IL-6 and PRG4 represent potential novel tissue biomarkers in conjunctival fibrosis post glaucoma surgery

Cynthia Yu-Wai-Man, PhD FRCOphth1*; Aristides D. Tagalakis, PhD2; Jinhong Meng, PhD2; Yann Bouremel, PhD1,4; Richard M.H. Lee, MSc FRCOphth1; Alex Virasami3, Stephen L. Hart, PhD2; Peng T. Khaw, PhD FMedSci1

1. National Institute for Health Research (NIHR) Biomedical Research Centre at Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology, London, United Kingdom

2. Experimental and Personalised Medicine Section, UCL Great Ormond Street Institute of Child Health, London, United Kingdom

3. Department of Histopathology, Great Ormond Street Hospital for Children NHS Foundation Trust, London, United Kingdom

4. UCL Department of Mechanical Engineering, London, United Kingdom

Word Count
Abstract = 330, Main text = 3000
Number of figures = 5, Number of supplementary tables = 3
Corresponding Author

Cynthia Yu-Wai-Man
UCL Institute of Ophthalmology
11-43 Bath Street
London EC1V 9EL, UK
Tel: +44 20 7608 6887; Fax: +44 20 7608 6887
E-mail: c.yu-wai-man@ucl.ac.uk
Key Points

**Question:** Are IL-6 and PRG4 potential novel tissue biomarkers of conjunctival fibrosis post glaucoma surgery?

**Findings:** In this cross-sectional study, IL-6 and PRG4 expression were upregulated and downregulated, respectively, in fibrotic conjunctiva and correlated strongly with the number of glaucoma surgeries and logMAR visual acuity.

**Meaning:** These results support IL-6 and PRG4 as potential novel tissue biomarkers of disease severity and prognosis in conjunctival fibrosis post glaucoma surgery. Longitudinal studies are needed to validate these potential biomarkers of fibrosis in the future.
Abstract

Importance: Post-surgical fibrosis is the critical determinant of the long-term surgical success in glaucoma surgery but there are currently no reliable biomarkers to stratify the risk of scarring.

Objective: To compare the clinical phenotype of patients with conjunctival fibrosis post glaucoma surgery with candidate gene expression tissue biomarkers of fibrosis.

Design, Setting, and Participants: In this cross-sectional study, 42 patients were recruited at the time of glaucoma surgery from September 2014 to September 2016. The participants were divided into fibrotic participants (n = 28, 43.8 ± 3.6 years, 57% females, 79% Caucasians) and non-fibrotic participants (n = 14, 47.7 ± 6.9 years, 29% females, 64% Caucasians).

Main outcomes and measures: Genotype-phenotype correlations of the IL-6 or PRG4 gene and detailed clinical phenotype. The IL-6 and PRG4 protein expression in conjunctival tissues was also assessed using in situ immunohistochemistry.

Results: The fibrotic participants had marked bleb scarring and vascularization and worse logMAR visual acuity. The central bleb area, bleb height, and bleb vascularity were 1.4 ± 0.1, 1.4 ± 0.1 and 3.4 ± 0.2, respectively. The IL-6 gene was upregulated in fibrotic cell lines (0.040) compared to non-fibrotic cell lines (0.011), difference = 0.029, 95% CI: 0.015-0.043, p = .003. The PRG4 gene was also downregulated in fibrotic cell lines (0.002) compared to non-fibrotic cell lines (0.109), difference =
0.107, 95% CI: 0.104-0.110, \( p = .033 \). We found a strong correlation between the \( IL-6 \) gene and the number of glaucoma surgeries (\( r = .94, p < .001 \)) and logMAR visual acuity (\( r = .64, p = .026 \)). There was also a moderate correlation between the \( PRG4 \) gene and the number of glaucoma surgeries (\( r = -.72, p = .005 \)) and logMAR visual acuity (\( r = -.62, p = .026 \)).

**Conclusions and relevance:** IL-6 and PRG4 represent potential novel tissue biomarkers of disease severity and prognosis in conjunctival fibrosis post glaucoma surgery. Future longitudinal studies with multiple post-operative measures are needed to validate the predictive value of these potential biomarkers of fibrosis.
**Introduction**

Fetal skin wound healing is scarless and fundamentally different from adult wound healing (1, 2). Scarless fetal wound healing is characterized by little inflammation, minimal fibroblast proliferation and decreased levels of potent inflammatory cytokines, such as IL-6 (3). IL-6 controls the effector characteristics of various T cell subsets, including Th17 cells, Th22 cells and certain IL-10 secreting subsets (4). IL-6 also plays an important role in pulmonary fibrosis (5), peritoneal fibrosis (6), renal interstitial fibrosis (7), and cancer-associated fibroblasts (8).

