	00015083
	ERYTHROMYCIN	ILOTYCIN		00015997
	CHLORAMPHENICOL	PENTAMYCETIN	10MG	00024317
	OXYTETRACYCLINE HCL & POLYMYXIN B SULFATE	TERRAMYCIN		00024791
	BORIC ACID	BORIC ACID	NOT AVLE	00050504
	SULFISOXAZOLE DIOLAMINE	GANTRISIN	4%	00115460
	CHLORAMPHENICOL & HYDROCORTISONE ACETATE	CHLOROMYCETIN W/HC		00155977
	CHLORAMPHENICOL	PENTAMYCETIN	2.5MG	00163503
	ERYTHROMYCIN	PDP-ERYTHROMYCIN	5MG	00191275
	BACITRACIN & POLYMYXIN B SULFATE	NEO BACE		00221392
	CHLORAMPHENICOL	ISOPTO FENICOL	0.5%	00239879
	GRAMICIDIN & NEOMYCIN SULFATE & POLYMYXIN B SULFATE	NEOSPORIN	10MU	00243183
	SULFACETAMIDE SODIUM	SULFACETAMIDE	10%	00269069
	SULFACETAMIDE SODIUM	SULF-10	10%	00281867
	SULFACETAMIDE SODIUM	OPTOSULFEX	10%	00343986
	CHLORAMPHENICOL	MINIMS CHLORAMPHENICOL	0.5%	00387525
	FLUOROMETHOLONE & NEOMYCIN SULFATE	FML-NEO LIQUIFILM	0.1%	00395153
	CHLORAMPHENICOL	SOPAMYCETIN		00402974
	BORIC ACID & SODIUM BORATE	COLLYRE HYGIENIQUE SOKER	11.1MG & 1.89MG	00425745
	CHLORAMPHENICOL	SOPAMYCETIN	2MG	00438650
	CHLORAMPHENICOL	SOPAMYCETIN	50MG	00438677
	CHLORAMPHENICOL	PENTAMYCETIN	2.5MG	00446521
	CHLORAMPHENICOL	PENTAMYCETIN	45MG	00504785
	ALLANTOIN & BORIC	OPTREX		00520101

ACID & HAMAMELIS & SALICYLIC ACID & SODIUM BORATE & ZINC SULFATE			
ALLANTOIN & BORIC ACID & GLYCEROL & HAMAMELIS & SODIUM BORATE	OPTREX		00520128
BORIC ACID	EYE WASH	2.5%	00524468
SULFACETAMIDE SODIUM	SULF-10	10%	00527963
BORIC ACID	BORIC ACID	2.5%	00540994
SULFACETAMIDE SODIUM	SULFEX	10%	00554022
BORIC ACID & SODIUM BORATE	EYE EZE	1.11%	00581836
GRAMICIDIN & NEOMYCIN SULFATE & POLYMYXIN B SULFATE	NEOSPORIN	10MU	00601659
CEFAZOLIN SODIUM	CEFAZOLIN	NOT AVLE	00622360
CHLORAMPHENICOL	AK-CHLOR	0.5%	00622958
SULFACETAMIDE SODIUM	AK-SULF	10%	00622966
CHLORAMPHENICOL	AK-CHLOR	1%	00627720
BORIC ACID & BUTACAINE SULFATE	EYE LOTION	2.3% & 0.14%	00629014
GRAMICIDIN & NEOMYCIN SULFATE & POLYMYXIN B SULFATE	AK-SPOR	1.75MG	00635065
ERYTHROMYCIN	AK-MYCIN	5MG	00641324
GRAMICIDIN & POLYMYXIN B SULFATE	OPTIMYXIN	10MU	00701785
CHLORAMPHENICOL	PENTAMYCETIN	10MG	00704571
CHLORAMPHENICOL	SPERSANICOL	0.5%	00725528
CHLORAMPHENICOL	SPERSANICOL	1%	00725536
SULFACETAMIDE SODIUM	SPERSACET	10%	00729299
SULFACETAMIDE SODIUM	PMS-SULFACETAMIDE	30%	00762059
CHLORAMPHENICOL	CEBENICOL	0.4%	00763454
CHLORAMPHENICOL	SOPAMYCETIN	2MG	00763535
ERYTHROMYCIN	ERYTHROTOPIC	5MG	00772305
SULFACETAMIDE SODIUM	BALSULPH	10%	00772313
SULFACETAMIDE SODIUM	SULAMYD	10%	00778053
SULFACETAMIDE SODIUM	SULAMYD	30%	00778061
SULFACETAMIDE SODIUM	SULAMYD	10%	00778347
ERYTHROMYCIN	ERYTHROMYCIN	5MG	00785725
SULFACETAMIDE	BALSULPH	10%	00790842
TETRACYCLINE HCL	TETRACYCLINE	1%	00792594
GRAMICIDIN & NEOMYCIN SULFATE & POLYMYXIN B SULFATE	OPTIMYXIN PLUS	10MU	00807435

BACITRACIN ZINC & NEOMYCIN SULFATE & POLYMYXIN B SULFATE	BACITRACIN-NEOMYCIN-POLY	10MU	00811971
SULFACETAMIDE SODIUM	SULFACETAMIDE SODIUM	10%	00811998
CHLORAMPHENICOL	SOPAMYCETIN	2MG	00837369
SULFACETAMIDE SODIUM	PMS-SULFACETAMIDE	10%	00838934
CHLORAMPHENICOL	PMS-CHLORAMPHENICOL	0.5%	00861383
OXYMETAZOLINE HCL	VISINE L.R.		00892025
GRAMICIDIN & POLYMYXIN B SULFATE	POLYSPORIN	10MU	00898805
POLYMYXIN B SULFATE	BIODERM	10000U/500G	00899380
AMIKACIN SULFATE	AMIKACIN EYE DROPS	NOT AVLE	00903067
CEFAZOLIN SODIUM	CEFAZOLIN	NOT AVLE	00903081
CYCLOSPORINE	CYCLOSPORIN	NOT AVLE	00903083
VANCOMYCIN HCL	VANCOMYCIN	NOT AVLE	00903088
CLARITHROMYCIN	CLARITHROMYCIN	NOT AVLE	00903092
CYCLOSPORINE	RESTASIS	0.05%	00903127
ERYTHROMYCIN	ILOTYCIN		00904082
ERYTHROMYCIN	ERYTHROMYCIN	5MG/G	00906840
ERYTHROMYCIN	PDP-ERYTHROMYCIN	5MG	00912755
OXYMETAZOLINE HCL	VISINE L.R.		01942484
BORIC ACID	EYE WASH	1.2%	01943464
BACITRACIN ZINC & NEOMYCIN SULFATE & POLYMYXIN B SULFATE	DIOSPORIN		02023792
SULFACETAMIDE SODIUM	DIOSULF	10%	02023830
CHLORAMPHENICOL	DIOCHLORAM	0.5%	02023857
PHENIRAMINE MALEATE & PHENYLEPHRINE HCL & POLYVINYL ALCOHOL	DIOROUGE	0.5%	02026511
ERYTHROMYCIN	DIOMYCIN	5MG/GM	02141574
CHLORAMPHENICOL	MINIMS CHLORAMPHENICOL	0.5%	02148374
BACITRACIN ZINC & POLYMYXIN B SULFATE	OPTIMYXIN	10MU	02160889
CHLORAMPHENICOL	PENTAMYCETIN	0.5%	02164051
ALLANTOIN & BORIC ACID & GLYCEROL & HAMAMELIS & SODIUM BORATE	OPTREX		02185482
ALLANTOIN & BORIC ACID & HAMAMELIS & SALICYLIC ACID & SODIUM BORATE & ZINC SULFATE	OPTREX		02186853
ERYTHROMYCIN	ERYTHROMYCIN	0.5%	02212935
BORIC ACID	EYE WASH	1.2%	02212994
GRAMICIDIN & POLYMYXIN B SULFATE	POLYCIDIN	10MU	02229903
BACITRACIN ZINC & POLYMYXIN B SULFATE	POLYCIDIN	10MU	02230193

ERYTHROMYCIN	ERYTHROMYCIN	5MG	02237041
POLYMYXIN B SULFATE & TRIMETHOPRIM SULFATE	SANDOZ-POLYTRIMETHOPRIM	1%	02239234
POLYMYXIN B SULFATE & TRIMETHOPRIM SULFATE	PMS-POLYTRIMETHOPRIM	1%	02240363
AZITHROMYCIN	AZASITE	1%	02321661
ERYTHROMYCIN	OPHTH ERYTHROMYCIN	5MG	02326663
BESIFLOXACIN HCL	BESIVANCE	0.6%	02336847
AZITHROMYCIN	AZASITE	1%	02345498
CYCLOSPORINE	RESTASIS	0.05%	02355655
VANCOMYCIN HCL	COMPOUND - VANCOMYCIN	NOT AVLE	22123297
CYCLOSPORINE	CYCLOSPORIN	4%	66123446
ERYTHROMYCIN STEARATE	ERYTHROMYCINE	0.5%	99100766
OFLOXACIN	APO-OFLOXACIN	0.3%	02248398
OFLOXACIN	PMS-OFLOXACIN	0.3%	02252570
NORFLOXACIN	NOROXIN	0.3%	00908294
NORFLOXACIN	NOROXIN	0.3%	01908294
NORFLOXACIN	NOROXIN	0.3%	01918294
OFLOXACIN	OPHTHO-FLOX	.30%	02243025
OFLOXACIN	SANDOZ-OFLOXACIN	0.3%	02247189
MOXIFLOXACIN HCL	VIGAMOX	0.5%	02252260
CIPROFLOXACIN HCL	PMS-CIPROFLOXACIN	0.3%	02253933
GATIFLOXACIN	ZYMAR	0.3%	02257270
CIPROFLOXACIN HCL	APO-CIPROFLOX	0.3%	02263130
CIPROFLOXACIN HCL	NU-CIPROFLOX	.3%	02333228
CIPROFLOXACIN HCL	SANDOZ-CIPROFLOXACIN	0.3%	02387131
MOXIFLOXACIN HCL	SANDOZ MOXIFLOXACIN	0.5%	02411520
PHENYLEPHRINE HCL & SODIUM BORATE & BORIC ACID	OCCU-CAL	0.2MG/ML & 1.9MG/ML & 11.16MG/ML	00540935
BETAMETHASONE SODIUM PHOSPHATE & GENTAMICIN SULFATE	SANDOZ-PENTASONE	3MG/ML	02244999
DEXAMETHASONE & FRAMYCETIN SULFATE & GRAMICIDIN	SANDOZ-OPTICORT	5MG	02247920
PREDNISOLONE ACETATE & SULFACETAMIDE SODIUM	BLEPHAMIDE LIQUIFILM	10%	00807788
PREDNISOLONE ACETATE & SULFACETAMIDE SODIUM	CETAPRED	10%	00042617
PREDNISOLONE ACETATE & SULFACETAMIDE SODIUM	CETAPRED	10%	00042625

PREDNISOLONE ACETATE & SULFACETAMIDE SODIUM	ISOPTO CETAPRED	10%	00042633
DEXAMETHASONE & NEOMYCIN SULFATE & POLYMYXIN B SULFATE	MAXITROL	0.1%	00042668
DEXAMETHASONE & NEOMYCIN SULFATE & POLYMYXIN B SULFATE	MAXITROL	0.1%	00042676
PREDNISOLONE ACETATE & SULFACETAMIDE SODIUM	BLEPHAMIDE LIQUIFILM	10%	00045632
BACITRACIN ZINC & HYDROCORTISONE & NEOMYCIN SULFATE & POLYMYXIN B SULFATE	CORTISPORIN	1%	00068772
DEXAMETHASONE & NEOMYCIN SULFATE	NEO-DECADRON	0.1%	00140732
CHLORAMPHENICOL & HYDROCORTISONE ACETATE	OPHTHOCORT	10MG	00156175
HYDROCORTISONE & OXYTETRACYCLINE HCL	TERRA-CORTRIL		00158178
CHLORAMPHENICOL & HYDROCORTISONE ACETATE	PENTAMYCETIN HC	10MG	00163511
CHLORAMPHENICOL & HYDROCORTISONE ACETATE	PENTAMYCETIN HC	10MG	00163538
DEXAMETHASONE & FRAMYCETIN SULFATE & GRAMICIDIN	SOFRACORT	5MG	00173592
DEXAMETHASONE & FRAMYCETIN SULFATE & GRAMICIDIN	SOFRACORT	5MG	00173606
PREDNISOLONE ACETATE & SULFACETAMIDE SODIUM	METIMYD	0.5%	00177024
HYDROCORTISONE ACETATE & NEOMYCIN SULFATE	NEO-CORTEF	0.5%	00194883
HYDROCORTISONE ACETATE & NEOMYCIN SULFATE	NEO-CORTEF	1.5%	00194921
HYDROCORTISONE ACETATE & NEOMYCIN SULFATE	NEO-CORTEF	1.5%	00194948
PREDNISOLONE DISODIUM PHOSPHATE & SULFACETAMIDE SODIUM	VASOCIDIN	0.25%	00218812
HYDROCORTISONE & NEOMYCIN SULFATE &	CORTISPORIN		00243140

