Aqueous Cytokine Levels are Associated with Progression of Peripheral Anterior Synechiae after Descemet Stripping Automated Endothelial Keratoplasty

Yuki Kusano
Tokyo Dental College Ichikawa General Hospital
Takefumi Yamaguchi (yamaguchit@tdc.ac.jp)
Tokyo Dental College Ichikawa General Hospital
Sota Nishisako
Tokyo Dental College Ichikawa General Hospital
Takehiro Matsumura
Tokyo Dental College Ichikawa General Hospital
Masaki Fukui
Tokyo Dental College Ichikawa General Hospital
Kazunari Higa
Tokyo Dental College Ichikawa General Hospital
Toshihiro Inoue
Kumamoto University
Jun Shimazaki
Tokyo Dental College Ichikawa General Hospital

Research Article

Keywords: Glaucoma, Peripheral anterior synechiae (PAS), aqueous humor (AqH), Descemet stripping automated endothelial keratoplasty (DSAEK)

Posted Date: April 22nd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-436836/v1

Abstract

Glaucoma is a serious complication after corneal transplantation that can lead to permanent loss of vision. Peripheral anterior synechiae (PAS) leads to severe glaucoma, which is refractory to medications, however, the exact mechanism of PAS development after corneal transplantation is still elusive. Recent studies revealed significant alterations in cytokine levels in aqueous humor (AqH) in glaucoma. Hence, we quantified PAS area using three dimensional OCT and evaluated the association between cytokine levels in AqH and PAS area after Descemet stripping automated endothelial keratoplasty (DSAEK). We first found that the total protein level in AqH was significantly higher in eyes with PAS progression than those without, and was positively correlated with the progression of PAS area after DSAEK. Furthermore, the postoperative PAS area was significantly correlated with the AqH levels of interleukin-8, interferon-γ, and soluble intercellular adhesion molecule-1. Multivariate analyses showed that the total protein levels in AqH and presence of preoperative PAS were significant risk factors for increased PAS area after DSAEK. The PAS development was associated with intraocular pressure increase after DSAEK. In conclusion, these findings suggest that pathological alterations in the AqH can cause PAS progression and glaucoma after DSAEK.

Introduction

Glaucoma is a serious complication after corneal transplantation that can lead to permanent loss of vision. The incidence of glaucoma varies among surgical procedures; for example, it has been calculated to be 9–35% after penetrating keratoplasty (PK)[1–4], 0–4.5% after anterior lamellar keratoplasty (ALK)[5–7] and 2–14% after endothelial keratoplasty (EK)[8–12]. The trends of postoperative transient intraocular pressure (IOP) increase also vary among these procedures, that is, 29–80% after PK, 17–36% after ALK, and 31–60% after EK[1–12]. Various mechanisms are involved in elevated IOP, including intraoperative viscoelastic material, pupillary block due to air tamponade, response to topical steroids, damage to outflow mechanisms, and angle-closure due to peripheral anterior synechiae (PAS)[1,9]. PAS is known to cause refractory glaucoma after corneal transplantation[13–15], iridocorneal endothelial (ICE) syndrome[16,17], and uveitis[18]. However, the exact mechanism of PAS development remains poorly understood.

Recent advances in anterior segment optical coherence tomography (AS-OCT) have enabled non-invasive and accurate quantification of PAS[13–15, 19,20]. Recently, we have shown that alterations in the aqueous humor (AqH) microenvironment, such as elevated total protein/cytokine levels, significantly influence the long-term prognosis of corneal endothelial cells after PK and Descemet stripping automated endothelial keratoplasty (DSAEK)[21–23]. Through these AqH investigations, we noticed that PAS developed after corneal transplantation in eyes with high AqH total protein/cytokine levels. Thus, we hypothesized that high AqH total protein/cytokine levels are associated with PAS formation after DSAEK, leading to an increase in IOP and refractory glaucoma. In the current study, we first measured AqH protein/cytokine levels before DSAEK and analysed PAS alterations after DSAEK using AS-OCT. Second, we evaluated the

correlations between AqH total protein/cytokine levels and PAS alterations. Third, we evaluated the association between PAS and the incidence of IOP increase after DSAEK.

Results

Clinical Results

A total of 146 consecutive patients who underwent DSAEK at Tokyo Dental College Ichikawa General Hospital between November 2015 and March 2019 were included in this study (Table 1). All the DSAEK surgeries were successful. The logarithm of minimal angle resolution (logMAR) significantly improved from 1.30 ± 0.71 preoperatively to 0.45 ± 0.40 at 3 months, 0.38 ± 0.39 at 6 months, and 0.36 ± 0.46 at 12 months (all, $P < 0.0001$). Corneal endothelial cell density (cells/mm^2) of the graft decreased from 2746 ± 249 to 1566 ± 592 at 3 months, 1433 ± 556 at 6 months, and 1253 ± 600 at 12 months (all, $P < 0.0001$). Postoperative complications occurred as follows: pupillary block due to air tamponade in three eyes (2%) and graft detachment requiring air injection in five eyes (4%), all of which were treated appropriately.

Table 1
Demographics of patients

Eyes (n)	**146**
Sex, n (%)	
Male	61(42%)
Female	85 (58%)
Age (years)	72.9 ± 10.5
Range	(27–95)
Axial length (mm)	23.4 ± 1.80
Number of previous intraocular surgeries	1.7 ± 1.5
Mean ± SD	
SD: standard deviation, BSCVA: best spectacle-corrected visual acuity, logMAR: logarithm of minimal angle resolution, IOP: intraocular pressure, CCT: central corneal thickness, NA: not available	
*Chi-squared test	

Time course alteration of PAS Indices

PAS developed in 10 eyes (6.8%) without preoperative PAS and progressed in 22 eyes (15.1%, Fig. 1a-d) with preoperative PAS and did not progress in 15 eyes (10.3%) with preoperative PAS. Table 2 shows the time-course alterations of the PAS indices in 146 eyes. The PAS %degree increased from 17.8 ± 32.1% preoperatively to 18.8 ± 31.2% at 3 months, 20.2 ± 34.1% at 6 months, and 22.6 ± 33 .6% at 12 months

after DSAEK (P = 0.89, 0.45, and 0.33, respectively). PAS maximum height increased from 0.46 ± 0.90 mm preoperatively to 0.51 ± 0.92 mm at 3 months, 0.53 ± 1.00 mm at 6 months, and 0.58 ± 1.05 mm at 12 months after DSAEK (P = 0.11, 0.12, and 0.17, respectively). PAS area increased from 2.00 ± 4.42 mm^2 preoperatively to 2.10 ± 4.86 mm^2 at 3 months, 2.71 ± 6.47 mm^2 at 6 months, and 3.00 ± 6.85 mm^2 at 12 months after DSAEK (P = 0.13, 0.16, and 0.06 respectively). Compared to the preoperative value, the PAS %degree increased by 1.96 ± 19.0 % at 3 months, 3.93 ± 18.9 % at 6 months, and 4.21 ± 19.0 % at 12 months postoperatively (Table 3). Maximum height increased by 0.07 ± 0.60 mm at 3 months, 0.11 ± 0.61 mm at 6 months, and 0.16 ± 0.60 mm at 12 months postoperatively. PAS area increased by 0.14 ± 4.27 mm^2 at 3 months, 0.89 ± 4.88 mm^2 at 6 months, and 0.98 ± 5.43 mm^2 at 12 months after DSAEK.