Scarless fetal wound healing is also associated with high levels of hyaluronic acid (9), which increases the expression of proteoglycan 4 (PRG4) (10). PRG4, also known as lubricin, is a lubricating mucin-like glycoprotein that has been detected at the ocular surface (11). PRG4 is downregulated in palmar fascia fibroblasts from patients with Dupuytren’s contracture (12). Moreover, increasing PRG4 expression using recombinant human PRG4 (rhPRG4) treatment decreases alpha smooth muscle actin expression in lens epithelial cells activated with TGFβ2 and may prevent the full myofibroblast phenotype in posterior capsular opacification (13).

Detailed clinical phenotyping and effective biobanking of large patient cohorts will be critical to study putative biomarkers of disease severity and prognosis in ocular fibrosis (14). Afro-Caribbean patients have a strong genetic predisposition to ocular and keloid scarring (15). Dupuytren’s contracture is also a familial disorder that is highly prevalent in individuals of Northern European descent (16). However, there is a current lack of reliable biomarkers to stratify the risk of scarring in the eye. Being able to predict patients’ risk of scarring and to tailor the antifibrotic treatment regimen to each individual patient will be an extremely useful tool clinically to prevent
undertreating or exposing them to unnecessary treatments with potential side effects.

In this study, correlation of the clinical phenotype of patients with conjunctival fibrosis with altered gene expression revealed IL-6 and PRG4 as potential novel tissue biomarkers of fibrosis.
Methods

Patient recruitment
We recruited glaucoma patients at the time of surgery at the Moorfields Eye Hospital (London, UK) from September 2014 to September 2016. All patients were recruited according to local ethics approval (REC reference 10/H0808/127) and the tenets of the Declaration of Helsinki. All participants gave written informed consent after explanation of the nature and possible consequences of the study. The inclusion criteria were: age (over 18 years) and patients planned to have glaucoma tube surgery. The exclusion criterion was previous conjunctival surgery other than glaucoma surgery.

Clinical examination
The participants were divided into two groups: participants who had previous glaucoma surgery and participants with no previous glaucoma surgery. All study participants underwent a standardized ophthalmic examination prior to surgery. This included best corrected visual acuity, intraocular pressure (IOP), lens status, and cup disc ratio. We also collected detailed patient demographics including age, gender, ethnicity, and type of glaucoma.

Moorfields Bleb Grading
We assessed each study participant with previous glaucoma surgery using the Moorfields bleb grading system (17). Central bleb area, maximal bleb area, and bleb height were graded on a scale of 1 to 5 (1 = 0%, 2 = 25%, 3 = 50%, 4 = 75%, 5 =
100%). Bleb vascularity was graded on a scale of 1 to 5 (1 = avascular, 2 = normal, 3 = mild, 4 = moderate, 5 = severe hyperaemia).

Medical and surgical treatment
We collected the number of anti-glaucoma eye drops, including beta blockers, prostaglandin analogues, carbonic anhydrase inhibitors, alpha agonists, pilocarpine, and the number of patients on oral acetazolamide. We also recorded any previous surgical and laser treatments, including the number of trabeculectomies, glaucoma tube surgeries, and diode laser treatments.

Fibroblast cell lines
We established fibrotic fibroblast (FF) and non-fibrotic fibroblast (NF) primary cell lines from conjunctival tissues collected from study participants with previous glaucoma surgery and study participants with no previous glaucoma surgery, respectively. The conjunctival tissues were mechanically dispersed and the tissue fragments were placed in tissue culture dishes with Dulbecco’s modified Eagle’s medium (DMEM, Invitrogen), 10% fetal calf serum, 100 U/ml penicillin, 100 µg/ml streptomycin, and 2 mM L-glutamine at 37°C with 5% CO₂ (18). Following outgrowth from the explant, the fibroblasts were trypsinized and cultured routinely in the above medium. Fibroblast cell lines in early passages 1-2 were used in the experiments.