POLYMYXIN B SULFATE			
BETAMETHASONE & SULFACETAMIDE SODIUM	CELESTONE-S	3MG	00271012
PREDNISOLONE DISODIUM PHOSPHATE & SULFACETAMIDE SODIUM	VASOCIDIN	0.25%	00281824
PREDNISOLONE ACETATE & SULFACETAMIDE SODIUM	BLEPHAMIDE S.O.P.	10%	00307246
DEXAMETHASONE & NEOMYCIN SULFATE & POLYMYXIN B SULFATE	MAXITROL	0.1%	00358177
PREDNISOLONE ACETATE & SULFACETAMIDE SODIUM	BLEPHAMIDE LIQUIFILM	10%	00395145
CHLORAMPHENICOL & HYDROCORTISONE ACETATE	SOPAMYCETIN HC	2MG	00438669
HYDROCORTISONE & OXYTETRACYCLINE HCL	TERRA-CORTRIL		00443298
BACITRACIN ZINC & HYDROCORTISONE & NEOMYCIN SULFATE & POLYMYXIN B SULFATE	CORTISPORIN	1%	00520322
PREDNISOLONE DISODIUM PHOSPHATE & SULFACETAMIDE SODIUM	VASOCIDIN	0.25%	00527998
BETAMETHASONE & SULFACETAMIDE SODIUM	CELESTONE-S	3MG	00575194
BETAMETHASONE SODIUM PHOSPHATE & GENTAMICIN SULFATE	GARASONE	0.3%	00586692
BETAMETHASONE SODIUM PHOSPHATE & GENTAMICIN SULFATE	GARASONE	0.3%	00586706
DEXAMETHASONE & NEOMYCIN & POLYMYXIN B SULFATE	AK-TROL	10MU	00626597
PREDNISOLONE ACETATE & SULFACETAMIDE SODIUM	AK-CIDE	10/0.5%	00630640
HYDROCORTISONE & NEOMYCIN SULFATE & POLYMYXIN B SULFATE	CORTISPORIN		00666211
BACITRACIN ZINC & HYDROCORTISONE & NEOMYCIN SULFATE & POLYMYXIN B SULFATE	CORTISPORIN	1%	00701904
CHLORAMPHENICOL &	PENTAMYCETIN HC	10MG	00704563

HYDROCORTISONE ACETATE			
CHLORAMPHENICOL & HYDROCORTISONE ACETATE	CORTIPHENAL H	1% & 1%	00750670
PREDNISOLONE DISODIUM PHOSPHATE & SULFACETAMIDE SODIUM	VASOCIDIN	0.25%	00760056
CHLORAMPHENICOL & HYDROCORTISONE ACETATE	SOPAMYCETIN HC	2MG	00763543
PREDNISOLONE ACETATE & SULFACETAMIDE SODIUM	BLEPHAMIDE S.O.P.	10%	00806617
PREDNISOLONE ACETATE & SULFACETAMIDE SODIUM	BLEPHAMIDE LIQUIFILM	10%	00807982
PREDNISOLONE DISODIUM PHOSPHATE & SULFACETAMIDE SODIUM	VASOCIDIN	0.25%	00808199
PREDNISOLONE ACETATE & SULFACETAMIDE SODIUM	ISOPTO CETAPRED	10%	00810894
CHLORAMPHENICOL & HYDROCORTISONE ACETATE	SOPAMYCETIN HC	10MG	00837350
PREDNISOLONE DISODIUM PHOSPHATE & SULFACETAMIDE SODIUM	VASOCIDIN	0.25%	00843741
PREDNISOLONE ACETATE & SULFACETAMIDE SODIUM	BLEPHAMIDE LIQUIFILM	10%	00903124
PREDNISOLONE DISODIUM PHOSPHATE & SULFACETAMIDE SODIUM	VASOCIDIN	0.25%	00903477
CHLORAMPHENICOL & HYDROCORTISONE ACETATE	PENTAMYCETIN HC	10MG	01980572
CHLORAMPHENICOL & HYDROCORTISONE ACETATE	PENTAMYCETIN HC	10MG	01980580
DEXAMETHASONE & FRAMYCETIN SULFATE & GRAMICIDIN	SOFRACORT	5MG	01987720
DEXAMETHASONE & NEOMYCIN SULFATE & POLYMYXIN B SULFATE	DIOPTROL	3.5MG	02023806
PREDNISOLONE	DIOPTIMYD	5MG	02023814

	ACETATE & SULFACETAMIDE SODIUM			
	DEXAMETHASONE & NEOMYCIN SULFATE & POLYMYXIN B SULFATE	DIOPTROL	3.5MG	02023849
	PREDNISOLONE DISODIUM PHOSPHATE & SULFACETAMIDE SODIUM	VASOCIDIN	0.25%	02133342
	DEXAMETHASONE & FRAMYCETIN SULFATE & GRAMICIDIN	SOFRACORT	5MG	02224631
	GENTAMICIN SULFATE & BETAMETHASONE SODIUM PHOSPHATE	DOM-GENTAMICIN-BETAMETHASONE	0.3% & 0.1%	02238818
	GENTAMICIN SULFATE & BETAMETHASONE SODIUM PHOSPHATE	PMS-GENTAMICIN-BETAMETHASONE	0.3% & 0.1%	02238819
	BACITRACIN & HYDROCORTISONE & NEOMYCIN SULFATE & POLYMYXIN B SULFATE	SANDOZ-CORTIMYXIN	10MU	02242485
	GENTAMICIN SULFATE & BETAMETHASONE SODIUM PHOSPHATE	BETAMYCIN	3MG/ML & 1MG/ML	02247446
	SULFACETAMIDE SODIUM & PHENYLEPHRINE HCL	VASOSULF	15% & .125%	00191965
	PHENYLEPHRINE HCL & SULFACETAMIDE SODIUM	VASOSULF	15%	00265152
	PHENYLEPHRINE HCL & SULFACETAMIDE SODIUM	VASOSULF	15%	00760048
Aminoglycoside	TOBRAMYCIN SULFATE	SANDOZ-TOBRAMYCIN	0.3%	02241755
	TOBRAMYCIN	APO-TOBRAMYCIN	0.3%	02245698
	FRAMYCETIN SULFATE	SOFRAMYCIN	5MG	02224887
	FRAMYCETIN SULFATE	SOFRAMYCIN	5MG	02224895
	GENTAMICIN SULFATE	GARAMYCIN	0.3%	00028126
	GENTAMICIN SULFATE	GARAMYCIN	0.3%	00512912
	GENTAMICIN SULFATE	GENTAMYTREX	5MG/ML	00717940
	GENTAMICIN SULFATE	GENTAMYTREX	5MG/GM	00717959
	GENTAMICIN	GENTROSULF	3%	00772429
	GENTAMICIN SULFATE	PMS-GENTAMICIN	0.5%	00777781
	GENTAMICIN SULFATE	OPHTAGRAM	0.3%	00789100
	GENTAMICIN	GENTROSULF	3%	00790753
	GENTAMICIN SULFATE	OPHTAGRAM	0.3%	00794317
	GENTAMICIN	GENTACIDIN	0.3%	00810282
	GENTAMICIN SULFATE	GENTAK	0.3%	00832162
	GENTAMICIN	RATIO-GARATEC	3MG	00880191
	TOBRAMYCIN	TOBRAMYCIN	-	00903080
	GENTAMICIN SULFATE	GENTAMICIN FORTIFIED	-	00903082

	NETILMICIN SULFATE	NATACYN	5%	00910911
	GENTAMICIN	CIDOMYCIN	0.3%	01932330
	GENTAMICIN	CIDOMYCIN	0.3%	01932349
	GENTAMICIN SULFATE	GENTAMICIN	0.3%	01933299
	GENTAMICIN	OCUGRAM	3MG	01987461
	GENTAMICIN	OCUGRAM	3MG	01987488
	FRAMYCETIN SULFATE	SOFRAMYCIN	5MG	01987666
	GENTAMICIN SULFATE	GENTAK	0.3%	01989073
	GENTAMICIN SULFATE	MINIMS GENTAMICIN SULF	0.3%	02009900
	GENTAMICIN SULFATE	GENTAMICIN SULFATE	0.3%	02014548
	GENTAMICIN SULFATE	DIOGENT	0.3%	02023776
	GENTAMICIN SULFATE	GENTAMICIN SULFATE	0.3%	02024500
	GENTAMICIN	GENTACIDIN	0.3%	02133245
	GENTAMICIN SULFATE	MINIMS GENTAMICIN SULF	0.3%	02148404
	GENTAMICIN SULFATE	GENTAMICIN SULFATE	0.3%	02229440
	GENTAMICIN SULFATE	GENTAMICIN SULFATE	0.3%	02230888
	GENTAMICIN SULFATE	PMS-GENTAMICIN	0.3%	02237689
	TOBRAMYCIN	CROWN AK-TOBRA	0.3%	02238710
	TOBRAMYCIN	TOBREXAN	0.3%	02261243
	TOBRAMYCIN SULFATE	COMPOUND - TOBRAMYCIN	-	22123296
	TOBRAMYCIN	TOBRAMYCIN	300MG	99100845
Antifungal	VORICONAZOLE	VORICONAZOLE	1%	09854663
	VORICONAZOLE	VORICONAZOLE	-	00903740
Antiviral	INTERFERON	INTERFERON	1MU/ML	09852751
	INTERFERON	INTERFERON	1MU/ML	00903448
	IDOXURIDINE	STOXIL	1MG/ML	00027014
	TRIFLURIDINE	VIROPTIC	1%	00589055
	TRIFLURIDINE	APO-TRIFLURIDINE	1%	02248119
	TRIFLURIDINE	SANDOZ-TRIFLURIDINE	1%	02248529
Mydriatic	ATROPINE SULFATE	ISOPTO ATROPINE	1%	00000639
	ATROPINE SULFATE	SMP ATROPINE	1%	00028800
	ATROPINE SULFATE	MINIMS ATROPINE	1%	00269255
	ATROPINE SULFATE	SMP ATROPINE	1%	00281603
	ATROPINE SULFATE	SMP ATROPINE	0.5%	00281611
	ATROPINE SULFATE	SMP ATROPINE	2%	00281638
	ATROPINE SULFATE	SMP ATROPINE	1%	00344842
	ATROPINE PLUS	ISOPTO MYDRAPRED	1%	00358215
	ATROPINE SULFATE	SMP ATROPINE	0.5%	00527939
	ATROPINE SULFATE	SMP ATROPINE	1%	00527947
	ATROPINE SULFATE	SMP ATROPINE	2%	00527955
	ATROPINE SULFATE	ATROPINE AK	1%	00622907
	ATROPINE SULFATE	ATROPINE SULFATE	1%	00811963
	ATROPINE SULFATE	ATROPINE	1%	01901311
	ATROPINE SULFATE	ATROPINE	1%	01948601
	ATROPINE SULFATE	ATROPINE	1%	02023695