Table 2
Time course alteration in PAS indices

PAS	Preop	3 months	6 months	12 months
%Degree	17.8 ± 32.1	18.8 ± 31.2	20.2 ± 34.1	22.6 ± 33.6
P value*		0.89	0.45	0.33
Maximal height (mm)	0.46 ± 0.90	0.51 ± 0.92	0.53 ± 1.00	0.58 ± 1.05
P value*		0.11	0.12	0.17
Area (mm^2)	2.00 ± 4.42	2.10 ± 4.86	2.71 ± 6.47	3.00 ± 6.85
P value*		0.13	0.16	0.17
*P values compared with preoperative values (N = 146 eyes) Mean ± SD				
PAS: peripheral anterior synechiae, SD: standard deviation				

Table 3
Progression of PAS indices

	3 months	6 months	12 months
Δ%Degree	1.96 ± 19.0	3.93 ± 18.9	4.21 ± 19.0
ΔMaximal height (mm)	0.07 ± 0.60	0.11 ± 0.61	0.16 ± 0.60
ΔArea (mm^2)	0.14 ± 4.27	0.89 ± 4.88	0.98 ± 5.43
Mean ± SD			
PAS: peripheral anterior synechiae, SD: standard deviation			
Δ%Degree = (Postoperative PAS degree) - (Preoperative PAS degree)			
ΔMaximal height = (Postoperative PAS maximal height) - (Preoperative PAS maximal height)			
ΔArea = (Postoperative PAS area) - (Preoperative PAS area)			

Correlation Between Total Protein Level in Aqueous Humor and PAS

The total protein level of the AqH was 1.14 ± 0.77 mg/dL. The mean protein level was significantly higher in 47 eyes with PAS or PAS progression than in those without (1.45 ± 1.03 mg/mL vs. 1.00 ± 0.57 mg/dL, P = 0.04). The total protein levels in the AqH were significantly correlated with the preoperative PAS %degree (Table 4, r = 0.180, P = 0.0258), maximum height (r = 0.246, P = 0.0021), and area (r = 0.155, P = 0.055). Furthermore, the total protein levels in the AqH were significantly correlated with the PAS %degree (r = 0.362, P < 0.0001 at 3 months, r = 0.310, P = 0.0001 at 6 months, and r = 0.356, P = 0.0002 at 12 months), maximum height (r = 0.376, P < 0.0001 at 3 months, r = 0.338, P = 0.0001 at 6 months, and r = 0.403, P < 0.0001 at 12 months), and area (r = 0.375, P < 0.0001 at 3 months, r = 0.338, P = 0.0001 at 6 months, and r = 0.391, P < 0.0001 at 12 months) after DSAEK.

Table 4
Correlations between total protein levels in aqueous humor and PAS indices

PAS indices	Preop		3 months		6 months		12 months	
	r (95% CI)	P value	r (95% CI)	P value	r (95% CI)	P value	r (95% CI)	P value
%Degree	0.180 (0.022 to 0.306)	0.026	0.362 (0.209 to 0.497)	< 0.0001	0.310 (0.170 to 0.486)	0.0001	0.356 (0.176 to 0.514)	0.0002
Maximum height	0.246 (0.091 to 0.390)	0.0021	0.376 (0.225 to 0.509)	< 0.0001	0.338 (0.170 to 0.486)	0.0001	0.403 (0.228 to 0.552)	< 0.0001
Area	0.155 (-0.004 to 0.306)	0.055	0.375 (0.225 to 0.509)	< 0.0001	0.338 (0.170 to 0.486)	0.0001	0.391 (0.215 to 0.543)	< 0.0001
Δ%Degree			0.242 (0.080 to 0.391)	0.0037	0.197 (0.020 to 0.361)	0.029	0.242 (0.052 to 0.415)	0.013
ΔMaximal height			0.163 (-0.002 to 0.319)	0.053	0.197 (0.020 to 0.361)	0.029	0.209 (0.017 to 0.386)	0.033
ΔArea			0.257 (0.097 to 0.405)	0.002	0.311 (0.142 to 0.462)	0.005	0.342 (0.160 to 0.502)	0.0004

*Spearman's correlation analysis, PAS: peripheral anterior synechiae, CI: confidence interval

Total protein levels in the AqH were significantly positively correlated with Δ%degree (r = 0.242, P = 0.0037 at 3 months, r = 0.197, P = 0.029 at 6 months, and r = 0.242, P = 0.0134 at 12 months), Δ maximum height of PAS (r = 0.163, P = 0.053 at 3 months, r = 0.197, P = 0.029 at 6 months, and r = 0.209, P = 0.033 at 12 months), and ΔPAS area (r = 0.257, P = 0.002 at 3 months, r = 0.311, P = 0.005 at 6 months, and r = 0.342, P = 0.0004 at 12 months).

Multivariate Analysis

Although these data indicated an association between total protein levels in the AqH and PAS progression after DSAEK, other clinical factors, such as the presence of preoperative PAS, larger grafts, and shorter axial length (shallow anterior chamber), can also be potential confounding factors. Thus, to identify the risk factors for the progression of PAS, we conducted a multivariate analysis. We selected the ΔPAS area as it represents a three-dimensional alteration of PAS (%degree and maximum height are two-dimensional parameters) and had the greatest standard correlation coefficients in the correlation analyses with the total protein levels in the AqH. Multivariate analyses showed that the total protein levels in the AqH and the presence of preoperative PAS factors were significantly associated with the progression of PAS area (β = 0.193–0.574, all P < 0.02), whereas axial length, graft size, and patient age were not (Table 5).

Table 5
Multivariate analysis for clinical factors associated with progression of PAS area

Δ PAS Area	**3 months**		**6 months**		**12 months**	
	β	**P value**	**β**	**P value**	**β**	**P value**
Preop total protein levels	**0.221**	**0.002**	**0.226**	**0.003**	**0.193**	**0.020**
Preop PAS (0 or 1) *	**0.551**	**0.000**	**0.574**	**0.000**	**0.574**	**0.000**
Axial length (mm)	-0.003	0.967	-0.002	0.976	0.031	0.704
Graft size (mm)	-0.054	0.444	-0.064	0.410	-0.033	0.679
Age	-0.068	0.333	-0.100	0.191	-0.073	0.365
Adjusted R^2	0.443		0.454		0.452	
VIF = 1.11 ~ 1.12						
*Presence of preoperative PAS was dichotomized as categorical variables for multivariate regression analysis as follows: presence of preoperative PAS, absence = 0, presence = 1						
PAS: peripheral anterior synechiae, VIF: variance inflation factor						

Association between PAS and Intraocular Pressure Increase

At 12 months, PAS was successfully observed using AS-OCT in 96 of the 146 eyes. Among these 96 eyes, an IOP increase greater than 21 mmHg was observed in 14 (48.3%) of 29 eyes with PAS and in 15 (23.1%) of 65 eyes without PAS. The incidence of IOP increase greater than 21 mmHg was significantly higher in eyes with PAS than in those without PAS (Table 6, P = 0.026).