Real-Time quantitative PCR
RT-qPCR reactions were performed using a Platinum quantitative PCR master mix (ThermoFisher Scientific, Hemel Hempstead, UK) on a CFX Real-Time PCR detection
system (Bio-Rad, Hemel Hempstead, UK). The Taqman gene expression assays were IL-6 (Hs00985639_m1), PRG4 (Hs00981633_m1), and GAPDH (Hs02758991_g1) (ThermoFisher Scientific, UK). All mRNA values were normalized relative to that of GAPDH and triplicate experiments were performed for each condition.

**Immunohistochemistry**

Fibrotic and non-fibrotic human conjunctival tissues were fixed in formalin for 24 hours. The samples were dehydrated through an alcohol gradient (70%, 95%, 100% Industrial Methylated Spirit IMS), followed by chloroform clearance on a Leica Peloris II tissue processing machine. The samples were embedded in paraffin wax using a Sakura Tissue-Tek TEC embedding machine. Tissue sections were cut on a Thermo Scientific HM340E manual rotary microtome at a section thickness of 3 µm using Feather S35 microtomy blades. The sections were mounted on Leica Xtra Adhesive slides, air dried for 60 minutes, and heated for 60 minutes at 70°C prior to staining. The sections were dewaxed and stained on a Leica Bond-Max automated immunostainer. The sections were incubated with IL-6 (1:400, rabbit polyclonal ab6672, Abcam, UK) or PRG4 (1:200, polyclonal rabbit HPA028523, Atlas, UK) antibody for 30 minutes at room temperature.

**Immunofluorescence**

FF and NF cell lines were seeded onto coverslips at 1 x 10^5 cells/well in 6-well plates. The cells were fixed with 4% paraformaldehyde for 15 minutes and incubated with blocking solution (PBS containing 10% normal goat serum/0.03% Triton X100) for 30 minutes. The cells were then incubated with primary antibodies against IL-6 (1:100,
ab6672, Abcam, UK) or PRG4 (1:100, HPA028523, Atlas, UK) for 2 hours, followed by Alexa 488-conjugated goat anti-rabbit IgG (H+L) (1:500, Thermo Fisher Scientific, UK) for 1 hour. The coverslips were then rinsed with PBS and mounted onto slides with mounting medium (DAKO, UK) containing 10 μg/ml 4',6-diamidino-2-phenylindole (DAPI). Three randomly chosen fields per sample were imaged at 20x magnification using a Leica DM4000B microscope. Stained areas of IL-6 and PRG4 were calculated using a bespoke image processing algorithm written in MATLAB. Each image was first converted into a greyscale image ranging from a level of 0 for black to 255 for white. The maximum background greyscale level of each image was then identified and any greyscale value above it was considered as staining.

**Statistical analysis**

All graphs display mean and standard error of the mean (SEM). Statistical analysis was performed using the Student’s t-test to calculate differences in individual p values. We performed genotype-phenotype comparisons using Spearman’s correlation of the IL-6 or PRG4 gene versus clinical parameters, such as bleb area, bleb height, bleb vascularity, number of glaucoma surgeries, age, intraocular pressure, logMAR visual acuity, cup disc ratio, and number of anti-glaucoma eye drops.
Results

Patient demographics
A total of 42 patients were recruited during the study period: 28 participants (67%) had previous glaucoma surgery (fibrotic group) and 14 participants (33%) had no previous glaucoma surgery (non-fibrotic group). Most study participants were white Caucasians: 22 white Caucasians (79%), 4 Asians (14%), and 2 Afro-Caribbeans (7%) in the fibrotic group; 9 white Caucasians (64%), 4 Asians (29%), and 1 Afro-Caribbean (7%) in the non-fibrotic group (see eTable 1 in the Supplement). There were no differences judged to be relevant in age with a mean age of 43.8 ± 3.6 years in the fibrotic participants and 47.7 ± 6.9 years in the non-fibrotic participants (p = .578). There were 43% males and 57% females in the fibrotic group and 71% males and 29% females in the non-fibrotic group.