ATROPINE SULFATE	MINIMS ATROPINE	1%	02148358
ATROPINE SULFATE	ATROPINE	1%	02212951
ATROPINE SULFATE & HYDROCORTISONE	HC-ATROPINE	1%	00062251
ATROPINE SULFATE & HYDROCORTISONE	HC-ATROPINE	1%	00062278
SCOPOLAMINE	ISOPTO HYOSCINE	0.25%	00000957
TROPICAMIDE	MYDRIACYL	0.5%	00000981
HOMATROPINE HBR	HOMATROPINE HBR	5%	00028916
PHENYLEPHRINE HCL	NEO-SYNEPHRINE	0.125%	00033502
PHENYLEPHRINE HCL	NEO-SYNEPHRINE	10%	00033529
CYCLOPENTOLATE HCL	CYCLOGYL	1%	00252506
CYCLOPENTOLATE HCL	CYCLOGYL	0.5%	00252549
PHENYLEPHRINE HCL	PHENYLEPHRINE HCL	10%	00269123
HOMATROPINE HBR	MINIMS HOMATROPINE	2%	00269158
CYCLOPENTOLATE HCL	CYCLOPENTOLATE	0.05%	00269204
PHENYLEPHRINE HCL	PHENYLEPHRINE	10%	00281808
PHENYLEPHRINE HCL	PREFRIN LIQUIFILM	0.12%	00385161
PHENYLEPHRINE HCL & PYRILAMINE MALEATE	PREFRIN-A LIQUIFLM	0.1%	00400408
PHENYLEPHRINE HCL	NEO-SYNEPHRINE	0.125%	00482684
CYCLOPENTOLATE HCL	CYC	1%	00506230
CYCLOPENTOLATE HCL	MYDPLEGIC	1%	00527580
HYPROMELLOSE	MURO TEARS		00544787
TROPICAMIDE	TROPICAMIDE	1%	00544825
PHENYLEPHRINE HCL	NEO-SYNEPHRINE	0.125%	00561886
PHENYLEPHRINE HCL	NEO-SYNEPHRINE	10%	00561894
TROPICAMIDE	TROPICACYL	1%	00622885
PHENYLEPHRINE HCL	AK-DILATE	2.5%	00622915
CYCLOPENTOLATE HCL	AK-PENTOLATE	1%	00626627
CYCLOPENTOLATE HCL	AK-PENTOLATE	0.5%	00626635
TROPICAMIDE	TROPICACYL	0.5%	00627704
PHENYLEPHRINE HCL & TROPICAMIDE	PHENYLTROPE	5%	00629693
CYCLOPENTOLATE HCL	SPERSAPENTOL	1%	00751758
TROPICAMIDE	TROPICAMIDE	1%	00751774
PHENYLEPHRINE HCL	SPERSAPHRINE	2.5%	00751820
TROPICAMIDE	PMS-TROPICAMIDE	1%	00872946
CYCLOPENTOLATE HCL	PMS-CYCLOPENTOLATE HCL	1%	00878189
TROPICAMIDE	TROPICAMIDE	1%	00896446
CYCLOPENTOLATE HCL	DIOPENTOLATE	1%	02023644
TROPICAMIDE	DIOTROPE	0.5%	02023660
TROPICAMIDE	DIOTROPE	1%	02023679
CYCLOPENTOLATE HCL	DIOPENTOLATE	0.5%	02023687
PHENYLEPHRINE HCL & TROPICAMIDE	DIOPHENYL-T	0.8%	02023717
PHENYLEPHRINE HCL	DIONEPHRINE	0.12%	02026473
PHENYLEPHRINE HCL	DIONEPHRINE	2.5%	02027100
CYCLOPENTOLATE HCL	MINIMS CYCLOPENTOLATE	0.5%	02148331
CYCLOPENTOLATE HCL	MINIMS CYCLOPENTOLATE	1%	02148382

	HOMATROPINE HBR	MINIMS HOMATROPINE	2%	02148420
	PHENYLEPHRINE HCL	MINIMS PHENYLEPHRINE	2.5%	02148447
	PHENYLEPHRINE HCL	MINIMS PHENYLEPHRINE	10%	02148455
	TROPICAMIDE	MINIMS TROPICAMIDE	1%	02148536
	CYCLOPENTOLATE HCL	DOM-CYCLOPENTOLATE HCL	1%	02181436
	TROPICAMIDE	TROPICAMIDE	1%	02212919
NSAID	KETOROLAC TROMETHAMINE	KETOROLAC TROMETHAMINE	0.5%	02247461
	KETOROLAC TROMETHAMINE	KETOROLAC TROMETHAMINE	0.5%	02245821
	KETOROLAC TROMETHAMINE	KETOROLAC TROMETHAMINE	0.45%	02369362
	DICLOFENAC SODIUM	DICLOFENAC SODIUM	0.1%	00903755
	DICLOFENAC SODIUM	DICLOFENAC SODIUM	0.1%	00940414
	KETOROLAC TROMETHAMINE	KETOROLAC TROMETHAMINE	0.5%	00961272
	SUPROFEN	SUPROFEN	1%	02132710
	INDOMETHACIN	INDOMETHACIN	0.1%	02219506
	DICLOFENAC SODIUM	DICLOFENAC SODIUM	0.1%	02238145
	KETOROLAC TROMETHAMINE	KETOROLAC TROMETHAMINE	0.4%	02248722
	NEPAFENAC	NEPAFENAC	0.1%	02308983
	KETOROLAC TROMETHAMINE	KETOROLAC TROMETHAMINE	0.5%	02336693
	NEPAFENAC	NEPAFENAC	0.3%	02411393
	BROMFENAC SODIUM	BROMFENAC SODIUM	0.7%	02439123
Steroids	DEXAMETHASONE	MAXIDEX	1MG	00000698
	BETAMETHASONE	BETNESOL	0.1%	00012173
	HYDROCORTISONE ACETATE	CORTRIL	0.5%	00024775
	HYDROCORTISONE ACETATE	CORTRIL	2.5%	00024783
	MEDRYSONE & POLYVINYL ALCOHOL	HMS LIQUIFILM	1%	00036676
	PREDNISOLONE ACETATE	PREDNICON	1%	00252492
	FLUOROMETHOLONE	FML-NEO	0.1%	00259454
	PREDNISOLONE DISODIUM PHOSPHATE	INFLAMASE	0.125%	00281735
	PREDNISOLONE DISODIUM PHOSPHATE	INFLAMASE FTE	1%	00281743
	HYDROCORTISONE ACETATE	CORTAMED	2.5%	00283231
	HYDROCORTISONE	CORTAMED		00283258

ACETATE			
METHYLPREDNISOLONE	MEDROL	0.1%	00358711
FLUOROMETHOLONE	SANDOZ-FLUOROMETHOLONE	0.1%	00432814
PREDNISOLONE DISODIUM PHOSPHATE	INFLAMASE	0.125%	00526452
PREDNISOLONE DISODIUM PHOSPHATE	INFLAMASE FTE	1%	00526460
HYDROCORTISONE ACETATE	CORTAMED	2.5%	00704458
PREDNISOLONE ACETATE	ULTRACORTENOL	0.5%	00727466
DEXAMETHASONE	DEXAMETHASONE	0.1%	00739839
DEXAMETHASONE SODIUM	CEBEDEX	0.1%	00741752
PREDNISOLONE ACETATE	BALPRED	1%	00764639
PREDNISOLONE ACETATE	PREDOLONE FORTE	1%	00801186
PREDNISOLONE ACETATE	PREDOLONE	1%	00809152
FLUOROMETHOLONE & POLYVINYL ALCOHOL	FML LIQUIFILM	0.1%	00893188
FLUOROMETHOLONE & POLYVINYL ALCOHOL	FML LIQUIFILM	0.1%	00894931
PREDNISOLONE ACETATE	PRED FORTE	1%	00894958
PREDNISOLONE ACETATE	AK-TATE	1%	00896144
FLUOROMETHOLONE	FML FORTE	0.25%	00897469
PREDNISOLONE ACETATE	OPHTHO-TATE	1%	00898732
PREDNISOLONE DISODIUM PHOSPHATE	INFLAMASE FTE	1%	00907626
PREDNISOLONE ACETATE	R.O.-PREDPHATE FORTE	1%	00908266
PREDNISOLONE ACETATE	PREDNISOLONE ACETATE	-	00961264
PREDNISOLONE ACETATE	PREDNISOLONE ACETATE	0.12%	01916181
PREDNISOLONE ACETATE	PREDNISOLONE ACETATE	1%	01916203
PREDNISOLONE SODIUM PHOSPHATE	PREDNISOLONE	1%	01924400
DEXAMETHASONE SODIUM PHOSPHATE	DEXAMETHASONE	0.1%	01947044
PREDNISOLONE SODIUM PHOSPHATE	PMS-PREDNISOLONE SOD/PHO	1%	01954237
HYDROCORTISONE ACETATE	CORTAMED	2.5%	01980661
DEXAMETHASONE SODIUM PHOSPHATE	OCUDEX	0.1%	01995022
BETAMETHASONE	BETNESOL	0.1%	02060868
PREDNISOLONE SODIUM PHOSPHATE	MINIMS PREDNISOLONE SOD	0.5%	02148498
RIMELOXONE	VEXOL	1%	02163691

PREDNISOLONE SODIUM PHOSPHATE	PREDNISOLONE	1%	02213079
FLUOROMETHOLONE	PMS-FLUOROMETHOLONE	0.1%	02238568
PREDNISOLONE SODIUM PHOSPHATE	SAB-PREDNASE	1%	02245858
LOTEPREDNOL ETABONATE	ALREX	0.2%	02320924
LOTEPREDNOL ETABONATE	LOTEMAX	0.5%	02321114
LOTEPREDNOL ETABONATE	LOTEMAX OINTMENT	5MG	02421941
PREDNISOLONE ACETATE	PRED FORTE	1%	99100258
PREDNISOLONE SODIUM PHOSPHATE	PREDNISOLONE	1%	99100839

Appendix G: Benzalkonium Chloride Content of Anti-Glaucoma Drugs

Generic Name	Trade Name	Strength	DIN	BAK (%vol.)
PILOCARPINE HCL	ISOPTO CARPINE	0.5%	00000833	0
PILOCARPINE HCL	MINIMS PILOCARPINE	2%	00269107	0
PILOCARPINE HCL	MINIMS PILOCARPINE	2%	02148463	0
PILOCARPINE HCL	MINIMS PILOCARPINE	4%	02148471	0
LATANOPROST	SANDOZ-LATANOPROST	50MCG/ML	02367335	0
TRAVOPROST	TEVA-TRAVOPROST Z	0.004%	02412063	0
TRAVOPROST	TEVA-TRAVOPROST Z	0.004%	09857504	0
TIMOLOL MALEATE	APO-TIMOP	0.25%	00755826	0
TIMOLOL MALEATE	APO-TIMOP	0.5%	00755834	0
TIMOLOL MALEATE	TIMOPTIC-XE	0.25%	02171880	0
TIMOLOL MALEATE	TIMOPTIC-XE	0.5%	02171899	0
DORZOLAMIDE & TIMOLOL	CO DORZOTIMOLOL	2/0.5%	02404389	0.0001
LEVOBUNOLOL HCL	BETAGAN	0.5%	00637661	0.004
LEVOBUNOLOL HCL	BETAGAN	0.25%	00751286	0.004
LEVOBUNOLOL	RATIO-LEVOBUNOLOL	0.25%	02031159	0.004
LEVOBUNOLOL	RATIO-LEVOBUNOLOL	0.5%	02031167	0.004
DIPIVEFRINE HCL	PROPINE	0.1%	00529117	0.004
EPINEPHRINE HCL	EPIFRIN	2%	00001112	0.004
DIPIVEFRINE HCL & LEVOBUNOLOL HCL	PROBETA	0.5%	02209071	0.004
LEVOBUNOLOL HCL	SANDOZ-LEVOBUNOLOL	0.5%	02241716	0.004
LEVOBUNOLOL HCL	SANDOZ-LEVOBUNOLOL	0.25%	02241715	0.004
CARBACHOL	ISOPTO CARBACHOL	1.5%	00000655	0.005
CARBACHOL	ISOPTO CARBACHOL	3%	00000663	0.005
EPINEPHRINE HCL	EPIFRIN	0.5%	00001090	0.005
EPINEPHRINE HCL	EPIFRIN	1%	00001104	0.005
BRIMONIDINE TARTRATE	ALPHAGAN	0.2%	02236876	0.005
DIPIVEFRINE HCL	DPE	0.1%	02152525	0.005
DIPIVEFRINE HCL	APO-DIPIVEFRIN	0.1%	02242232	0.005
BRIMONIDINE TARTRATE	RATIO-BRIMONIDINE	0.2%	02243026	0.005
BIMATOPROST	LUMIGAN	0.03%	02245860	0.005
BRIMONIDINE TARTRATE & TIMOLOL MALEATE	COMBIGAN	0.2/0.5%	02248347	0.005
BRIMONIDINE TARTRATE	ALPHAGAN P	0.15%	02248151	0.005
BRIMONIDINE TARTRATE	PMS-BRIMONIDINE	0.2%	02246284	0.005
BRIMONIDINE TARTRATE	APO-BRIMONIDINE	0.2%	02260077	0.005
BRIMONIDINE TARTRATE & TIMOLOL MALEATE	COMBIGAN	0.2/0.5%	09857298	0.005