Table 6
Association between presence of PAS and IOP increase after DSAEK

	PAS (+)	PAS (-)	Total
IOP increase (+)	14	15	29
IOP increase (-)	17	50	67
Total	31	65	96
No of eyes			
Post-DSAEK IOP increase was defined as an increase in intraocular pressure above 21 mmHg			
PAS: peripheral anterior synechiae, IOP: intraocular pressure			
P value = 0.026 (Fisher's exact test)			

Cytokine Levels in Eyes with and without PAS

We showed that the preoperative protein level in AqH is a relevant factor for PAS formation after DSAEK; however, the exact reasons remain elusive. Therefore, we hypothesized that inflammation in the AqH plays a pivotal role in PAS formation, leading to an IOP increase after DSAEK. We measured and compared the inflammatory cytokine levels in the AqH between the eyes with and without the presence of PAS after DSAEK (Table 7). The levels of IL-6 ($P = 0.043$), IL-8 ($P = 0.003$), IL-17A ($P = 0.002$), MCP-1 ($P = 0.045$), IFN-γ ($P < 0.001$), E-selectin ($P = 0.015$), and sICAM-1 ($P < 0.001$) were significantly higher in 47 eyes with PAS or PAS progression than in those without.

Table 7
Preoperative aqueous total protein/cytokine levels stratified by presence of PAS after DSAEK

	PAS (-) (N = 99)	PAS (+) (N = 47)	*P* value†
Total protein	1.00 ± 0.57	1.45 ± 1.03	**0.036**
IL-1α	46.1 ± 65.4	51.3 ± 93.1	0.926
IL-1β	3.51 ± 6.37	4.79 ± 6.48	0.095
IL-4	31.8 ± 50.3	43.7 ± 64.7	0.815
IL-6	947 ± 1620	1270 ± 1150	**0.043**
IL-8	64.7 ± 93.3	122 ± 149	**0.003**
IL-10	5.91 ± 14.4	10.2 ± 29.6	0.148
IL-12p70	12.1 ± 7.56	20.8 ± 17.0	0.100
IL-13	8.05 ± 4.28	7.99 ± 4.92	0.711
IL-17A	11.1 ± 14.7	20.8 ± 14.3	**0.002**
MIP-1α	32.5 ± 80.2	21.1 ± 33.8	0.203
MIP-1β	130 ± 202	90.8 ± 154	0.740
MCP-1	913 ± 612	1120 ± 643	**0.045**
TNF-α	59.2 ± 62.3	56.0 ± 65.5	0.985
IFN-α	2.71 ± 2.54	1.96 ± 2.92	0.228
IFN -γ	110 ± 161	238 ± 184	**< 0.001**
E-Selectin	3720 ± 2290	7000 ± 6620	**0.015**
P-Selectin	6970 ± 5570	12300 ± 1700	0.161
sICAM-1	3340 ± 3970	7440 ± 5770	**< 0.001**
IP10	236 ± 340	136 ± 123	0.220
GM-CSF	15.3 ± 13.7	14.9 ± 8.25	0.706

Mean ± SD Protein:(mg/ml), Cytokines:(pg/ml)

†Mann-Whitney U test, compared between eyes with and without PAS after DSAEK.

PAS: peripheral anterior synechiae, IL: interleukin, MIP: macrophage inflammatory protein, MCP: monocyte chemotactic protein, TNF: tumor necrosis factor, GM-CSF: granulocyte-macrophage colony-stimulating factor, IFN: interferon, sICAM: soluble intracellular adhesion molecule, IP10: interferon gamma-induced protein 10

Correlation Between Cytokine Level in Aqueous Humor and PAS Indices

To substantiate these results, we further evaluated the correlations between cytokine levels in the AqH and PAS areas (Table 8). We selected the PAS area for the same reason as aforementioned. The results showed that the preoperative PAS area was significantly positively correlated with preoperative levels of IL-8 ($r = 0.314$, $P = 0.001$), IL-17A ($r = 0.409$, $P = 0.005$), IFN-γ ($r = 0.448$, $P < 0.001$), E-selectin ($r = 0.326$, $P = 0.002$), and sICAM-1($r = 0.308$, $P < 0.001$). Furthermore, the PAS area at 3 months was significantly correlated with the preoperative AqH levels of IL-8 ($r = 0.282$, $P = 0.004$), IL-17A ($r = 0.410$, $P = 0.006$), IFN-γ ($r = 0.445$, $P < 0.001$), E-selectin ($r = 0.265$ $P = 0.015$), and sICAM-1 ($r = 0.336$, $P < 0.001$). The PAS area at 6 months was significantly correlated with the preoperative AqH levels of IL-1β ($r = 0.291$, $P = 0.050$), IL-8 ($r = 0.350$, $P = 0.001$), IL-17A ($r = 0.430$, $P = 0.009$), IFN-γ ($r = 0.466$, $P < 0.001$), E-selectin ($r = 0.299$ $P = 0.013$), and sICAM-1 ($r = 0.304$, $P = 0.003$). The PAS area at 12 months was significantly correlated with the preoperative AqH levels of IL -8 ($r = 0.252$, $P = 0.021$), IFN-γ ($r = 0.318$, $P = 0.009$), and sICAM-1 ($r = 0.292$, $P = 0.004$).

Table 8
Correlations between preoperative cytokine levels and PAS area after DSAEK

	Preop PAS area		PAS area at 3 months		PAS area at 6 months		PAS area at 12months	
	r	P value*	r	P value*	r	P value*	r	P value*
IL-1α	0.0002	0.999	0.008	0.938	0.031	0.782	-0.012	0.914
IL-1β	0.220	0.104	0.258	0.062	**0.291**	**0.050**	0.182	0.221
IL-4	0.115	0.259	0.046	0.657	0.081	0.494	0.018	0.876
IL-6	0.121	0.198	0.198	0.037	0.231	0.029	0.186	0.078
IL-8	**0.314**	**0.001**	**0.282**	**0.004**	**0.350**	**0.001**	**0.252**	**0.021**
IL-10	0.104	0.340	0.173	0.121	0.162	0.190	0.211	0.085
IL-12p70	0.230	0.097	0.169	0.236	0.282	0.070	0.170	0.281
IL-13	0.173	0.353	0.061	0.749	0.324	0.123	0.031	0.879
IL-17A	**0.409**	**0.005**	**0.410**	**0.006**	**0.430**	**0.009**	0.318	0.055
MIP-1α	-0.146	0.275	-0.187	0.171	-0.186	0.222	-0.210	0.166
MIP-1β	0.019	0.840	-0.006	0.947	0.026	0.808	-0.054	0.616
MCP-1	0.146	0.111	0.151	0.106	0.190	0.065	0.087	0.397
TNF-α	0.107	0.395	-0.007	0.959	-0.028	0.845	-0.172	0.223
IFN-α	0.107	0.395	-0.148	0.316	-0.231	0.141	-0.233	0.147
IFN -γ	**0.448**	**0.0003**	**0.445**	**0.0004**	**0.466**	**0.0007**	**0.318**	**0.009**
E-Selectin	**0.326**	**0.002**	**0.265**	**0.015**	**0.299**	**0.013**	0.197	0.096
P-Selectin	0.151	0.100	0.126	0.178	0.082	0.429	0.023	0.825
sICAM-1	**0.308**	**0.0007**	**0.336**	**0.0003**	**0.304**	**0.003**	**0.292**	**0.004**
IP10	-0.095	0.303	-0.089	0.344	-0.073	0.477	-0.087	0.397
GM-CSF	0.057	0.829	-0.032	0.905	-0.093	0.732	0.055	0.856

*Spearman's correlation analysis, Protein:(mg/ml), Cytokines:(pg/ml)