Clinical phenotype
All participants in the previously operated group had marked bleb scarring and vascularization (Figure 1A). Using the Moorfields Bleb Grading system, the central bleb area, maximal bleb area, bleb height, and bleb vascularity were 1.4 ± 0.1, 1.4 ± 0.1, 1.4 ± 0.1 and 3.4 ± 0.2, respectively (Figure 1B). The fibrotic participants had multiple glaucoma surgeries with a mean of 1.5 (range = 1 to 3). Among the fibrotic group, there were 13 participants (47%) who had trabeculectomy surgery, 4 participants (14%) who had glaucoma tube surgery, and 11 participants (39%) who had multiple glaucoma surgeries (see eTable 1 in the Supplement). The study participants in the fibrotic group had worse best-corrected visual acuity with a mean logMAR vision of 0.58 compared to 0.31 in the non-fibrotic group, difference
= 0.27, 95% CI: 0.11-0.42, \( p = .037 \) (Figure 2A). The fibrotic and non-fibrotic participants had a mean pre-operative IOP of 22.3 and 27.1 mmHg, respectively, difference = 4.8, 95% CI: 1.8-9.1, \( p = .133 \) (Figure 2B). The majority of participants had advanced disc cupping with a cup disc ratio of 0.87 and 0.84 in the fibrotic and non-fibrotic groups, respectively, difference = 0.03, 95% CI: -0.01-0.07, \( p = .350 \) (Figure 2C). Most study participants were also on multiple anti-glaucoma eye drops with a mean of 3.6 in the fibrotic group and 3.8 in the non-fibrotic group, difference = 0.2, 95% CI: -0.2-0.7, \( p = .550 \) (Figure 2D). A high percentage of fibrotic and non-fibrotic participants was on topical beta blockers, prostaglandin analogues, carbonic anhydrase inhibitors, alpha agonists and oral acetazolamide (Figure 2E).

**Genotype-phenotype correlations of the IL-6 and PRG4 genes in fibrotic and non-fibrotic conjunctival fibroblasts**

We next established fibrotic fibroblast (FF3, FF13, FF14, FF15, FF16, FF17) and non-fibrotic fibroblast (NF1, NF4, NF7, NF8) primary cell lines from conjunctival tissues collected from ten patients in the study (see eTable 2 in the Supplement). We performed real-time quantitative PCR to compare the IL-6 and PRG4 genes in FF and NF cell lines. The IL-6 gene was upregulated in FF cell lines (0.040) compared to NF cell lines (0.011), difference = 0.029, 95% CI: 0.015-0.043, \( p = .003 \). FF14 and FF16 had the highest IL-6 gene expression while NF4 and NF7 had the lowest IL-6 gene expression (Figure 3A). The PRG4 gene was also downregulated in FF cell lines (0.002) compared to NF cell lines (0.109), difference = 0.107, 95% CI: 0.104-0.110, \( p = .033 \). NF1 and NF4 had the highest PRG4 gene expression while FF13 and FF15 had the lowest PRG4 gene expression (Figure 3B).
We also performed Spearman’s correlation between the IL-6 or PRG4 gene and the detailed clinical phenotype of patients. The Spearman’s correlation coefficient r ranges in value from -1 to +1. The larger the absolute value of the coefficient, the stronger the correlation between the variables. A positive r indicates a positive relationship between the two variables whereas a negative r indicates a negative relationship. We found a strong correlation between the IL-6 gene and the number of glaucoma surgeries (r = .94, p < .001) (Figure 3C) and logMAR visual acuity (r = .64, p = .026) (Figure 3D). There was also a moderate correlation between the PRG4 gene and the number of glaucoma surgeries (r = -.72, p = .005) (Figure 3E) and logMAR visual acuity (r = -.62, p = .026) (Figure 3F). However, we found no correlations between the IL-6 or PRG4 gene and the central bleb area, maximal bleb area, bleb height, and bleb vascularity (see eTable 3 in the Supplement). There were also no correlations between the IL-6 or PRG4 gene and the patient age, IOP, cup disc ratio, and number of anti-glaucoma eye drops (see eTable 3 in the Supplement).

**Fibrotic conjunctival tissues express increased IL-6 and decreased PRG4 proteins**

We further stained fibrotic and non-fibrotic conjunctival tissues from glaucoma study participants for IL-6 and PRG4 protein expression. Fibrotic conjunctival tissues post glaucoma surgery undergo marked histopathological changes compared to non-fibrotic conjunctival tissues. Similar to the gene expression patterns, fibrotic conjunctival tissues expressed increased IL-6 and decreased PRG4 protein staining compared to non-fibrotic conjunctival tissues (Figures 4A and 4B).