BRIMONIDINE	SANDOZ-BRIMONIDINE	0.2%	02305429	0.005
BRIMONIDINE TARTRATE	APO-BRIMONIDINE P	0.15%	02301334	0.005
PILOCARPINE HCL	RATIO-PILOCARPINE	1%	02229393	0.0075
DORZOLAMIDE HCL & TIMOLOL MALEATE	COSOPT	20MG/5MG/ML	02258692	0.0075
DORZOLAMIDE	SANDOZ-DORZOLAMIDE	2%	02316307	0.0075
DORZOLAMIDE & TIMOLOL	SANDOZ-DORZOL/TIMOL	1%	02344351	0.0075
TIMOLOL MALEATE	TIMOLOL MALEATE-EX	0.5%	02242276	0.01
BRINZOLAMIDE & TIMOLOL MALEATE	AZARGA	1%/0.5%	02331624	0.01
TIMOLOL MALEATE	TIMOPTIC	0.5%	00451207	0.01
BETAXOLOL HCL	BETOPTIC	0.5%	00695688	0.01
TIMOLOL MALEATE	TIMOPTIC	0.25%	00451193	0.01
TIMOLOL MALEATE	MYLAN-TIMOLOL	0.5%	00893781	0.01
TIMOLOL MALEATE	MYLAN-TIMOLOL	0.25%	00893773	0.01
BETAXOLOL	BETOPTIC S	0.25%	01908448	0.01
TIMOLOL MALEATE	SANDOZ-TIMOLOL	0.5%	02166720	0.01
TIMOLOL MALEATE	SANDOZ-TIMOLOL	0.25%	02166712	0.01
TIMOLOL MALEATE	PMS-TIMOLOL	0.5%	02083345	0.01
TIMOLOL MALEATE	PMS-TIMOLOL	0.25%	02083353	0.01
BRINZOLAMIDE	AZOPT	2% & .5%	02238873	0.01
PILOCARPINE HCL	ISOPTO CARPINE	1%	00000841	0.01
PILOCARPINE HCL	ISOPTO CARPINE	2%	00000868	0.01
PILOCARPINE HCL	ISOPTO CARPINE	4%	00000884	0.01
PILOCARPINE HCL	PILOPINE HS	4%	00575240	0.01
PILOCARPINE HCL	ISOPTO CARPINE	6%	00000892	0.01
PILOCARPINE HCL	MINIMS PILOCARPINE	4%	00269085	0.01
DIPIVEFRINE HCL	DPE	0.1%	02145324	0.01
TIMOLOL MALEATE	TIMOLOL MALEATE-EX	0.25%	02242275	0.01
TRAVOPROST	TRAVATAN	0.004%	02244896	0.015
TIMOLOL MALEATE & TRAVOPROST	DUOTRAV PQ	0.004/0.5%	02278251	0.015
TRAVOPROST	TRAVATAN Z	0.004%	02318008	0.015
TRAVOPROST	TRAVATAN Z	0.004%	09857332	0.015
TIMOLOL MALEATE & TRAVOPROST	DUOTRAV PQ	0.004/0.5%	09857333	0.015
TIMOLOL MALEATE & TRAVOPROST	DUOTRAV PQ	0.004/0.5%	09857512	0.015
TIMOLOL MALEATE & TRAVOPROST	DUOTRAV PQ	0.004/0.5%	09857513	0.015
LATANOPROST	XALATAN	0.005%	02231493	0.02
LATANOPROST & TIMOLOL	XALACOM	5MG/5MCG/ML	02246619	0.02
BIMATOPROST	LUMIGAN RC	0.01%	02324997	0.02
BIMATOPROST	LUMIGAN RC	0.01%	09857368	0.02
LATANOPROST	APO-LATANOPROST	50MCG/ML	02296527	0.02
BIMATOPROST	LUMIGAN RC	0.01%	09857398	0.02
LATANOPROST & TIMOLOL	SDZ-	5MG/5MCG/ML	02394685	0.02

TRAVOPROST	LATANOP/TIMOLOL SANDOZ TRAVOPROST	0.004%	02413167	0.02

Risk factors for secondary surgical intervention after primary glaucoma filtration surgery: an Ontario population-based study
Armstrong et. al. 2018

		CS HR (primary outcome)[1]		CS HR (death)[1]		SD HR[1]	
Covariables	**Reference**	**Hazard Ratio**	**P-Value**	**Hazard Ratio**	**P-Value**	**Hazard Ratio**	**P-Value**
Age 66-74	Age 75+	1.35	0.0056	0.46	<.0001	1.35	0.0055
Corneal Transplant	No corneal transplant	1.95	0.066	0.00	0.96	1.96	0.044
Surgeries on the Iris	No surgeries on Iris	0.70	0.043	1.26	0.21	0.70	0.045
History of Antibiotics	No history of antibiotics	1.48	0.015	0.91	0.73	1.48	0.01
History of Aminoglycosides	No history of aminoglycosides	3.21	<.0001	0.39	0.35	3.21	<.0001
History of Mydriatics	No history of mydriatics	2.19	<.0001	0.75	0.49	2.20	<.0001
History of NSAIDs	No history of NSAIDs	0.63	0.0081	0.99	0.97	0.63	0.0089
Filtering Surgery w/IDD	Filtering procedure (solo-procedure)	0.67	0.064	1.03	0.9	0.67	0.056
Combined w/cateract extraction only	Filtering procedure (solo-procedure)	0.45	<.0001	0.82	0.32	0.45	<.0001
combined w/cateract extraction and IDD	Filtering procedure (solo-procedure)	0.15	0.0012	1.17	0.6	0.15	0.0012

[1]CS HR: Cause-Specific Hazard Ratios, SD HR: Subdistribution Hazard Ratios

Hazard ratios are consistent across all tests (Cox, Cause-Specific, and Subdistribution).

Conclusion: no immortality bias

*type of filtration surgery is our main exposure of interest

Cumulative incidence function for death (competing risk)

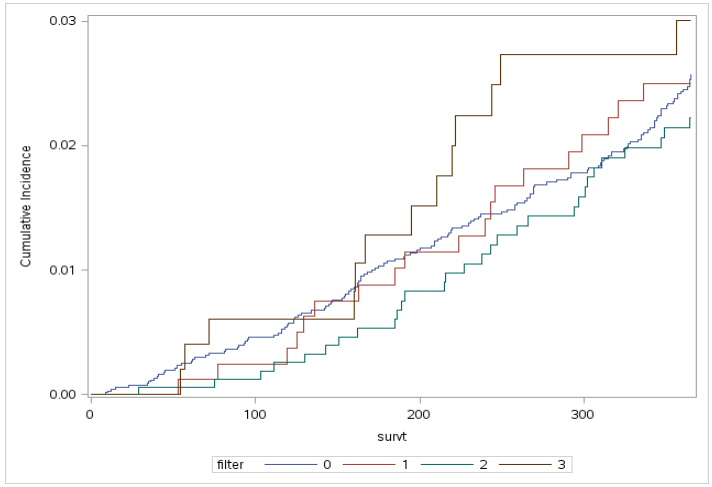


Figure to left shows that across all types of filtration sugeries the outcome death is equalily distributed. The p-value is not significant.

Gray's test for equality of cumulative incidence functions- p-value=0.7403, not significant

Legend for Filtration Surgeries:

Filter 0= filtration surgery w. no combination

Filter 1= filtration surgery w. cateract extraction

Fitler 2 = filtration surgery w. seton

Filter 3= filtration surgery w. seton conbined with cateract extraction

survt=days

Cumulative incidence function for primary outcome

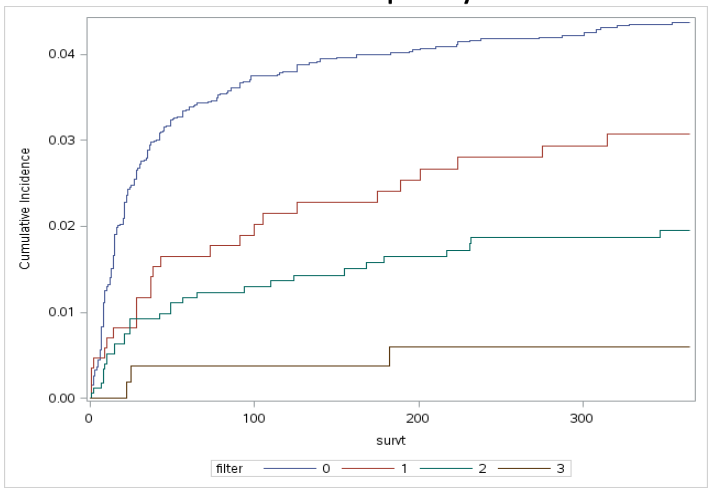


Figure to left shows that across all types of filtration sugeries the primary outcome varies. The p-value is significant.

Gray's test for equality of cumulative incidence functions- p-value=<.0001, significant

Appendix I:

Protocol / Funded Grant: An Investigator-Initiated Multi-Center Prospective Clinical Trial to Examine the Efficacy of Peri-Operative NSAID vs. Steroid Treatment in Trabeculectomy Wound Management

Given the results described in Chapter 2 and Chapter 3, a randomized clinical trial comparing the effects of steroidal and NSAID anti-inflammatory modalities after glaucoma surgery is warranted. There was a desire to “close the translational loop” and bring our retrospective clinical data-inspired *in vitro* findings back to the clinical setting, however the duration of my PhD studies denies the opportunity to collect any clinical trial results. Hence, the results of this RCT are outside the scope of my PhD, however, the following RCT protocol is included. It was submitted as a grant to the Glaucoma Research Society of Canada and funded in 2018. Fifteen glaucoma surgeons from across Canada have been recruited and patient enrollment is scheduled to begin summer 2019.

Introduction, Purpose & Objectives

Perioperative inflammation control is essential to both the short and long-term success of glaucoma surgery. Currently, this is accomplished with the use of topical steroidal anti-inflammatory drugs, such as dexamethasone or floromethalone. Although these drugs have proven anti-inflammatory action, there are associated adverse effects such as steroid associated intraocular pressure (IOP) spikes and inhibition of wound healing.[175,270] As elevation of IOP following glaucoma surgery is undesirable, there is a need to explore alternatives to corticosteroids with similar efficacy in inflammation control.

There have been recent studies to suggest non-steroidal anti-inflammatory drugs (NSAIDs), such as diclofenac, may be a promising alternative to corticosteroids following glaucoma surgery.[90,175] A recent study by Yuen et al. compared treatment with corticosteroids versus NSAIDs after Ahmed glaucoma valve surgery and found the steroid group showed a greater mean IOP at all measured follow-up points postoperatively.[90] Yuen et al. conducted a similar comparison and found that although there were no significant group differences in IOP at any of the follow-up visits, there was a clinical trend toward lower IOP values in the NSAID-treated group compared to the steroid-treated cohort.[90] More interestingly however, Levkovitch-Verbin et al. observed that the average number of glaucoma medications being taken at the eight-month follow-up point was significantly greater in the cohort treated with steroids post-phacotrabeculectomy compared to the NSAID-treated group.

This study aims to examine the efficacy of bromfenac (0.07%) relative to dexamethasone (0.1%) anti-inflammatory therapy in trabeculectomy wound management. Previous *in vitro* results suggested that indomethacin was preferable to dexamethasone for sub-conjunctival wound healing.[223] The novel ophthalmic NSAID bromfenac, contains bromine hydrophilic moieties such that its penetration into sub-conjunctival and sub-corneal tissues is higher.[271,272] It is hypothesized that substitution of bromfenac (0.07%) NSAID anti-inflammatory therapy one week after trabeculectomy will result in non-inferior IOP control with the need for fewer post-operative interventions compared to patients remaining on steroid only.

Study Objectives

- To compare the effects of steroid only to steroid followed by NSAID peri-operative therapy in achieving target IOP success following trabeculectomy
- To compare event-free survival time with an event defined as a post-op intervention required to achieve target IOP in the two patient groups
- To compare the bleb morphology in the two patient groups using the standardized Moorfields bleb grading scale

Study Design & Outcomes

Description of Study Design

This will be a multi-center randomized prospective clinical trial. Figure I-1 illustrates the study treatment schedule.