IL: interleukin, MIP: macrophage inflammatory protein, MCP: monocyte chemotactic protein, TNF: tumor necrosis factor, GM-CSF: granulocyte-macrophage colony-stimulating factor, IFN: interferon, sICAM: soluble intracellular adhesion molecule, IP10: interferon gamma-induced protein 10

Discussion

Glaucoma is a serious complication of corneal transplantation. The visual outcome significantly worsens in patients with post-keratoplasty glaucoma owing to unrecoverable loss of the visual field and increased risk of graft failure[24]. The development of post-keratoplasty glaucoma has been reported to be associated with trauma, inflammation, and aphakic BK[25]. Among the various clinical findings of post-keratoplasty glaucoma, the presence or absence of PAS is relevant, as clinicians classify patients into different diagnostic categories, such as primary angle-closure, open-angle, or secondary glaucoma. Previous studies have emphasized that AS-OCT is useful for assessing PAS and angle structure in cases with the opaque cornea, where the details of PAS are not visible[13–15, 19,20]. In previous reports, the incidence of PAS differed from 11–56%, presumably due to different aetiologies[15,26]. Maier et al. evaluated PAS using AS-OCT and reported that PAS was significantly associated with the development of post-keratoplasty glaucoma both after PKP and DSAEK[26].

Clinically, PAS is believed to be associated with chronic inflammation in the anterior chamber, decentered graft, history of narrow-angle, breakdown of the blood-aqueous barrier (BAB), loss of iris integrity in ICE syndrome, and significant structural alteration after PKP[27]; however, the mechanism of PAS formation remains elusive. Moriarty et al. evaluated the breakdown of BAB in 25 patients undergoing pan-retinal photocoagulation (PRP) for proliferative diabetic retinopathy using a laser-flare photometer and PAS formation for 8 weeks after PRP and did not find an association between the breakdown of BAB and PAS formation after PRP[28]. They also evaluated BAB breakdown in 25 glaucomatous eyes for 8 weeks after diode-laser trabeculoplasty (DLT) and found that PAS developed in only four patients[29]. In these reports, the mean flare photon levels reached a peak approximately 24 hours after PRP or DLT and returned to normal levels 3–4 days later. The absence of PAS formation in these studies might be attributable to a temporal breakdown of the BAB, which was not chronic.

In contrast, PAS formation after 1 year of DSAEK was significantly correlated with high protein levels in the AqH. It should be noted that the high total protein levels in the AqH found in the current study can reflect a chronic breakdown of the BAB due to iris damage in BK eyes[30,31]. The preoperative high protein levels in the AqH were significantly correlated with the chronic reduction of ECD overtime after PKP and DSAEK[21–23], suggesting that the condition of high protein and cytokine levels in the AqH can be irreversible after surgery and adversely affect the transplanted corneal endothelial cells chronically. As seen in these studies, we speculate that chronic breakdown of the BAB (high total protein level) can cause PAS progression after DSAEK.

Glaucoma due to PAS formation does not seem to be a common complication after DSAEK, compared to PKP, particularly in Western countries where FECD is the major aetiology of endothelial keratoplasty[32]. In contrast, in Asian countries, where complicated PBK or LI-BK is the cause of 60–70% of BK cases[33,34], we observed PAS formation and progression in some patients after DSAEK. However, in a recent study conducted in New York City (New York, USA), which included 353 DSAEK procedures (FECD in 40%,

PBK/aphakic BK in 40%, failed PKP in 13.3%, and post-glaucoma surgery in 6.7%), Wu et al. reported that PAS occurred in 37 patients (10.5%) after DSAEK, although AS-OCT was not used for PAS evaluation[35]. Furthermore, a previous report from Germany showed that IOP rise after DSAEK was significantly greater in PBK than in FECD[26], although they did not compare PAS between patients with PBK and FECD. Thus, we believe that PAS formation and IOP rise after DSAEK are clinically relevant issues in both Western and Asian countries.

AqH has a unique composition that includes proteins, ascorbate, glutathione, complement factors, and other biologically active substances. In the current study, we evaluated total protein and cytokine levels that could be easily measured at low cost. However, we did not measure the AqH levels of specific substances, myocilin[36], osteopontin[37], and endogenous prostaglandins[38], which are reportedly associated with IOP rise. Thus, the elevated total protein/cytokine levels measured in the current study might not be the direct cause of PAS formation. In the future, we will need to identify the therapeutic molecules that prevent PAS formation. Proteomics is a powerful tool that can measure more than 200–1000 protein levels in the AqH[23,39]. Using bioinformatics such as gene ontology and open databases, we can analyse protein interactions and biological pathways, which are driven in the AqH of eyes with severe iris damage[23,39]. Further studies are necessary to elucidate the exact mechanism of PAS formation and IOP rise after corneal transplantation, using proteomic analysis of the AqH and genomics analysis of trabecular meshwork/peripheral iris tissues to identify alterations in the aqueous environment and the reaction of trabecular meshwork/peripheral iris tissues against it.

Among the cytokines measured in the current study, IL-8, IL-17A, IFN-γ, E-selectin, and sICAM-1 were significantly correlated with PAS formation. In general, it is a well-known clinical phenomenon that long-standing inflammation in the anterior chamber causes PAS in such eyes with anterior uveitis. Regarding cytokine levels in the anterior chamber, John et al. found that the concentration of IL-8 in the AqH was significantly elevated in eyes with primary open-angle glaucoma compared to normal controls[40]. Furthermore, they reported that patients with severe visual defects had higher AqH levels of IL-8[40]. IFN-γ is a strong pro-inflammatory cytokine that strengthens the Th-1 immune response[41]. Previous studies have suggested that serum IFN-γ level can be a risk factor for various systemic diseases, such as angina pectoris and sepsis, predicting major coronary events and mortality[42 43]. In the field of ophthalmology, numerous reports evaluated IFN-γ level in AqH in various eye diseases, and found its significant elevations in uveitis, age-related macular degeneration (AMD), bullous keratopathy, and eyes with graft immunological rejection[23,44–46]. Maier et al. shown that IFN-γ level in AqH before penetrating keratoplasty was higher in eyes with postoperative immune reactions than those without immune reactions[46]. We previously revealed that IFN-γ levels in AqH were elevated in eyes with bullous keratopathy[23]. However, this is the first report to demonstrate the association between PAS formation and IFN-γ levels in AqH. However, eyes with AMD and BK usually do not develop PAS; thus, the mechanism of PAS formation needs to be investigated in the future, by identifying the source of increased IFN-γ levels and evaluating the influence of disrupted BAB of the iris stroma. IL-17A + Th17 cells produce IFN-γ and mediate ocular surface autoimmunity.