In addition, we stained the FF and NF cell lines to compare the distribution of the IL-6 and PRG4 proteins in fibrotic and non-fibrotic conjunctival fibroblasts. FF cell lines
had a higher proliferative rate than NF cell lines but there were overall no differences in cell morphology between the two groups. There was a mixture of nuclear and cytoplasmic IL-6 and PRG4 staining pattern in both FF and NF cell lines (Figures 5A and 5B). FF cell lines showed increased IL-6 staining compared to NF cell lines. FF14 and FF16 had the highest IL-6 staining while NF1 and NF4 had the lowest IL-6 staining (Figure 5C). We, however, did not find any differences in PRG4 staining between FF and NF cell lines (Figure 5D). This could potentially be due to the sensitivity and specificity of the PRG4 antibody used in the study.
Discussion

Deep clinical phenotyping and the use of specific tissue biomarkers represent key aspects of personalized medicine in ocular fibrosis (14). In a previous study, we have shown that there is a distinct fibrosis gene signature in the conjunctiva after glaucoma surgery and the RNA signature revealed an upregulation and downregulation of the \textit{IL-6} and \textit{PRG4} genes, respectively, in fibrotic human conjunctival fibroblasts (19). Using detailed genotype-phenotype comparisons, we show here that there is a strong correlation between the \textit{IL-6} or \textit{PRG4} gene with the number of glaucoma surgeries and logMAR visual acuity. Fibrotic conjunctival tissues post glaucoma surgery also express increased IL-6 and decreased PRG4 protein staining compared to non-fibrotic conjunctival tissues.

Our results are consistent with important mechanistic pathways in conjunctival fibrosis, namely extracellular matrix remodelling and the inflammatory response. The extracellular matrix component PRG4 is a serum response factor (SRF) target gene (20). SRF is a master regulator of cytoskeletal gene expression (20, 21) and the Myocardin-related transcription factor/ Serum response factor (MRTF/SRF) pathway has been linked to ocular (22-24), vascular (25), skin (26), and lung fibrosis (27). PRG4 also binds to Toll-like receptors (TLRs) and plays an important anti-inflammatory role in downstream signalling pathways, such as IL-6 (28-30). Multiple TLRs can initiate an IL-6 transcriptional response (31). IL-6 is a potent inflammatory cytokine and signal transduction involves the activation of JAK (Janus kinase) tyrosine kinase family members, leading to the activation of transcription factors of the STAT (signal transducers and activators of transcription) family (32). Another major
signalling pathway for IL-6 is the Ras-MEK (MAPK/ERK kinase)-MAPK (mitogen-activated protein kinase) cascade (32).

Inflammation is a major risk factor for scarring after trabeculectomy surgery and increased inflammatory cells in the conjunctival tissues of patients with previous glaucoma surgery is associated with an increased risk of conjunctival scarring (33, 34). Afro-Caribbean patients also represent a high-risk group for conjunctival scarring and the increased number of conjunctival macrophages may partially explain the tendency for a lower success rate of filtration surgery in this group of patients (35). In addition, long-term treatment with multiple anti-glaucoma eye drops has been identified as a significant risk factor for failure in trabeculectomy as it causes subclinical conjunctival inflammation with increased macrophages and lymphocytes in the conjunctival epithelium (36, 37).

We report here an upregulation of the IL-6 gene in fibrotic human conjunctival fibroblasts after glaucoma filtration surgery. Higher IL-6 levels also correlated strongly with multiple failed glaucoma surgeries and worse visual acuities in glaucoma patients. Trachoma is another conjunctival scarring disease and IL-6 is overexpressed in scarring trachoma fibroblasts (38). Moreover, IL-6 is an important immune mediator and a genome-wide association study further suggests that the genetic associations with trachoma scarring might be focussed on processes relating to the immune system (39). Similarly, increased IL-6 has been found in dermal interstitial blister fluid from systemic sclerosis patients (40). High IL-6 expression in early diffuse cutaneous systemic sclerosis patients is also associated with more severe skin involvement at three years and worse long-term survival than in those without elevated IL-6 levels (41).
In this study, we also found a downregulation of the PRG4 gene in fibrotic human conjunctival fibroblasts after glaucoma filtration surgery. Lower PRG4 levels correlated moderately with multiple failed glaucoma surgeries and worse visual acuities in patients. PRG4 is an important biological modifier that regulates processes such as tissue development, homeostasis, inflammation, innate immune response, and wound healing (42, 43). Chronic obstructive pulmonary disease (COPD) is characterized by a progressive loss of lung function that is caused by repeated pulmonary inflammation (44). Other studies have shown that serum PRG4 correlated strongly with the one-year change in predicted lung forced vital capacity and is a diagnostic biomarker in COPD patients (45).