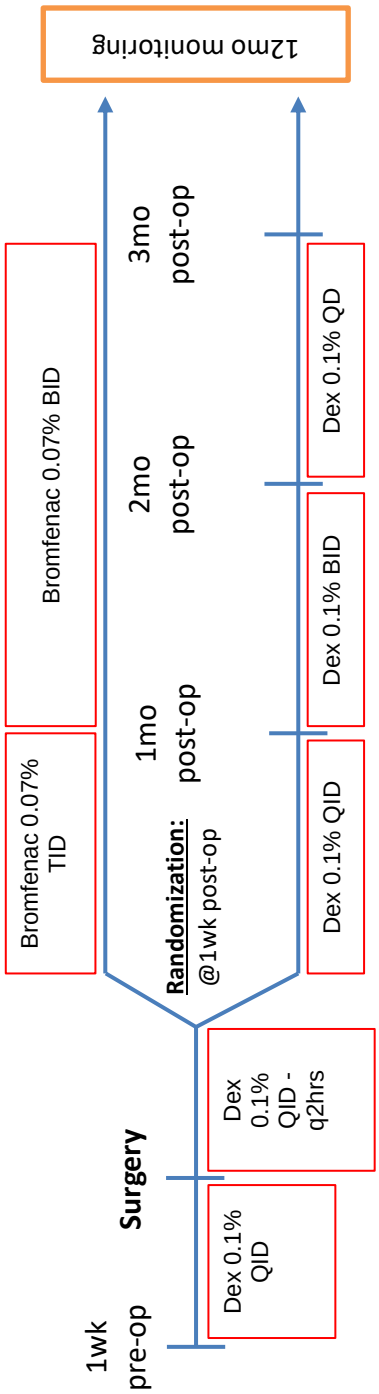


Figure I-1: RCT Drug Treatment Protocol

Study Outcomes

Main Outcome

The primary outcome is difference in event free survival (minimal and significant events; Table 0-1) between the two arms over the first post-operative year. An event is defined as a post-operative (secondary) intervention that is necessary to achieve a patient's target IOP.

Secondary Outcomes

Absolute intra-ocular pressure and percent reduction from baseline will be monitored at all follow up visits to assess non-inferiority between experimental groups in terms of IOP control.

Additional survival curves will be prepared to illustrate the difference in event free survival between treatment groups – curves will be prepared based on time to either a significant or minimal secondary intervention (Table 0-1).

Table I-1. Post-operative (secondary) interventions qualifying as minimal or significant

QUALIFICATION GROUP	DEFINITION
Minimal **Medical** Intervention	Require fewer (but non-zero) glaucoma medications post-op than pre-op
Minimal **Surgical** Intervention	Require needling ± 5-FU, etc.
Significant **Medical** Intervention	Require more glaucoma medications than pre-op to achieve target IOP
Significant **Surgical** Intervention	Require a new glaucoma surgical intervention – i.e. tube shunt, trab at new clock hour, etc.
Notes: laser suture lysis is not considered a secondary intervention	

Sample Size Analysis

The primary outcome is difference in event (defined within Table I-1) free survival between the two arms over the first post-operative year. We will power our study according to the primary outcome – an event from any qualification group occurring. Secondary outcomes (time to the specific type of secondary intervention) will be calculated based on the sample size attained – and are expected to require more statistical power than the primary outcome due to their lower individual occurrence rates. The logrank test will be used to compare the survival curves generated for the primary and each secondary outcome. The closest matching previous study was published by Levkovitch-Verbin et. al. 2013 in the Journal of Glaucoma, with 21 patients per arm. Ten percent of the dexamethasone treated patients' surgeries failed and 5% of the NSAID treated patients' surgeries failed.[175] This gives an effective hazard of 0.1 for the control arm and 0.05 for the experimental arm – with hazard ratio of 2. This was a relatively large treatment effect reported by this study with others reporting smaller differences to no significant difference – likely an artifact of all these studies' small sample sizes. This would suggest the true effect lies somewhere in the middle, with a hazard ratio of 1 to 2. We want to power our study sufficiently to detect a more modest/realistic treatment effect, that is still clinically relevant, so we intend to use a more conservative estimated hazard ratio of 1.25 within our sample size calculations. This will give us the ability to be more accurate than the previously mentioned studies. Using the Rubinstein et al. 1981 approach to the

logrank test sample size estimation (using: alpha = 0.05, beta = 0.8, minimum hazard ratio = 1.25, equal randomization, and estimated mean survival time of 8 months) we calculated approximately 74 patients necessary per group.[273]

Study Enrollment & Withdrawal

Participant Inclusion Criteria

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Adult patient over the age of 18 years
2. Uncontrolled open angle glaucoma
3. Scheduled to undergo stand-alone trabeculectomy
4. No previous:
   a. incisional glaucoma surgery
   b. vitrectomy
   c. strabismus surgery
   d. extracapsular cataract extraction (ECCE)
5. No ocular surgery of any kind in prior 6 months

Participant Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Steroids contraindicated
2. NSAIDs contraindicated
3. Poor corneal epithelial health

Participant Withdrawal or Termination

Reasons for Withdrawal or Termination

Participants are free to withdraw from participation in the study at any time upon request. An investigator may terminate participation in the study if:

- Any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant.
- The participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.

Handling of Participant Withdrawals or Termination

Participants will be asked to notify their study doctor in the event they wish to withdraw their consent and discontinue study participation. Participants will be able to withdraw data pertaining to their study participation up until dissemination of study results (e.g. study publication). In the event study results have already been disseminated, the participant will be reassured that any published study data are completely non-identifiable.

In the event a participant must be terminated from the study, the participant will be contacted by their treating study team and the reason for termination will be clearly disclosed. If the termination is pertaining to a medical issue, the participant will be directly followed by the study team and/or referred for additional treatment, if required.

Study Drugs

Overall Study Drug Regimen

Pre-Operative Course

All study participants will complete a one-week course of topical dexamethasone 0.1% QID prior to the scheduled trabeculectomy.

Post-Operative Course

On the day after trabeculectomy (Day 1), all participants will resume dexamethasone 0.1% QID for one additional week. At the investigator's discretion, the dexamethasone dose frequency may be increased up to q2h during the one-week postoperative period.

At the Day 7 visit, participants will be randomized to one of two treatment groups:

Table I-2. Randomized Study Drug Regimen

Group One: Bromfenac 0.07%	**Group Two: Continue Dexamethasone 0.1%**
TID to one month post-op	QID to one month post-op
BID for two months (from 1 month post-op to 3 months post-op)	BID for one month
	QD for one month

Participants will dose with their assigned study treatment until three months post-trabeculectomy.

Antibiotic Use

Participants will also complete a one-week course of fluoroquinolone antibiotic (gatifloxacin ophthalmic solution 0.3%, QID) during the one-week postoperative

period. Antibiotic use is considered standard of care for trabeculectomy management.

Dosing and Administration

Study participants, or their caregiver, will administer study drug. Study participants, and/or caregiver, will be instructed on study drug administration to encourage proper study drug use.

Study participants will be provided with a Dosing Guide which outlines the study drug regimen. This guide will be reviewed with each study participant to encourage proper study drug dosing.

Route of Administration

Topical dexamethasone and bromfenac will be used for this study (eye drops).

Duration of Therapy

Study participants will dose with study drug for a total duration of three months plus one week.

Study Procedures & Schedule

Schedule of Events Table

Table I-3. Schedule of Events Table	**Screening / Baseline**	**Surgery (Day 0)**	**Day 1**	**Week 1**	**Week 2**	**Month 1**	**Month 3**	**Month 6**	**Month 9**	**Month 12 or Early Term.**
Informed Consent	x									
Inc/Exclu. Criteria	x									
Demographics[1]	x									
Medical History[2] *including medications*	x									
IOP	x[3]		x	x	x	x	x	x	x	x
BCVA	x[4]		x	x	x	x	x	x	x	x
Slit Lamp Exam	x		x	x	x	x	x	x	x	x
Bleb Grading Scale (Moorfields)			x	x	x	x	x	x	x	x
C/D Ratio	x									x
Humphrey Perimetry	x									x
Trabeculectomy		x								
Review Concomitant Meds/Procedures[5]			x	x	x	x	x	x	x	x
Review AEs			x	x	x	x	x	x	x	x
GQL-15	x					x		x		x
GSS	x			x			x	x		

[1]Including: age, sex and race
[2]Inclusive of use and number of IOP-lowering medication(s)
[3]Baseline IOP: mean of 3 Goldmann applanation readings taken on different days
[4]Best-corrected visual acuity (VA) by the Snellen chart and converted to logarithm of the minimum angle of resolution VA
[5]Inclusive of all postoperative medications, procedures and surgeries

Visit Windows

Study visits may occur within the following acceptable visit windows:

Table I-4. Study Visit Windows

STUDY VISIT	ACCEPTABLE WINDOW
Day 1	Must occur on the day after trabeculectomy
Week 1	Must occur one week from trabeculectomy
Week 2	+/- 3 days
Month 1	+/- 7 days
Month 3	+/- 7 days
Month 6	+/- 14 days
Month 9	+/- 14 days
Month 12	+/- 14 days

Study Procedures & Evaluations

Study procedures and evaluations conform to the standards set out by the World Glaucoma Association for the Design and Reporting of Glaucoma Surgical Trials.[274]

Informed Consent

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation will be provided to the participants. Consent forms will be ethics-approved prior to use and the participant will be asked to read and review the document. The Investigator, or their delegate, will explain the research study to the participant and answer any questions that may arise.

Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participant will sign the informed consent document prior to any procedures being done specifically for the study.

The participants may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study

Inclusion/Exclusion Criteria

The inclusion and exclusion criteria will be reviewed following the Informed Consent discussion to determine if the participant is eligible to continue study participation. In the event a participant is deemed ineligible, the reason for Screen Failure will be documented.

Demographics

Patient demographics will be collected including age, sex and race.

IOP

IOP is to be measured using Goldmann applanation. The individual reading the tonometer dial and recording the numerical value should not be the same person who is manipulating the Goldmann tonometer.

The baseline IOP will be the mean of three Goldmann readings taken on different days. These readings should occur within a maximum period of one month.

For each Goldmann applanation reading, two measurements should be taken and averaged to determine the mean IOP. Three measurements should be taken if the first two measurements are greater than 3 mmHg difference. If more than two measurements are taken, the median (rather than mean) IOP value will be used.

Trabeculectomy & Mitomycin C Use

The following information pertaining to trabeculectomy is to be recorded for each study participant:

- MMC dose: 0.2 – 0.4 mg/mL
- Limbal or fornix approach
- Suture type to close conjunctiva

Study participants will receive MMC (0.2 – 0.4 mg/mL) delivered to the subconjunctival space using injection.

Concomitant Medications & Procedures

The use of any concomitant medications or procedures will be assessed at each study visit following the trabeculectomy. All concomitant medications and procedures will be recorded on a designated study source worksheet.

Concomitant medications to be recorded include concomitant prescription medications, over-the-counter medications and non-prescription medications.

It will be documented whether the concomitant medication or procedure was initiated as an intervention toward target IOP.

Adverse Events

The occurrence of any adverse events will be assessed at each study visit following the trabeculectomy. If a Serious Adverse Event (SAE) has occurred, the investigator will follow the reporting procedures as outlined in section 4.6.

Assessment of Safety

Specification of Safety Parameters

Definition of Adverse Events (AEs)

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention related.

Definition of Serious Adverse Events (SAEs)

An AE or suspected adverse reaction is considered "serious" if, in the view of the investigator, it results in any of the following outcomes:

- death
- a life-threatening adverse event
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- a congenital anomaly/birth defect

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Reporting Procedures

Adverse Event Reporting

All adverse events will be recorded within the designated source worksheets and Case Report Form (CRF).

Serious Adverse Event Reporting

The site investigator will complete a SAE Form within the following timelines:

- All deaths and immediately life-threatening events, whether related or unrelated to the study intervention, will be recorded on the SAE Form and submitted to the Lead Site within 24 hours of site awareness, if reasonably possible.
- Other SAEs will be submitted to the Lead Site within 72 hours of site awareness.

All SAEs will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the adherence to be stable.

The site investigator will determine if the SAE is reportable per the guidelines of their overseeing ethics committee and institution. A report to all necessary parties will be prepared and submitted within the institution-specified timeline.

Safety Oversight

Safety oversight will be under the direction of the Lead Site study team. The Lead Site will meet quarterly to review and assess safety data on each arm of the study. The Lead Site will be responsible for distributing quarterly safety reports and notifying study investigators of any ongoing study safety issues.

Curriculum Vitae

Education

Post-Secondary

2014 - present **MD/PhD**, Schulich School of Medicine and Dentistry, London, Ontario
Modulating Inflammation and Wound Healing in the Surgical Management of Glaucoma
Laboratory of: Dr. Cindy Hutnik, Ivey Eye Institute, Departments of Ophthalmology and Pathology, Schulich School of Medicine

2013 **Honors Bachelor of Science.** *Major in Medical Cell Biology and Major in Biology*, Western University, London, Ontario

Other

2016 **CSTAR Inter-Professional Summer Surgery School 2016**
Schulich School of Medicine, Arthur Labatt Family School of Nursing and Fanshawe College School of Nursing

Master Class on Writing Research for Publication 2016
Center for Education Research and Innovation (CERI), Schulich School of Medicine, Continuing Professional Development

Research Interests

Wound healing, inflammation and fibrosis
Microinvasive glaucoma surgery
Fibroproliferative and myofibroblast pathophysiology
Tissue engineering for disease modeling applications
Systematic review and meta-analysis
Electronic health records big data health analytics (ICES-Western)
Machine learning applications to medical record keeping and quality indicators
Blockchain applications for distributed, private medical record keeping
Clinical practice guideline development, methodological quality and their implementation

Research Profiles

Mendeley https://tinyurl.com/JJA-Mendeley

Scopus Author ID: 57061621900

OrcID https://orcid.org/0000-0002-0848-7496

Research Gate https://www.researchgate.net/profile/James_Armstrong14

Research Grants/Scholarships (successful in bold)

2018 **Glaucoma Research Society of Canada**. Funding to support a randomized controlled trial evaluating the efficacy NSAIDs vs steroidal anti-inflammatory therapy after trabeculectomy – awarded $19,818

The McGrath Research Scholarship. Funding to provide graduate students with an opportunity to undertake vision science research projects with established researchers in an environment that provides strong mentorship - awarded $8,000.