In eye disorders such as Coats disease[47], diabetic retinopathy[48], central ocular vein occlusion[49], elevation of ICAM-1 levels in AqH have been reported, suggesting its contribution to pathogenesis. ICAMs are members of the immunoglobulin superfamily that are involved in generating and maintaining cell connections and comprise an extensive cell-cell and cell-matrix network. ICAM expression is induced by inflammatory cytokines and may play a crucial role in inflammatory mechanisms. MCP-1 is the main chemotactic factor for the migration of macrophages and the pathogenesis of chronic inflammation[21]. In patients with open-angle glaucoma, Inoue et al. showed that a higher preoperative MCP-1 level was associated with poorer outcomes of trabeculectomy in eyes with open-angle glaucoma[50]. Furthermore, Ohira et al. reported that MCP-1 levels were higher in uveitic glaucoma than in open-angle glaucoma[51]. Although they did not evaluate PAS in their study, we postulated that the MCP-1 level can directly result in PAS formation, followed by IOP elevation after trabeculectomy or uveitic glaucoma. However, as shown in previous studies in humans and animals, MCP-1 levels in AqH are elevated after cataract surgery as proliferated lens epithelial cells on the capsule secrete MCP-1 overtime after phacoemulsification[52]. The current study included 32 eyes that underwent simultaneous cataract surgery, which might have influenced the results as preoperative lens condition can affect MCP-1 levels. However, there were no differences in MCP-1 levels between solitary DSAEK and those combined with cataract surgery (P = 0.196). Moreover, a multivariate analysis that evaluated the influence of cataract surgery showed that preoperative phacoemulsification (β = 0.011, P = 0.926), age (β = 0.067, P = 0.518), and preoperative iris damage (β=-0.095, P = 0.427) were not, but preoperative steroid use (β = 0.215, P = 0.049) was associated with the MCP-1 level in the AqH before DSAEK.

This study had some limitations. First, the different graft sizes (7.75–8.5mm) may have had some effect on PAS formation, as previously reported[26]. However, multivariate analyses showed that the different graft sizes did not correlate with the PAS parameters after DSAEK. Thus, we consider its influence to be minimal. Second, this study included heterogeneous aetiologies for BK, which might have caused bias. Therefore, we conducted a subgroup analysis including only PBK, which showed similar results. Only one patient developed PAS in the eyes with FECD. Thus, the results of the current study may not apply to patients with FECD. Third, although the results showed moderate to strong correlations between AqH total protein/cytokine levels and PAS progression, we should be careful not to overinterpret the results as elevated levels of AqH total protein/cytokine may not be the direct cause of PAS formation. Further translational studies are valuable for investigating the exact mechanism.

In conclusion, we evaluated PAS using AS-OCT and identified PAS in 47 eyes (32.2%) after DSAEK. We also showed that preoperative total protein levels were significantly correlated with PAS progression after DSAEK. Additionally, the incidence of IOP increase was significantly greater in eyes with PAS than in those without PAS after DSAEK, suggesting that PAS directly caused an IOP increase. Furthermore, elevated levels of specific cytokines, such as IL-8, IL-17A, IFN-γ, E-selectin, and sICAM-1 were significantly correlated with PAS formation after DSAEK. MCP-1 and IL-6 levels were significantly higher in eyes with PAS than in those without PAS. These results suggest that microenvironmental changes in AqH cause progression of PAS as a result of chronic inflammation with elevated levels of specific cytokines.

Methods

This prospective study adhered well to the tenets of Declaration of Helsinki. This study was approved by the institutional ethics review board of Tokyo Dental College Ichikawa General Hospital (acceptance number: I-15-42R). The corneal grafts were provided from SightLife (Seattle USA) or Cornea Center Eye Bank (Chiba, Japan), which have relevant regulations taking care to not violate the privacy of donors. No grafts were procured from prisoners. Written informed consent was obtained from all the participants before the intervention.

Participants

The aetiologies of DSAEK in the studied eyes included pseudophakic BK (PBK, 67 eyes), post-laser iridotomy BK (LI-BK, 25 eyes), FECD (20 eyes), post-trabeculectomy BK (12 eyes), uveitis (five eyes), post-PK endothelial decompensation (four eyes), BK due to birth injury (four eyes), and other causes (nine eyes).

Surgical Technique

DSAEK surgery was performed using the double-glide technique[53,54]. After sub-Tenon anesthesia using an injection of 2% lidocaine, a 5.0-mm temporal corneoscleral incision was made. An anterior chamber maintenance cannula was inserted through paracentesis at 2 o'clock or 10 o'clock position, and Descemet stripping was performed using a reverse-bent Sinsky hook (Asico, Westmont, IL, USA). The recipient's endothelium and Descemet's membrane were carefully removed using forceps. Pre-cut donor grafts were trephinated, and the endothelial surface of the donor lenticle was coated with a small amount of viscoelastic material. Donor tissue was gently inserted into the anterior chamber using Busin glide (Asico). The air was carefully injected into the anterior chamber to unfold the graft. At 10 min after air injection, half of the air was replaced with a balanced salt solution (Alcon, Fort Worth, TX, USA). At the end of the surgery, 2 mg of subconjunctival betamethasone was administered. In patients with significant lens opacity (32 eyes), standard phacoemulsification and aspiration were performed with implantation of an intraocular lens (IOL), followed by DSAEK. Therefore, we performed solitary DSAEK in 111 eyes, DSAEK combined with simultaneous cataract surgery in 32 eyes, and DSAEK combined with simultaneous IOL suture in three eyes. All DSAEK procedures were successful and uneventful, without the need for excessive intraoperative manipulation.

Postoperative Care

After DSAEK, patients were prescribed topical eye drops of levofloxacin (Cravit, Santen, Osaka, Japan) and betamethasone 0.1% (Sanbetazon, Santen) five times a day. Topical betamethasone eye drops were tapered over the following 6 months. Starting at 6 months after DSAEK, we prescribed fluorometholone 0.1% eye drops (Flumetholon 0.1, Santen) three times a day for up to 12 months after surgery. Forty patients experienced mild IOP elevation $\geq$ 21 mmHg, which resolved with cessation of topical steroids

and/or topical anti-glaucoma agents. There were no cases of graft rejection during the 12-month follow-up.

Anterior Segment Optical Coherence Tomography Imaging

All patients underwent AS-OCT examination preoperatively and at 3, 6, and 12 months postoperatively. AS-OCT (SS-1000, CASIA, TOMEY, Nagoya, Japan) is a type of Fourier-domain OCT that uses a 1320 nm wavelength scanning laser source and a photodetector to detect wavelength-resolved interference signals. All eyes were imaged by trained technicians using an “angle analysis” protocol, which was composed of 128 radial B-scans, each with 512 A-scans (16-mm scan length). This allowed 360° imaging of the whole anterior segment in 2.4 second and showed time-course alterations of clear three-dimensional images after DSAEK (Figure 1a-d). Eyes receiving topical medications that affected pupil size were not included.

Measurement of Area, Degree, and Maximum Height of PAS

To examine the PAS, the angle structure was analysed using AS-OCT. The extent of PAS in each meridian was measured using the built-in software after manual detection of the scleral spur and anterior irido-angle adhesion (iris endpoint, Figure 2a and b). The software automatically aligned the scleral spur (solid red line in the polar plot, Figure 2c and d) and the iris endpoint (dotted green line in the polar plot) of individual cross-sectional OCT images and then computed the PAS area (mm^2), which was defined as the area bound below the iris endpoint and above the scleral spur (blue area in Figure 2c and d). The parameters were automatically calculated: PAS %degree (%), that is, the percentage of degrees that presented with PAS of the total angle degrees analysed, maximum PAS height (mm), that is, the maximum distance from the scleral spur to the iris endpoint, and PAS area (mm^2). In the present study, angle analysis could not be performed in the upper or lower areas as the eyelids obstructed angle imaging in some patients. Therefore, the PAS was measured at a total range of 90° on the nasal and temporal sides of the horizontal line, 45° each, which were imaged at all time points in all patients.

Aqueous Humor Samples

AqH samples containing 70–300 µL were obtained under sterile conditions at the beginning of surgery after topical anesthesia in DSAEK surgery. First, paracentesis was performed on the clear cornea. AqH samples were obtained using a 27–gauge needle, taking care not to touch the iris, lens, or corneal endothelium. The samples were centrifuged at 3,000 × g for 5 min. The soluble fractions were collected and stored at -80°C until measurements.