New biomarkers of fibrosis will provide sensitive and reproducible means of targeting and personalizing therapy, as well as assessing disease response in the future. The drug pirfenidone decreased IL-6 levels (46) and reduced disease progression in patients with idiopathic pulmonary fibrosis (47). Tocilizumab, a monoclonal antibody against IL-6, did not show a significant reduction in skin thickening but there was evidence of reduced decline in lung forced vital capacity in a Phase 2 clinical trial in systemic sclerosis patients (48).

**Limitations**

As this is a cross-sectional study, it is not possible to determine if the observed differences in IL-6 and PRG4 expression in fibrotic and non-fibrotic cell lines precede fibrosis, rather than being the consequence of developing fibrosis. A future longitudinal study of first-time surgery patients with multiple post-operative measures of tissue biomarkers will be necessary to validate the predictive relationship desired for
clinically useful biomarkers. Another limitation is the relatively small sample size and we are setting up a fibrosis biobank of tissues to validate our results in larger longitudinal studies in the future.

**Conclusions**

In this cross-sectional study, we have shown that IL-6 and PRG4 are upregulated and downregulated, respectively, in fibrotic human conjunctiva post glaucoma filtration surgery, and correlated strongly with the number of glaucoma surgeries and logMAR visual acuity. Confirmation from future longitudinal studies are needed to have greater confidence that IL-6 and PRG4 are potential novel tissue biomarkers of disease severity and prognosis in conjunctival fibrosis after glaucoma surgery.
Author Contributions: Dr Yu-Wai-Man had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Yu-Wai-Man.

Acquisition, analysis, and/or interpretation of data: Yu-Wai-Man, Tagalakis, Meng, Bouremel, Lee, Virasami.

Drafting of the manuscript: Yu-Wai-Man.


Critical revision of the manuscript for important intellectual content: All authors.


Obtained funding: Yu-Wai-Man, Khaw.

Administrative, technical, or material support: Yu-Wai-Man, Tagalakis, Meng

Study supervision: Yu-Wai-Man, Khaw, Hart.

Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none were reported.

Funding/Support: This research was supported by the National Institute for Health Research (NIHR) Biomedical Research Centre at Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology, the Medical Research Council, and Moorfields Eye Charity.

Role of the Funder/Sponsor: The funding sources had no role in the design or conduct of the study; collection, management, analysis, and interpretation of the data;
preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.
**Figure legends**

**Figure 1.** All patients in the previously operated group had marked bleb scarring and vascularization.

A, Representative scarred and vascularized blebs after glaucoma filtration surgery. B, Central bleb area, maximal bleb area, and bleb height were graded on a scale of 1 to 5 (1 = 0%, 2 = 25%, 3 = 50%, 4 = 75%, 5 = 100%). Bleb vascularity was graded on a scale of 1 to 5 (1 = avascular, 2 = normal, 3 = mild, 4 = moderate, 5 = severe hyperaemia). Results show mean ± SEM.

**Figure 2.** Clinical phenotype of fibrotic (n = 28) and non-fibrotic (n = 14) patients.


**Figure 3.** Genotype-phenotype correlations of *IL-6* and *PRG4* genes in fibrotic and non-fibrotic conjunctival fibroblasts.

A, *IL-6* and B, *PRG4* genes were measured by real-time quantitative PCR. All mRNA values were normalized relative to that of GAPDH and triplicate experiments were performed for each condition. Results show mean ± SEM. Spearman’s correlation r between C, *IL-6* gene and number of glaucoma surgeries. D, *IL-6* gene and logMAR visual acuity. E, *PRG4* gene and number of glaucoma surgeries. F, *PRG4* gene and logMAR visual acuity. The values in more than one cell line were the same for *PRG4* gene expression.
Figure 4. Fibrotic conjunctival tissues express increased IL-6 and decreased PRG4 proteins compared to non-fibrotic conjunctival tissues.

Sections of fibrotic and non-fibrotic conjunctiva tissues were stained for A, IL-6 and B, PRG4 proteins. Black arrows indicate areas of high IL-6 and PRG4 staining. Scale bar = 50 μm.

Figure 5. Fibrotic fibroblast cell lines express increased IL-6 protein compared to non-fibrotic cell lines.

FFs and NFs were seeded onto coverslips and stained for A, IL-6 and B, PRG4. Scale bar = 30 μm. Fluorescent intensity of C, IL-6 and D, PRG4 staining were calculated using a bespoke image processing algorithm written in MATLAB. Three randomly chosen fields were imaged per condition. Results show mean ± SEM.
References