AMOSO Innovation Fund. Lipid mediators of inflammation and resolution: investigating the association with abnormal wound healing and surgical outcome – Awarded $146,560 over 2yrs

2017 **Nomination -** Vanier Canada Doctoral Scholarship. The proposal I put forward was nominated by the University of Western Ontario's Vanier Selection Committee for the 2018 competition – nominated to receive $150,000. Ultimately ranked 75 of 100 national finalists, with the top 55

receiving funding

Investigator initiated proposal – Alcon Canada. Modeling Aqueous Drainage through the Supraciliary Space: a Tissue Engineering and Microfluidics Approach – applied for $56,000

Glaucoma Research Society of Canada. Funding to support Tenon's capsule tissue mimetic model validation. – awarded $20,000.

Private Fundraising Initiative. Funding to support ICES database research costs. Assessing neuroprotective effects of Trazodone and other promising agents in an Ontario-based retrospective cohort - raised $10,000 in private donations.

2016 **The McGrath Research Scholarship.** Funding to provide graduate students with an opportunity to undertake vision science research projects with established researchers in an environment that provides strong mentorship. - awarded $8,000.

Lawson Internal Research Fund. Funding to investigate patient and surgeon risk factors associated with trabeculectomy failure – awarded $15,000.

Allergan Educational Grant. PhD stipend supplementation for research in novel methods of wound healing modulation for glaucoma surgical patients - awarded $18,500.

Ivey Eye Institute. PhD stipend supplementation for research on microinvasive glaucoma surgery optimization - $18,500/year for 3 years.

2015 **Schulich Summer Research Training Program.** Supported for one summer of epidemiological research and a subsequent summer of tissue engineering and fibrosis related research - awarded $9,000.

Patents / Provisional Patents

2018 **Armstrong, J.J.** and Hutnik, C.M.L. 2018. Compositions and methods for treating ocular inflammation and ocular scarring. U.S. Provisional Patent Application No. 62/677,284, filed May 2018. Provisional status until May 2019.

Peer-Reviewed Publications

2019 **Armstrong, J.J.**, Denstedt, J., Trelford, C., Li, E.A. and Hutnink, C.M.L. Differential effects of dexamethasone and indomethacin on Tenon's capsule fibroblasts: implications for glaucoma filtration surgery. *Experimental Eye Research,* Volume 182, May 2019; 65-73.
DOI: https://doi.org/10.1016/j.exer.2019.03.015

Contribution: Developed research question with CH, designed experimental methods, executed experiments with JC, CT and EL. Drafted manuscript, with edits from JD, CT, EL and CH.

Kiatos, E., **Armstrong, J.J.**, Tsioros, S., Hutnik, C.L.M., Malvankar-Mehta, M. and Hodge, W.G. Of phaco and pain management: our review and meta-analysis of intravenous sedation in modern cataract surgery. *Ophthalmology Management* Jan 2019, 46-54.

Contribution: Assisted with data collection and analysis, and drafting of manuscript

2018 **Armstrong, J.J.**, Welk, B., Reid, J.N.S., Kansal, V. and Hutnik, C.M.L. Secondary surgical intervention after primary glaucoma filtration surgery: an Ontario population-based study. *Canadian Journal of Ophthalmology,* June 2018.
DOI: https://doi.org/10.1016/j.jcjo.2018.04.004

Contribution: Originator of research question, recruited authors, drafted database coding strategy, first authored ICES DCP, created experimental groups, participated in data analysis, and first authored manuscript.

Armstrong, J.J. and Hutnik, C.M.L. Comment re: Selective laser trabeculoplasty as replacement therapy in medically controlled glaucoma patients. *International Glaucoma Review* 2018;18:4 52-3.
http://www.e-igr.com/ES/index.php?issue=184&ComID=1799

Contribution: completed first and contributed to final drafts after consultation with Dr. Hutnik and discussion of original article.

Kansal, V., **Armstrong, J.J.**, Pintwala, R. and Hutnik, C.M.L. Optical Coherence Tomography for Glaucoma Diagnosis: An Evidence Based Meta-Analysis. *PLoSOne*. Jan 2018.
DOI: https://doi.org/10.1371/journal.pone.0190621

Contribution: assisted in development of research question, recruited authors, developed database search strategy and screening questions, participated in screening, collected and analyzed data, edited manuscript.

2017 Michaelov, E., **Armstrong. J.J.**, Nguyen, M., Instrum, B., Lam, T., Denstedt, J. and Hutnik, C.M.L. Assessing the Methodological Quality of Glaucoma Clinical Practice Guidelines and their Recommendations on Microinvasive Glaucoma Surgery: a Systematic Review and AGREE II Assessment. *Journal of Glaucoma.*
DOI: 10.1097/IJG.0000000000000820

Contribution: Originator of research question, recruited authors, developed database search strategy and screening questions, assisted in screening and data analysis, edited manuscript.

***Awarded**:* Canadian Glaucoma Society best paper of 2017

Kiatos, E., **Armstrong, J. J.**, Hutnik, C.M.L., Tsioros, S., Malvankar-Mehta, M. S. and Hodge, W. G. The value of corneoscleral rim cultures in keratoplasty: a systematic review and cost-effectiveness analysis. *ClinicoEconomics and Outcomes Research* 2017:9 459-474.

Contribution: Participated in screening, data extraction and manuscript preparation.

Armstrong, J. J., Wasiuta, T., Kiatos, E., Malvankar, M. and Hutnik, C.M.L. The effects of phacoemulsification on intra-ocular pressure and topical medication use in patients with glaucoma: a systematic review and meta-analysis of three-year data, *Journal of Glaucoma* 2017;26:511-522.

Contribution: Originator of research question, recruited authors, developed database search strategy and screening questions, carried out screening, collected and analyzed data, drafted manuscript

2016 Rodrigues, I. B., **Armstrong, J. J.**, MacDermid, J. C. Facilitators and barriers to exercise adherence in patients with osteoporosis: a systematic review, *Osteoporosis International* 27(10):1-11 (2016).

*Contribution***:** Optimized database search strategy, refined inclusion criteria and screening questions, reference screening, GRADE quality assessment, edited manuscript

Armstrong, J. J., Goldfarb, A. M., Instrum, R. S., MacDermid, J. C. Improvement evident, but still necessary in clinical practice guideline quality: a systematic review, *Journal of Clinical Epidemiology* 81:13-2 (2016),
DOI: 10.1016/j.jclinepi.2016.08.005.

Contribution: Originator of research question, recruited authors, developed database search strategy and screening questions, carried out screening, collected and analyzed data, drafted manuscript

Armstrong, J. J., Rodrigues, I. B., Wasiuta, T. & MacDermid, J. C. Quality assessment of osteoporosis clinical practice guidelines for physical activity and safe movement: an AGREE II appraisal. *Arch. Osteoporos.* **11,** 6 (2016).

Contribution: Originator of research question, developed methods, recruited authors, collected and analyzed data, drafted manuscript

Publications Submitted/Under Review/In Press

2018 Trelford, C., Denstedt, J., **Armstrong, J.J.** and Hutnik, C.M.L. Using a 3D bioartificial tissue of human Tenon's capsule fibroblasts to assess the fibrogenic and inflammatory properties of the Tenon's Capsule. Submitted to: *Clinical and Experimental Ophthalmology,* Nov 2018.

Rejected and Re-Submitted Submissions

2018 Kansal, V., **Armstrong, J.J.**, and Hutnik, C.M.L. Canadian Trends in Glaucoma Filtration Procedures from 2003 to 2016: Potential Impact of Minimally Invasive Glaucoma Surgery. Submitted to *Canadian Journal of Ophthalmology,* Oct 2018.

Budure, A., **Armstrong, J.J.**, Belrose, J., Ly, C. and Hutnik, C.M.L. Incidence of Perioperative Hypertension in Phacoemulsification Surgery. Submitted to: *Journal of Cataract & Refractive Surgery*. Sept 2018.

Michaelov, E, **Armstrong, J.J.**, Kansal, V., Ly, C., Denstedt, J. and Hutnik. C.M.L. Evaluation of Micro-invasive Glaucoma Surgery Devices: A Systematic Review and Meta-analysis. Submitted to: *Canadian Journal of Ophthalmology / Journal Canadien d'Ophtalmologie*, December 2017. Rejected: March 2018.

Rejected and Abandoned Submissions

2017 **Armstrong, J. J.**, Pintwala, R., Michaelov, E. and Hutnik, C.M.L. Risk factors for bleb-forming glaucoma surgery failure: a systematic review and meta-analysis. *International Journal of Ophthalmology* (submitted Sept 1, 2017)

Contribution: Originator of research question, recruited authors, developed database search strategy and screening questions, carried out screening, collected and analyzed data, drafted manuscript.

Armstrong, J. J., Diaz, A. P., O'Gorman, D. B. Palmar fascia mimetics as novel models of Dupuytren's disease cord development, *Advances in Wound Care* (submitted, April 22, 2017)

Contribution: Collaboratively developed research question and methods with DO, carried out experimental work with AD, drafted the manuscript and finalized it collaboratively with DO.

Abstracts: Podium Presentations

2019 **Armstrong, J.J.**, Li, E., Vinokurtseva, A. and Hutnik, C.M.L. Repurposing COX-2: an immuno-resolving and anti-cicatrizing therapy. Presented at the Canadian Ophthalmological Society Annual Meeting & Exhibition 2019, June 13-16; Quebec City, Qc

Armstrong, J.J., Li, E., Vinokurtseva, A. Liu, H. and Hutnik, C.M.L. Acetylsalicylic acid reduces TGFb-induced myofibroblast transdifferentiation and activity. Presented at London Health Research Day 2019; April 30; London, On

Armstrong, J.J., Li, E. and Hutnik, C.M.L. Acetylsalicylic acid reduces collagen contraction, remodeling and myofibroblast proliferation in a 3D Tenon's capsule tissue mimetic. Presented at the American Glaucoma Society Annual Meeting 2019, March 14-17; San Francisco, CA, USA

Armstrong, J.J., Denstedt, J., Li, E., Dube, J., Trelford, C., Liu, H. and Hutnik, C.M.L. Modulating wound healing after glaucoma surgery. Presented at Lawson Health Research InstituteTalks on Fridays, February 1; London, On

Armstrong, J.J., Boyd, E. and Hutnik, C.M.L. Proposal for a Canadian Trial evaluating NSAIDs vs. Steroid post-glaucoma Surgery. Presented at the Canadian Glaucoma Society Annual Meeting 2019, January 8-19; Paradise Valley, AZ, USA

2018 **Armstrong, J.J.**, Denstedt, J., Li, E., Tingey, D. and Hutnik, C.M.L. Development of a novel acetylsalicylic acid-based adjuvant for glaucoma surgery. Presented at the Ivey Eye Institute Research Day 2018; November 2; London, On
**Awarded Best Oral Presentation by a Graduate Student ($1000)*

Denstedt, J., **Armstrong, J. J.** and Hutnik, C.M.L. Modulating wound healing in trabeculectomy with SB-431542. Presented at the Ivey Eye Institute Research Day 2018; November 2; London, On
**Awarded Best Oral Presentation by a Medical Student ($1000)*

Armstrong, J.J., Trelford, C., Denstedt, J. and Hutnink, C.M.L. Modulation of wound healing after glaucoma filtration surgery: a 3-minute thesis. Presented at the Schulich Summer Research Training Program/Summer Research Opportunities Program Symposium; August 14; London, On

Armstrong, J.J. and Hutnik, CML. Acetylsalicylic acid reduces collagen contraction, remodeling and myofibroblast proliferation in subconjunctival tissue mimetic. Presented at the Canadian Ophthalmological Society Annual Meeting; 2018 June 3; Toronto, On
**COS Award for Excellence in Ophthalmic Research, 2nd place ($2000)*