Total Protein and Cytokine Level Measurements

The total protein levels of the AqH samples were determined using a DC protein assay (Bio-Rad, Hercules, CA, USA)[30]. In brief, bovine serum albumin (BSA) was used as a standard in the range of 0.06 to 1.37 mg/mL. Samples (5 µL) of BSA and AqH were added to 96-well microplates, followed by the immediate

addition of a mixture containing 25 μL reagent A and 200 μL reagent C. After 15 min of incubation at room temperature in the dark, the microplates were read at 690 nm and 405 nm using a microplate reader (Model 550; Bio-Rad). The cytokine levels of interleukin (IL)-1α, IL-1β, IL-4, IL-6, IL -8, IL -10, IL -12p70, IL-13, IL-17A, macrophage inflammatory protein (MIP)-1α, MIP-1β, monocyte chemotactic protein (MCP)-1, tumor necrosis factor (TNF)-α, interferon (IFN)-α, IFN-γ, E-selectin, P-selectin, soluble intercellular adhesion molecule (sICAM)-1, interferon gamma-induced protein (IP)-10 and granulocyte-macrophage colony-stimulating factor (GM-CSF) in AqH samples were measured using Luminex (ProcaPlex kit, Luminex, San Antonio, TX, USA) beads-based multiplex immunoassay according to previous report[31]. Briefly, 50 μL of AqH samples were incubated with antibody-coated capture beads in an incubation buffer at room temperature. After a 2-h incubation, the beads were washed three times using washing buffer, and phycoerythrin-labeled streptavidin was added for 30 min in the dark at room temperature. After washing three times with washing buffer, the plates were resuspended in 150 μL of reading buffer, and the assays were performed using a Luminex 200.

Statistical Analysis

Data were analysed using STATA/IC 14.0, for iOS (StataCorp LP, College Station, TX, USA). The Shapiro–Wilk test was used to assess whether the data were normally distributed. Spearman's correlation analyses were used to evaluate the correlations between the total protein and cytokine levels in the AqH and PAS %degree, maximum height, and area. Fisher's exact test was used to assess the differences in the incidence of post-DSAEK IOP rise (≥ 21 mmHg) between the groups with and without PAS. For multivariate analyses of the clinical factors that were correlated with the progression of PAS area, we selected five independent clinical factors (preoperative total protein levels in AqH, preoperative PAS, axial length, graft size, and age; variance inflation factors= 1.11–1.12) and conducted multiple linear regression analyses. Data are expressed as mean ± standard deviation. Statistical significance was set at $P < 0.05$.

Declarations

Acknowledgments: We would like to thank Editage company for English language editing.

Funding: This study is supported by the Grant-in-Aid for Scientific Research 15K10906 from the Ministry of Education, Culture, Sports, Science and Technology (TY). The funding organization had no role in the design or conduct of this research.

Author contributions: T.Y. contributed to the study conception of this study. Y.K., T.Y., T.M., M.F., and J.S. acquired patient samples. T.Y., and K.H. measured protein and cytokine levels. Y.K. quantified PAS indices. Y.K., T.Y., and S.N. conducted statistical analyses. Y.K., T.Y., T.I. and J.S. contributed to the analyses and interpretation of the data. Y.K. and T.Y. wrote the paper.

Competing interests: We have no competing or financial interests to declare.

References

1. Goldberg, D. B., Schanzlin, D. J. & Brown, S. I. Incidence of increased intraocular pressure after keratoplasty. *Am J Ophthalmol.* **92**, 372–377 https://doi.org/10.1016/0002-9394(81)90527-4 (1981).
2. Karesh, J. W. & Nirankari, V. S. Factors associated with glaucoma after penetrating keratoplasty. *Am J Ophthalmol.* **96**, 160–164 https://doi.org/10.1016/s0002-9394(14)77783-9 (1983).
3. Chien, A. M. *et al.* Glaucoma in the immediate postoperative period after penetrating keratoplasty. *Am J Ophthalmol.* **115**, 711–714 https://doi.org/10.1016/s0002-9394(14)73636-0 (1993).
4. Foulks, G. N. Glaucoma associated with penetrating keratoplasty. *Ophthalmology.* **94**, 871–874 https://doi.org/10.1016/s0161-6420(87)33542-0 (1987).
5. Ogawa, A. *et al.* Aetiology-specific comparison of long-term outcome of deep anterior lamellar keratoplasty for corneal diseases. *Br J Ophthalmol.* **100**, 1176–1182 https://doi.org/10.1136/bjophthalmol-2015-307427 (2016).
6. Huang, O. S., Mehta, J. S., Htoon, H. M., Tan, D. T. & Wong, T. T. Incidence and Risk Factors of Elevated Intraocular Pressure Following Deep Anterior Lamellar Keratoplasty. *Am J Ophthalmol.* **170**, 153–160 https://doi.org/10.1016/j.ajo.2016.07.025 (2016).
7. Musa, F. U. *et al.* Long-term risk of intraocular pressure elevation and glaucoma escalation after deep anterior lamellar keratoplasty. *Clin Exp Ophthalmol.* **40**, 780–785 https://doi.org/10.1111/j.1442-9071.2012.02796.x (2012).
8. Vajaranant, T. S. *et al.* Visual acuity and intraocular pressure after Descemet's stripping endothelial keratoplasty in eyes with and without preexisting glaucoma. *Ophthalmology.* **116**, 1644–1650 https://doi.org/10.1016/j.ophtha.2009.05.034 (2009).
9. Maier, A. K. *et al.* Intraocular pressure elevation and post-DSEK glaucoma after Descemet`s stripping endothelial keratoplasty. *Graefes Arch Clin Exp Ophthalmol.* **251**, 1191–1198 https://doi.org/10.1007/s00417-012-2203-5 (2013).
10. Ozeki, N. *et al.* Intraocular pressure elevation after Descemet's stripping endothelial keratoplasty. *Jpn J Ophthalmol.* **56**, 307–311 https://doi.org/10.1007/s10384-012-0149-0 (2012).
11. Maier, A. K. *et al.* Intraocular pressure elevation and post-DMEK glaucoma following Descemet membrane endothelial keratoplasty. *Graefes Arch Clin Exp Ophthalmol.* **252**, 1947–1954 https://doi.org/10.1007/s00417-014-2757-5 (2014).
12. Muller, L. *et al.* Changes in intraocular pressure after descemet stripping automated endothelial keratoplasty: a retrospective analysis. *Cornea.* **34**, 271–274 https://doi.org/10.1097/ICO.0000000000000342 (2015).
13. Karadag, O. *et al.* Incidence of and risk factors for increased intraocular pressure after penetrating keratoplasty. *Cornea.* **29**, 278–282 https://doi.org/10.1097/ICO.0b013e3181b6eb9e (2010).
14. Memarzadeh, F. *et al.* Optical coherence tomography of the anterior segment in secondary glaucoma with corneal opacity after penetrating keratoplasty. *Br J Ophthalmol.* **91**, 189–192

https://doi.org/10.1136/bjo.2006.100099 (2007).