Kansal, V., **Armstrong, J.J.**, Pintwala, R. and Hutnik, CML. Optical Coherence Tomography for Glaucoma Diagnosis: an Evidence Based Meta-Analysis. Presented at the Canadian Ophthalmological Society Annual Meeting; 2018 June 3; Toronto, On

Denstedt, J., **Armstrong, J.J.**, Trelford, C. and Hutnik, CML. Assessing the effects of indomethacin and dexamethasone on wound healing using a 3D bioartificial tissue of human Tenon's capsule fibroblasts. Presented at the Canadian Ophthalmological Society Annual Meeting; 2018 June 3; Toronto, On

Kiatos, E., **Armstrong, J.J.**, Hodge, W., Malvankar-Mehta, M. and Hutnik, CML. A systematic review and meta-analysis of intravenous sedation in modern cataract surgery. Presented at the Canadian Ophthalmological Society Annual Meeting; 2018 June 3; Toronto, On

Armstrong, J.J. and Hutnik, C.L.M. Why MD/PhD and why ophthalmology: research and your future career. Co-presented to the Schulich School of Medicine MD/PhD students and prospective students; 2018 Jan 27; London, On

2017 Michaelov, E., **Armstrong. J.J.**, Nguyen, M., Instrum, B., Lam, T., Denstedt, J. and Hutnik, C.M.L. Assessing the Methodological Quality of Glaucoma Clinical Practice Guidelines and their Recommendations on Microinvasive Glaucoma Surgery: a Systematic Review and AGREE II Assessment. Presented at Ivey Eye Institute Departmental Research Day; 2017 Nov 3; London, On

Denstedt, J., **Armstrong, J.J.**, Trelford, C. and Hutnik, C. The Effects of Indomethacin and Dexamethasone on Tenon's Capsule Tissue Mimetics: avoidance of fibrotic outcomes. Presented at Ivey Eye Institute Departmental Research Day; 2017 Nov 3; London, On
**Awarded 1st place*

Armstrong, J.J., Welk, B., Reid, J.N.S., Kansal, V. and Hutnik, C.M.L. Risk Factors for Secondary Surgical Intervention after Glaucoma Filtration Surgery: a population-based study. Presented at Ivey Eye Institute Departmental Research Day; 2017 Nov 3; London, On

Kansal, V., **Armstrong, J. J.**, Pintwala, R. and Hutnik, C.M.L. Optical Coherence tomography for Glaucoma Diagnosis and Monitoring. Presented to the London Optometry Association; 2017 Sept. 20; London, On

Armstrong, J. J., Wasiuta, T., Kiatos, E., Malvankar, M. and Hutnik, C.M.L. The Effects of Phacoemulsification on Intra-Ocular Pressure and Topical Medication Use in Patients with Glaucoma: A Systematic Review and Meta-Analysis of Three Year Data. Presented at the Canadian Ophthalmological Society Annual Meeting; 2017 June 18; Montreal, Qb
**Designated 'Hot Topic'*

Kiatos, E., **Armstrong, J. J.**, Hutnik, C.M.L., Tsioros, S., Malvankar-Mehta, M. S. and Hodge, W. G. The value of corneoscleral rim cultures in keratoplasty: a systematic review and cost-effectiveness analysis. Presented at the Canadian Ophthalmological Society Annual Meeting; 2017 June 18; Montreal, Qb

Trelford, C. B., **Armstrong, J. J.,** Hutnik, C.M.L. Using transcriptome analysis to compare Tenon's capsule of patient derived samples, 2D monolayer cell culture and a novel bioartificial tissue construct. Presented at Lawson Health Research Institute "Talks on Friday's"; 2017 Jan 13; London,

On

2016 Trelford, C. B., **Armstrong, J. J.,** Hutnik, C.M.L. Validation of a novel bioartificial tissue model of Tenon's capsule through whole transcriptome comparison with *ex vivo* Tenon's capsule and Tenon's capsule fibroblasts in 2D monolayer cell culture. Presented at the Department of Pathology Honors Thesis Symposium; 2016 Dec 7; London, On

Armstrong, J. J., Wasiuta, T., Kiatos, E., Malvankar, M. and Hutnik, C.M.L. The Effects of Phacoemulsification on Intra-Ocular Pressure and Topical Medication Use in Patients with Glaucoma: A Systematic Review and Meta- Analysis of Three-Year Data. Presented at Ivey Eye Institute Departmental Research Day; 2016 Nov 4; London, On

Armstrong, J.J., Diaz, A. P., O'Gorman, D. B. Creation of an *in vitro* model of fibrosis using Dupuytren's disease patient explant tissues. Presented at: Schulich School of Medicine Summer Research Training Program (SRTP) Seminar Series; 2016 Aug 9; London, On

Armstrong J. J., Diaz, A. P., O'Gorman, D., B. Dupuytren's disease: toward an in vitro model of fibroproliferative disease. Presented at: 2016 Canadian Bone and Joint Conference; 2016 April 9; London, On
**Awarded top oral presentation (value $250)*

2015 **Armstrong, J. J.**, Rodrigues, I. B., Wasiuta, T. & MacDermid, J. C. Quality assessment of osteoporosis clinical practice guidelines for physical activity and safe movement: an AGREE II appraisal. Presented at: Schulich School of Medicine Summer Research Training Program (SRTP) Seminar Series; 2015 July 7; London, On

Abstracts: Poster Presentations

2019 Denstedt, J., **Armstrong, J.J.** and Hutnik, C.M.L. Improving glaucoma surgery with the small molecule ALK-5 inhibitor SB-431542. Presented at the Canadian Ophthalmological Society Annual Meeting & Exhibition 2019, June 13-16; Quebec City, Qc
**Awarded Second Place for Best Overall Poster*

Armstrong, J.J. and Hutnik, C.M.L. Repurposing COX-2: an immunoresolving therapy. Accepted to the Keystone Symposia for Lipidomics and Functional Metabolic Pathways in Disease 2019, March 31 – April 4; Steamboat Springs, Colorado, USA
**unpublished*

Armstrong, J.J., Li, E.A., Vinokurtseva, A. and Hutnik, C.M.L. Repurposing COX-2: an immuno-resolving and anti-cicatrizing therapy. Presented at the Schulich School of Medicine and Dentistry, Department of Pathology and Laboratory Medicine Research Day 2019, March 29; London, On

Li, E.A., **Armstrong, J.J.**, Vinodurtseva, A. and Hutnik, C.M.L. Acetylsalicylic acid inhibits inflammation induced myofibroblast transdifferentiation in human tenons capsule fibroblasts. Presented at the Schulich School of Medicine and Dentistry, Department of Pathology and Laboratory Medicine Research Day 2019, March 29; London, On

Denstedt, J., Michaelov, E., **Armstrong, J.J.** and Hutnik, C.M.L. A Systematic Review and Meta-Analysis of Minimally Invasive Glaucoma Surgery Devices. Presented at the American Glaucoma Society Annual Meeting 2019, March 14-17; San Francisco, CA, USA

2018 Budure, A., **Armstrong, J.J.**, Belrose, J. and Hutnik, C.M.L. The incidence of perioperative hypertension during routine cataract surgery. Presented at the Ivey Eye Institute Research Day 2018; November 2; London, On

Armstrong, J.J., Welk, B., Reid, J., Kansal, V. and Hutnik, C.M.L. Risk Factors for Secondary Surgical Intervention after Glaucoma Filtration Surgery: A Population-Based Study. Presented at the Canadian Ophthalmological Society Annual Meeting & Exhibition; June 1-3; Toronto, On

Armstrong, J.J., Pena-Diaz, A. and O'Gorman, D.B. Palmar fascia mimetics as novel models of Dupuytren's Disease cord development. Presented at the Canadian Connective Tissue Conference of Canada; May 25-27; Montreal, Qc

Denstedt, J., **Armstrong, J.J.** and Hutnik, C. The functional response of Tenon's capsule fibroblast cells to treatment with fibrotic growth factors in a 3D collagen lattice. Presented at London Health Research Day; May 10, 2018; London, Ontario

Armstrong, J. J., Denstedt, J and Hutnik, C. Acetylsalicylic acid reduces collagen contraction, remodelling and myofibroblast proliferation in subconjunctival tissue mimetic. Presented at ARVO; 2018 May 1; Honolulu, Hawaii

Denstedt, J., **Armstrong, J.J.**, Trelford, C. and Hutnik, C. The Effects of Indomethacin and Dexamethasone on Tenon's Capsule Tissue Mimetics: avoidance of fibrotic outcomes. Presented at ARVO; 2018 April 29; Honolulu, Hawaii

Armstrong, J. J., Denstedt, J. and Hutnik, C. M. L. Acetylsalicylic acid reduces collagen contraction, remodelling and myofibroblast proliferation in subconjunctival tissue mimetic. Presented at the Department of Pathology and Laboratory Medicine Research Day, Schulich School of Medicine; April 13; London, On

Hutnik, C. M. L., Michaelov, Evan, **Armstrong, J.J.**, Kansal, Vinay and Denstedt, James. Evaluation of Micro-invasive Glaucoma Surgery Devices: A Systematic Review and Meta-analysis. Presented at the American Glaucoma Society Annual Meeting; March 1-3; New York, NY

Armstrong, J.J., Welk, B., Reid, J.N.S., Kansal, V. and Hutnik, C.M.L. Risk Factors for Secondary Surgical Intervention after Glaucoma Filtration Surgery: a population-based study. Presented at the American Glaucoma Society Annual Meeting; March 1-3; New York, NY

Denstedt, J., **Armstrong, J.J.**, Trelford, C. and Hutnik, C. The Effects of Indomethacin and Dexamethasone on Tenon's Capsule Tissue Mimetics: avoidance of fibrotic outcomes. Presented at the American Glaucoma Society Annual Meeting; March 1-3; New York, NY

2017 Michaelov, E., **Armstrong. J.J.**, Nguyen, M., Instrum, B., Lam, T., Denstedt, J. and Hutnik, C.M.L. Assessing the Methodological Quality of Glaucoma Clinical Practice Guidelines and their Recommendations on Microinvasive Glaucoma Surgery: a Systematic Review and AGREE II Assessment. Presented at the American Academy of Ophthalmology Annual Meeting; November 11-14; New Orleans
**Awarded best poster*

Kansal, V., **Armstrong, J.J.**, Pintwala, R. and Hutnik, C.M.L. Optical Coherence Tomography for Glaucoma Diagnosis: An Evidence Based Meta-Analysis. Presented at the American Academy of Ophthalmology Annual Meeting; November 11-14; New Orleans

Armstrong, J. J., Dang, B., Liu, H., Tellios, V., Tellios, N., Cejic, N. Cronk, A. and Hutnik, C. Bio-modulation of Primary Human Tenon's Capsule Fibroblasts Using a Novel Application of Coated Magnesium. Presented at the Canadian Ophthalmological Society Annual Meeting & Exhibition; June 15-18; Montreal, Quebec

Armstrong, J. J. and Hutnik, C.M.L. Effect of Glaucomatous Aqueous Humor Cytokines on Tenon's Capsule Fibroblast Populated Collagen Lattices. Presented at The Department of Pathology and Laboratory Medicine Research Day; March 30; London, Ontario

Armstrong, J. J., Wasiuta, T., Kiatos, E., Malvankar, M. and Hutnik, C.M.L. The Effects of Phacoemulsification on Intra-Ocular Pressure and Topical Medication Use in Patients with Glaucoma: A Systematic Review and Meta-Analysis of Three Year Data. Presented at the London Health Research Day; March 28, 2017; London, Ontario

Trelford, C., **Armstrong, J. J.**, Hutnik, C.M.L. The Validation of a 3D Bioartificial Tissue of the Tenon's Capsule. Presented at the London Health Research Day; March 28, 2017; London, Ontario

Michaelov, E., **Armstrong, J. J.**, Nguyen, M. and Hutnik, C.M.L. The quality of ophthalmology clinical practice guidelines and a review of recommendations for micro-invasive glaucoma surgical procedures: an AGREE II appraisal. Presented at the London Health Research Day; March 28, 2017; London, Ontario

Kiatos, E., **Armstrong, J. J.**, Hutnik, C.M.L., Tsioros, S. M., Malvankar-Mehta, M. S. and Hodge, W. G. The Value of Corneoscleral Rim Cultures in Keratoplasty: A Systematic Review and Cost-Effectiveness Analysis. Presented at the London Health Research Day; March 28, 2017; London, Ontario

Trelford, C., **Armstrong, J. J.**, Hutnik, C.M.L. The Validation of a 3D Bioartificial Tissue of the Tenon's Capsule. Presented at The Department of Pathology and Laboratory Medicine Research Day; March 30; London, Ontario

Armstrong, J. J., Wasiuta, T., Kiatos, E., Malvankar, M. and Hutnik, C.M.L. The Effects of Phacoemulsification on Intra-Ocular Pressure and Topical Medication Use in Patients with Glaucoma: A Systematic Review and Meta-Analysis of Three-Year Data. Presented at the Canadian Society for Epidemiology and Biostatistics: 2017 Biennial Conference; May 30 – June 2; Banff, Alberta

2016 **Armstrong J. J.**, Diaz, A. P., O'Gorman, D., B. Dupuytren's disease: toward an in vitro model of fibroproliferative disease. Poster presented at: Canadian National Medical Students Research Symposium; 2016 June 8; Winnipeg, MB

Armstrong J. J., Diaz, A. P., O'Gorman, D., B. Dupuytren's disease: toward an in vitro model of fibroproliferative disease. Poster presented at: Canadian Connective Tissue Conference; 2016 June 3; Hamilton, On

2013 **Armstrong, J. J.**, Dragunas, A. J., Dickey, J. P. Early or late kick: a biomechanical comparison of two breaststroke underwater swimming techniques. Poster presented at: University of Western Ontario, in partial fulfillment of requirements for biology 4970G; 2013 April 10; London, On.
**Awarded a mark of 95%*

Training/Safety Policy Documents

2018 **Armstrong, J.J.** Safe Work Practices / Standard Operating Procedure for Persons Working with Varicella Zoster or Herpes Simplex Virus *in vitro*. Prepared for: Lawson Health Research Institute, London, On, Canada.

Student Supervision (co-supervision under Dr. Cindy Hutnik)

2019 A. Budure (MD3), J. Denstedt (MD3), M. Fung (MD2), E. Li (HBSc), G. Ge (MD4), E. Boyd (MD2), D. Rabinovitch (HBSc), A. Vinokurtseva (MD1), M. Wai (HBSc), C. Christian (High School Co-op)

2018 A. Budure (MD3), J. Denstedt (MD3), R. McInnis (MD2), M. Fung (MD2), E. Li (HBSc), G. Ge (MD4), E. Boyd (MD2), D. Rabinovitch (HBSc), A. Vinokurtseva (MD1), M. Wai (HBSc)

2017 A. Budure (MD2), J. Dube (HBSc), J. Denstedt (MD2), E. Michaelov (MD4), M. Nguyen (HBSc), S. Tsioros (HBSc), T. Lam (MD2), R. McInnis (MD1), M. Fung (MD1), E. Boyd (MD1)

2016 V. Kansal (MD4), J. Denstedt (MD1), E. Michaelov (MD3), C. Trelford (HBSc),

Ethics Applications

2019 Boyd, E., **Armstrong J. J.**, Denstedt, J. and Hutnik, C. A national multi-center randomized controlled trial evaluating efficacy of dexamethasone versus [NSAID] for wound modulation after trabeculectomy.

Armstrong J. J., Belrose, J., Hutnik, C. Incidence of perioperative hypertension and management strategies employed for patients undergoing cataract surgery.

2017 **Armstrong J. J.**, Denstedt, J., Hutnik, C. Growth factors in the aqueous humor and subsequent trabeculectomy failure

Armstrong J. J., Belrose, J., Hutnik, C. Incidence of perioperative hypertension and management strategies employed for patients undergoing cataract surgery.

Research Awards/Accolades

2018 **Ivey Eye Institute Best Presentation by a Graduate Student.** Awarded to the best presentation at research day as judged by the panel. Value: $1000

Canadian Glaucoma Society Best Paper Award – Medical Student Category 2018. Awarded to the best paper published by a Canadian medical student within the topic of Glaucoma. Value: $500
**Second Author*

Canadian Ophthalmological Society Paper Presentation Award 2018. Awarded for excellence in ophthalmic research. Value: $2,000.

2016 **Dr. Glen S. Wither Award for Research**. Awarded for outstanding participation and research excellence in the Schulich Summer Research Training program. Value: $600.

Research featured by The US Agency for Healthcare Research and Quality's Effective Healthcare Program's Scientific Resource Center (SRC) newsletter.

Research featured by The British Dupuytren's Society.
www.dupuytrens-society.org.uk/ongoing-research/

Top Oral Presentation. Novel Therapies: Biological Repair and Tissue Regeneration.
Canadian Bone and Joint Conference 2016. Value: $200.

Supervisors/Mentors

Dr. Cindy Hutnik, MD, PhD, Ivey Eye Institute, Departments of Ophthalmology and Pathology, Schulich School of Medicine

Dr. David O'Gorman, PhD, Roth|MacFarlane Hand and Upper Limb Center, Departments of Surgery and Biochemistry, Schulich School of Medicine

Dr. Blayne Welk, MD, MSc, Departments of Surgery and Epidemiology & Biostatistics, Schulich School of Medicine

Dr. Jim Lewis, MD, PhD, Director of Clinical Research Training, Schulich School of Medicine

Other Research Experience

Judge Pathology & Toxicology 4980E – Judge for honors thesis poster presentations, Schulich School of Medicine. March 27, 2017.

Reviewer *Osteoporosis International (3), Toxicology in Vitro (2), International Journal of Molecular Sciences (1), Canadian Journal of Ophthalmology (1), BMJ Open (1),*

Lab Manager *Import/export/customs for human, animal and cell culture derived specimens: infectious and non-infectious.*

Business / Industry Involvement

2018/19 **Appili Therapeutics**. Partnership encompassing mentorship and guidance to attain venture capital funding and build drug development pipeline for downstream applications of patented intervention.

Aequus Pharma. Consultation and development relating to novel ophthalmic applications for repurposed drugs. Work done on anti-fibrotic/anti-inflammatory surgical adjuvants. Preparation of pre-clinical development plan for a chitosan based thermosensitive injectable gel, loaded with an anti-inflammatory/anti-fibrotic agent, as an adjuvant for glaucoma filtration surgery.

2017 **Alcon Canada**. Expert opinion and intellectual contribution to wound healing as it relates to the Cypass glaucoma stent.

2016 **Allergan Canada**. Expert opinion and intellectual contribution to wound healing instructional presentation relating to the XEN glaucoma stent.

Medical Electives

2016 **Ophthalmology** (300hrs research)
Dr. Cindy Hutnik, St. Joseph's Hospital, London, Ontario
Meds 5010 Non-Credit Pre-Clerkship Summer Research Elective

2015 **Plastic Surgery** (100hrs Surgery/Clinic)
Dr. Brian Evans, University Hospital, London, Ontario
Meds 5010 Non-Credit Pre-Clerkship Summer Clinical Elective

Medical Observerships

2016 **Ophthalmology** (8hrs)
Dr. Devesh Varma, Prism Eye Institute, Credit Valley Hospital, Mississauga, Ontario
Ophthalmology (32hrs)
Dr. David Tingey, St. Joseph's Hospital, London, Ontario
Plastic Surgery (12hrs)
Dr. Brian Evans, University Hospital, London, Ontario
Oral and Maxillofacial Surgery (12hrs)
Dr. Joe Armstrong, University Hospital, London, Ontario

2015 **Plastic Surgery** (8hrs)
Dr. Bing Gan, St. Joseph's Health Care, London, Ontario
Plastic Surgery (32hrs)
Dr. Damir Matic, Victoria Hospital, London, Ontario
Neurosurgery (9hrs)
Dr. Stephen Lownie, University Hospital, London, Ontario
Oral and Maxillofacial Surgery (8hrs)
Dr. Joe Armstrong, University Hospital, London, Ontario
Emergency Medicine (12hrs)
Dr. Denis Atoe, Blenheim, Ontario
Family Medicine (8hrs)
Dr. Martha Clendenning, Blenheim, Ontario

2014 **Orthopedic Surgery** (35hrs)
Dr. Robert Litchfield, University Hospital, London, Ontario
Emergency Medicine (6hrs)
Dr. Behzad Hassani, University Hospital, London, Ontario
Radiology (6hrs)
Dr. Mark Landis, Victoria Hospital, London, Ontario

Employment Experience

2016-Present **Graduate Research Assistant.** Deps. of Ophthalmology and Pathology, Schulich School of Medicine
Private Tutor. Grade 11/12 physics.

2015-2016 **Security.** Belfort Nightclub.

2013-2014 **Personal Trainer**. Goodlife Fitness. #1 performing level 2 trainer – city wide, London, On

2013-2016 **Security.** Gatsby Soundhouse and Bar. Broke up 5 physical altercations without the use of force.

2011-2014 **Exam Proctor.** London Public Library.

Leadership and Community Involvement

2017-2018 **Polymerase Spiral Reaction Technology Group**, founding member. Coordinate weekly meetings for all Lawson affiliated labs currently working on diagnostic applications for novel PSR technology.

SMART committee, founding member. MD/PhD students mentoring MD students who wish to undertake research endeavors

MD Curriculum Development - Research Integration Experience: Pilot Project. Helped develop a pilot project to integrate MD students into health research labs, fostering inter-professional cooperation and the early development of critical research skills in medical students. Medical school-wide implementation schedule for 2019.

2015-2016 **Oral Symposium Chair,** Canadian National Medical Student Research Symposium.

Schulich Summer Research Training Program Committee, Student Representative.

Mentor, Altitude: Healthcare Mentorship.

Surgery Interest Group Executive, Schulich School of Medicine.

Inter-professional Summer Surgery School, Chair of Organizing Committee, Schulich School of Medicine.

2003-2014 **Emergency Department Volunteer**, London Health Sciences Center, University Hospital.

Athlete, London Aquatic Club.

Reading Tutor, London Public Library.

Team Captain (twice elected), Western Mustangs Swimming Team.

2008-2013 **Athlete**, Western Mustangs Swimming.

2011-2012 **Athletes Representative**, Swim Ontario Fundraising Committee.

Team Canada Member (Swimming), Pan American Games, Guadalajara, Mexico.

Team Canada Member (Swimming), Nations Cup, Montreal, Quebec.

2002-2015 **London Aquatic Club**, Athlete

Other Awards

Academic

2011-2013 **CIS Academic All Canadian**
Qualify for CIS championships and achieve an average greater than 80%.

2011-2013	**Deans Honor List**
	Obtain an average greater than 80%.
2008	**Western University Scholarship of Excellence**
	Given to students entering university with an average of 90% or more.
Athletic	
2013	**Western Mustangs Purple Blanket Award**
	Selected by Committee: Western athlete with greatest athletic and academic achievement.
2013	**OUA Graduating Athlete Award of Distinction**
	Selected by Committee.
2010-2013	**OUA All-Star (5 time)**
	Given to athletes who receive silver or better in one or more events.
2009-2013	**OUA Medalist (18 times) and CIS Medalist (3 times)**
	Western Mustangs Swimming Team.
2012	**Canada Games Aquatic Center Hall of Fame Inductee**
	Chosen by Committee.
2011	**Western Mustangs Bronze W Award**
	Given to athletes who qualify for their sports' OUA team three years in a row.
2009	**Western Mustangs Rookie of the Year**
	Top ranked swimmer in first year of varsity eligibility.
Volunteer	
2014	**Ontario Volunteer Service Award**
	Nomination then Chosen by Committee.
2011	**Volunteer of Distinction**, London Public Library.
	Chosen by Committee.

Extracurricular Activities

Memberships	CMA, since Aug 2014 OMA, since Aug 2014 Canadian Connective Tissue Society, since June 2016 Clinician Investigator Trainee Association of Canada, since June 2016 Canadian Society for Clinical Investigation, since June 2016
Sports	Ballroom Dancing – Fred Astaire Studios, Intermural Softball – Schulich Medicine Team, Intermural Inner Tube Water Polo – Schulich Medicine Team, Schulich Inter-Class Hockey League, Schulich Meds Golf League, Schulich Weightlifting Club
Clubs	Medical Skills Club, Microsurgery Interest Club, Neurology and Neurosurgery Interest Group, Oncology Interest Group, Ophthalmology Interest Group, Point and Shoot Billiards Club, Point of Care Ultrasound Interest Group, Radiology Interest Group, Sports and Rehabilitative Medicine Interest Group, Surgery Interest Group, Surgically Oriented Anatomy Prosectors (SOAP), Western Emergency Medicine Interest Group

Academic Affiliations

2014-2021	Student, Department of Undergraduate Medical Education, Schulich School of Medicine
2016-2019	Student, Department of Pathology and Laboratory Medicine, Schulich School of Medicine
2016-2019	Student, Department of Ophthalmology, Schulich School of Medicine
2016-2019	Student, Ivey Eye Institute, St. Joseph's Healthcare, London, On
2016-2019	Student, Institute for Clinical and Evaluative Sciences (ICES Western), LHSC, London, On