15. Fukuda, R. *et al.* Noninvasive observations of peripheral angle in eyes after penetrating keratoplasty using anterior segment fourier-domain optical coherence tomography. *Cornea.* **31**, 259–263 https://doi.org/10.1097/ICO.0b013e318226daa9 (2012).
16. Teekhasaenee, C. & Ritch, R. Iridocorneal endothelial syndrome in Thai patients: clinical variations. *Arch Ophthalmol.* **118**, 187–192 https://doi.org/10.1001/archopht.118.2.187 (2000).
17. Silva, L., Najafi, A., Suwan, Y., Teekhasaenee, C. & Ritch, R. The iridocorneal endothelial syndrome. *Surv Ophthalmol.* **63**, 665–676 https://doi.org/10.1016/j.survophthal.2018.01.001 (2018).
18. Daniel, E. *et al.* Risk of Ocular Hypertension in Adults with Noninfectious Uveitis. *Ophthalmology.* **124**, 1196–1208 https://doi.org/10.1016/j.ophtha.2017.03.041 (2017).
19. Lim, L. S. *et al.* Acute primary angle closure: configuration of the drainage angle in the first year after laser peripheral iridotomy. *Ophthalmology.* **111**, 1470–1474 https://doi.org/10.1016/j.ophtha.2004.01.036 (2004).
20. Sanchez-Parra, L., Pardhan, S., Buckley, R. J., Parker, M. & Bourne, R. R. Diurnal Intraocular Pressure and the Relationship With Swept-Source OCT-Derived Anterior Chamber Dimensions in Angle Closure: The IMPACT Study. *Invest Ophthalmol Vis Sci.* **56**, 2943–2949 https://doi.org/10.1167/iovs.14-15385 (2015).
21. Yazu, H. *et al.* Preoperative Aqueous Cytokine Levels are Associated With Endothelial Cell Loss After Descemet's Stripping Automated Endothelial Keratoplasty. *Invest Ophthalmol Vis Sci.* **59**, 612–620 https://doi.org/10.1167/iovs.17-23049 (2018).
22. Yagi-Yaguchi, Y. *et al.* Preoperative Aqueous Cytokine Levels Are Associated With a Rapid Reduction in Endothelial Cells After Penetrating Keratoplasty. *Am J Ophthalmol.* **181**, 166–173 https://doi.org/10.1016/j.ajo.2017.07.005 (2017).
23. Yamaguchi, T. *et al.* Pathological processes in aqueous humor due to iris atrophy predispose to early corneal graft failure in humans and mice. *Sci Adv.* **6**, eaaz5195 https://doi.org/10.1126/sciadv.aaz5195 (2020).
24. Irvine, A. R. & Kaufman, H. E. Intraolar pressure following penetrating keratoplasty. *Am J Ophthalmol.* **68**, 835–844 https://doi.org/10.1016/0002-9394(69)94577-2 (1969).
25. Ayyala, R. S. Penetrating keratoplasty and glaucoma. *Surv Ophthalmol.* **45**, 91–105 https://doi.org/10.1016/s0039-6257(00)00141-7 (2000).
26. Maier, A. K. *et al.* Anterior segment analysis and intraocular pressure elevation after penetrating keratoplasty and posterior lamellar endothelial keratoplasty. *Ophthalmic Res.* **53**, 36–47 https://doi.org/10.1159/000365252 (2015).
27. Prasanth, B., Dubey, S. & Mathur, U. IOP changes after DSEK. *Ophthalmology.* **117**, 1460–1461 author reply 1461–1462 https://doi.org/10.1016/j.ophtha.2010.03.021 (2010).
28. Moriarty, A. P., Spalton, D. J., Shilling, J. S., Ffytche, T. J. & Bulsara, M. Breakdown of the blood-aqueous barrier after argon laser panretinal photocoagulation for proliferative diabetic retinopathy. *Ophthalmology.* **103**, 833–838 https://doi.org/10.1016/s0161-6420(96)30607-6 (1996).

29. Moriarty, A. P. *et al.* Comparison of the anterior chamber inflammatory response to diode and argon laser trabeculoplasty using a laser flare meter. *Ophthalmology.* **100**, 1263–1267 https://doi.org/10.1016/s0161-6420(93)31495-8 (1993).
30. Yamaguchi, T. *et al.* Elevated Cytokine Levels in the Aqueous Humor of Eyes With Bullous Keratopathy and Low Endothelial Cell Density. *Invest Ophthalmol Vis Sci.* **57**, 5954–5962 https://doi.org/10.1167/iovs.16-20187 (2016).
31. Aketa, N. *et al.* Iris Damage Is Associated With Elevated Cytokine Levels in Aqueous Humor. *Invest Ophthalmol Vis Sci.* **58**, Bio42–bio51 https://doi.org/10.1167/iovs.17-21421 (2017).
32. Price, M. O. *et al.* Descemet's stripping automated endothelial keratoplasty outcomes compared with penetrating keratoplasty from the Cornea Donor Study. *Ophthalmology.* **117**, 438–444 https://doi.org/10.1016/j.ophtha.2009.07.036 (2010).
33. Ishii, N. *et al.* Factors associated with graft survival and endothelial cell density after Descemet's stripping automated endothelial keratoplasty. *Sci Rep.* **6**, 25276 https://doi.org/10.1038/srep25276 (2016).
34. Hayashi, T. *et al.* Topographic characteristics after Descemet's membrane endothelial keratoplasty and Descemet's stripping automated endothelial keratoplasty. *PLoS One.* **12**, e0188832 https://doi.org/10.1371/journal.pone.0188832 (2017).
35. Wu, E. I., Ritterband, D. C., Yu, G., Shields, R. A. & Seedor, J. A. Graft rejection following descemet stripping automated endothelial keratoplasty: features, risk factors, and outcomes. *Am J Ophthalmol* **153**, 949–957 e941, doi:10.1016/j.ajo.2011.10.004 (2012).
36. Howell, K. G., Vrabel, A. M., Chowdhury, U. R., Stamer, W. D. & Fautsch, M. P. Myocilin levels in primary open-angle glaucoma and pseudoexfoliation glaucoma human aqueous humor. *J Glaucoma.* **19**, 569–575 https://doi.org/10.1097/IJG.0b013e3181d13020 (2010).
37. Chowdhury, U. R., Jea, S. Y., Oh, D. J., Rhee, D. J. & Fautsch, M. P. Expression profile of the matricellular protein osteopontin in primary open-angle glaucoma and the normal human eye. *Invest Ophthalmol Vis Sci.* **52**, 6443–6451 https://doi.org/10.1167/iovs.11-7409 (2011).
38. Yamagishi-Kimura, R., Honjo, M. & Aihara, M. Contribution of prostanoid FP receptor and prostaglandins in transient inflammatory ocular hypertension. *Sci Rep.* **8**, 11098 https://doi.org/10.1038/s41598-018-29273-1 (2018).
39. Chowdhury, U. R., Madden, B. J., Charlesworth, M. C. & Fautsch, M. P. Proteome analysis of human aqueous humor. *Invest Ophthalmol Vis Sci.* **51**, 4921–4931 https://doi.org/10.1167/iovs.10-5531 (2010).
40. Kuchtey, J., Rezaei, K. A., Jaru-Ampornpan, P., Sternberg, P. Jr. & Kuchtey, R. W. Multiplex cytokine analysis reveals elevated concentration of interleukin-8 in glaucomatous aqueous humor. *Invest Ophthalmol Vis Sci.* **51**, 6441–6447 https://doi.org/10.1167/iovs.10-5216 (2010).
41. Zhang, Y., Yang, Q., Guo, F., Chen, X. & Xie, L. Link between neurodegeneration and trabecular meshwork injury in glaucomatous patients. *BMC Ophthalmol.* **17**, 223 https://doi.org/10.1186/s12886-017-0623-z (2017).

42. Pedersen, E. R. *et al.* Systemic markers of interferon-gamma-mediated immune activation and long-term prognosis in patients with stable coronary artery disease. *Arterioscler Thromb Vasc Biol.* **31**, 698–704 https://doi.org/10.1161/ATVBAHA.110.219329 (2011).
43. Kyriazopoulou, E. *et al.* Macrophage activation-like syndrome: an immunological entity associated with rapid progression to death in sepsis. *BMC Med.* **15**, 172 https://doi.org/10.1186/s12916-017-0930-5 (2017).
44. Spindler, J., Zandi, S., Pfister, I. B., Gerhardt, C. & Garweg, J. G. Cytokine profiles in the aqueous humor and serum of patients with dry and treated wet age-related macular degeneration. *PLoS One.* **13**, e0203337 https://doi.org/10.1371/journal.pone.0203337 (2018).
45. Bonacini, M. *et al.* Cytokine Profiling in Aqueous Humor Samples From Patients With Non-Infectious Uveitis Associated With Systemic Inflammatory Diseases. *Front Immunol.* **11**, 358 https://doi.org/10.3389/fimmu.2020.00358 (2020).
46. Maier, P., Heizmann, U., Bohringer, D., Kern, Y. & Reinhard, T. Distinct cytokine pattern in aqueous humor during immune reactions following penetrating keratoplasty. *Mol Vis.* **16**, 53–60 (2010).
47. Liang, T. *et al.* Aqueous humour cytokines profiles in eyes with Coats disease and the association with the severity of the disease. *BMC Ophthalmol.* **20**, 178 https://doi.org/10.1186/s12886-020-01421-0 (2020).
48. Hillier, R. J. *et al.* Aqueous Humor Cytokine Levels and Anatomic Response to Intravitreal Ranibizumab in Diabetic Macular Edema. *JAMA Ophthalmol.* **136**, 382–388 https://doi.org/10.1001/jamaophthalmol.2018.0179 (2018).
49. Mashima, A., Noma, H., Yasuda, K., Goto, H. & Shimura, M. Anti-vascular endothelial growth factor agent reduces inflammation in macular edema with central retinal vein occlusion. *J Inflamm (Lond).* **16**, 9 https://doi.org/10.1186/s12950-019-0214-2 (2019).
50. Inoue, T., Kawaji, T. & Tanihara, H. Monocyte chemotactic protein-1 level in the aqueous humour as a prognostic factor for the outcome of trabeculectomy. *Clin Exp Ophthalmol.* **42**, 334–341 https://doi.org/10.1111/ceo.12204 (2014).
51. Ohira, S., Inoue, T., Iwao, K., Takahashi, E. & Tanihara, H. Factors Influencing Aqueous Proinflammatory Cytokines and Growth Factors in Uveitic Glaucoma. *PLoS One.* **11**, e0147080 https://doi.org/10.1371/journal.pone.0147080 (2016).
52. Kawai, M. *et al.* Elevated levels of monocyte chemoattractant protein-1 in the aqueous humor after phacoemulsification. *Invest Ophthalmol Vis Sci.* **53**, 7951–7960 https://doi.org/10.1167/iovs.12-10231 (2012).
53. Yamaguchi, T. *et al.* Effect of anterior and posterior corneal surface irregularity on vision after Descemet-stripping endothelial keratoplasty. *J Cataract Refract Surg.* **35**, 688–694 https://doi.org/10.1016/j.jcrs.2008.11.062 (2009).
54. Yamaguchi, T. *et al.* The contribution of the posterior surface to the corneal aberrations in eyes after keratoplasty. *Invest Ophthalmol Vis Sci.* **52**, 6222–6229 https://doi.org/10.1167/iovs.11-7647 (2011).

Figures

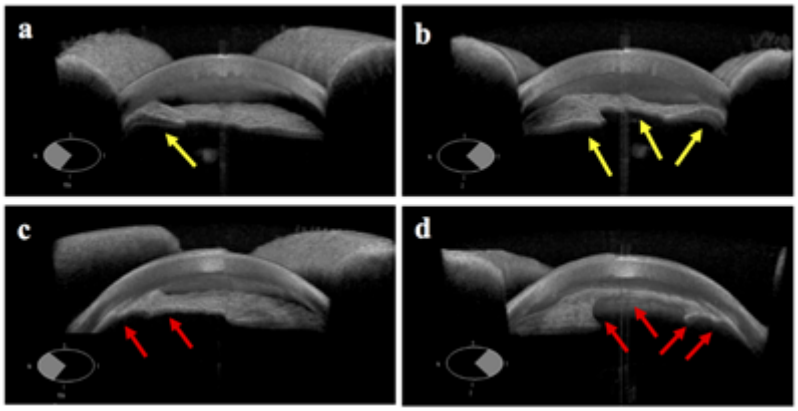

Figure 1

Three-dimensional imaging of PAS progression after DSAEK Three-dimensional images of PAS before (a: nasal, b: temporal, yellow arrows) and after (c: nasal, d: temporal, red arrows) DSAEK

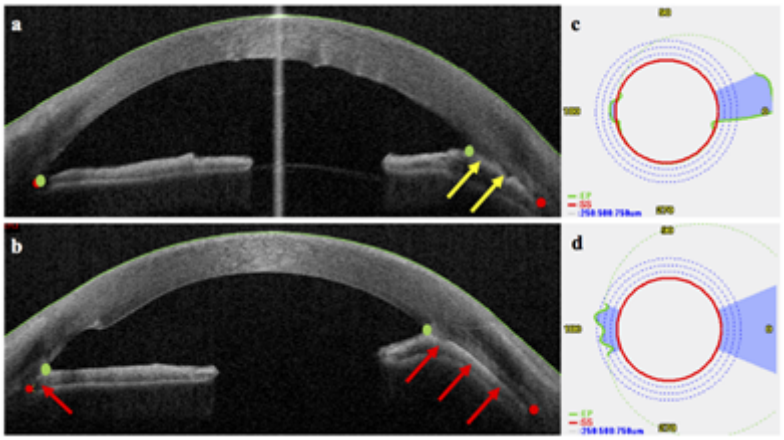

Figure 2

Anterior segment OCT analysis of PAS before and after DSAEK Anterior segment OCT images of a representative patient before (a) and after (b) DSAEK. (A-B) An 84-year-old man with LI-BK successfully underwent DSAEK. The total protein level in the aqueous humor was 3.46 mg/mL. Before DSAEK, the patient had a limited PAS on the temporal side (a: yellow arrows). After DSAEK, PAS developed on the nasal side and expanded on the temporal side (b: red arrows). Red points represent the scleral spur. Green points indicate the peripheral end point of the iris. (c–d) PAS analysis using AS-OCT. The inbuilt software automatically aligns the scleral spur (red polar plot), the iris end point (green polar plot), and the PAS area (blue area) based on the individual cross-sectional OCT images. The software calculates the PAS degree, maximum PAS height (mm), and PAS area (mm2). In this patient, the PAS degree increased from 65% to 100% after DSAEK. The maximum PAS height increased from 2.2 mm to 3.6 mm after DSAEK. The PAS area increased from 7.53 mm2 to 20.94 mm2. He lost light sensation due to refractory glaucoma at 15 months after DSAEK.