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Microcirugía de glaucoma con el implante Preserflo: fluídica experimental, morfología de la ampolla de filtración, cambios biométricos y corneales

MEMORIA PARA OPTAR AL GRADO DE DOCTOR

PRESENTADA POR

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Tesis doctoral

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Memoria para optar al grado de Doctor

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RESUMEN / SUMMARY

RESUMEN

Introducción

La reducción de la presión intraocular (PIO) ha demostrado ser el único tratamiento válido del glaucoma, principal causa de ceguera irreversible en el mundo[1]. En un estudio reciente realizado por GBD 2019 Blindness and Vision Impairment Collaborators[2], en 2020 la segunda causa principal de ceguera mundial entre los 33,6 millones de ciegos mayores de 50 años fue el glaucoma[3] (3,6 millones de casos, rango 2,8 a 4,4), por detrás de las cataratas.

Aunque la técnica quirúrgica de referencia para el tratamiento del glaucoma es la trabeculectomía, descrita y aplicada desde mediados de la década de los 60´, actualmente se vienen desarrollando nuevos métodos quirúrgicos menos invasivos cuyo objetivo es reducir las complicaciones. Entre ellos, uno de los dos nuevos dispositivos de microcirugía filtrante de glaucoma es el implante Preserflo (Santen Pharmaceutical Company Ltd, Osaka, Japón). Este dispositivo consiste en un tubo de drenaje de humor acuoso diseñado para ser implantado ab externo mediante la disección de la conjuntiva y de la cápsula de Tenon. El objetivo de este implante sería el de comunicar la cámara anterior con el espacio subconjuntival[4], creando una corriente contínua de humor acuoso hacia el plexo venoso episcleral.

El flujo a través de Preserflo aún no ha sido medido experimentalmente. Los propios investigadores implicados en el diseño del tubo se basaron en una hipotética falta de validez de la ecuación de Hagen-Poiseuille en diámetros de tubo tan pequeños y materiales tan extremadamente hidrofóbicos como el poli (bloque de estireno-isobutileno-bloque-estireno), "SIBS", para explicar la falta de evidencia experimental sobre la presión diferencial previa a su lanzamiento. A pesar de la falta de evidencia científica, algunos estudios publicados sostienen que este implante fue diseñado específicamente para evitar la hipotonía. Por último, se desconoce el efecto que el mecanismo de restricción del flujo asociado de manera característica con su diseño pudiera tener sobre la morfología de la ampolla, la refracción, la biometría y el endotelio.

Objetivos

1. Medir las dimensiones del implante Preserflo con microscopía electrónica de emisión de campo (FESEM) con el objetivo de confirmar que el diámetro interno del tubo (70 µm) es consistente con la medida aportada por el fabricante.
2. Calcular de manera teórica la resistencia al flujo (R_{THEO}) y la presión diferencial (Δp).
3. Medir experimentalmente el flujo (Q) por el implante Preserflo con un método gravitacional donde la presión hidrostática sea la única fuerza que empuje el flujo a través del tubo.
4. Realizar una clasificación clínica y morfológica, así como un análisis geométrico de las ampollas de filtración asociadas con este micro-dispositivo de drenaje en el postoperatorio temprano (24 horas a 3 meses).
5. Analizar los efectos que esta técnica quirúrgica pueda tener sobre la topografía y biometría en los tres primeros meses tras la cirugía.
6. Estudiar los cambios endoteliales asociados con este implante desde el postoperatorio temprano hasta el primer año.

Material Y Métodos

DINÁMICA DE FLUÍDOS

El diámetro interno del tubo fue medido con un microscopio electrónico de emisión de campo Quanta FEG 250. Las medidas se tomaron de borde a borde y de borde a sombra de la imagen, ya que el implante presentaba una ligera inclinación. Se tomaron a su vez medidas del grosor de la pared.

La ecuación de Hagen-Poiseuille[5] se utilizó para calcular la resistencia teórica al flujo (R_{THEO}, mmHg/(µL/min)) y la presión diferencial ($\Delta p = \Delta p$= p1-p2, mmHg) para un flujo volumétrico (Q) igual a la producción media de humor acuoso en el ser humano (2 µL/min)[6] y una viscosidad de 0.7185 centipoises (cP) a 36 °C en pacientes con glaucoma primario de ángulo abierto (GPAA)[7].

Configuración experimental: una muestra de Preserflo fue insertada en un tubo de un diámetro superior (DT=1 mm), con ambos extremos del tubo inmersos en suero salino fisiológico, utilizado para limitar la presencia de burbujas en el circuito. El circuito conectaba dos contenedores situados a diferentes alturas, el superior sellado para evitar también la formación de burbujas. Una vez el flujo atravesaba el implante, la masa era recogida en el contenedor inferior y medida con una balanza de precisión (Mettler-Toledo, PB 3002-S, Columbus Ohio, USA). La diferencia de altura entre los contenedores generaba la presión hidrostática (P), única fuerza de empuje del flujo de acuerdo con la Ley de Hagen Poiseuille. La temperatura se mantuvo a 21ºC. Una vez recogida la masa en función del tiempo (2 horas por experimento), los datos se analizaron con MATLAB (versión R2018a). Durante el experimento no observaron posibles alteraciones del circuito, como la formación de pequeñas gotas de agua.

CLASIFICACIÓN CLÍNICA Y MORFOLÓGICA. GEOMETRÍA DE LA AMPOLLA.

Para analizar la ampolla de filtración se diseñó un estudio prospectivo en el que se incluyeron 28 ojos de 28 pacientes consecutivos que íban a ser intervenidos de glaucoma mediante el implante Preserflo con el uso de mitomicina C (MMC) 0.2 mg/ml, en el mismo centro (Unidad de Glaucoma del Hospital HLA Moncloa, Oftalvist Madrid). Todos los pacientes habían sido diagnosticados previamente de glaucoma primario de ángulo abierto.

Las características preoperatorias se recogieron en todos los pacientes (demografía, cirugía combinada o no combinada, parámetros oculares y número de fármacos).

Durante el seguimiento, se recogieron datos de la PIO, la agudeza visual mejor corregida, análisis con tomografía de coherencia óptica de segmento anterior (AS-OCT) para medir la posición del tubo en cámara anterior y los diámetros horizontal y vertical de la cavidad de fluído epiescleral intra-ampolla (24 horas, una semana, un mes y tres meses). Se recogieron datos sobre el número de pacientes que presentaron hipotonía, número de fármacos y reintervenciones (revisión con aguja o revisión abierta en quirófano).

La morfología de la ampolla de filtración fue analizada clínicamente en la lámpara de hendidura por el mismo examinador que realizó la OCT. Se tomaron fotografías para realizar una clasificación clínica de acuerdo con la clasificación Indiana Bleb Grading Appearance Scale (IBAGS).

TOPOGRAFÍA Y BIOMETRÍA

Para el análisis de los cambios topográficos y biométricos se diseñó un estudio prospectivo en el que se incluyeron 29 ojos de 30 pacientes consecutivos que íban a ser intervenidos de cirugía de glaucoma con el implante Preserflo con MMC 0.2 mg/ml en el mismo centro (Unidad de Glaucoma del Hospital HLA Moncloa, Oftalvist Madrid).

Las características preoperatorias se recogieron en todos los pacientes (demografía, tipo de cirugía combinada o no combinada, parámetros oculares y número de fármacos).

Se recogieron además los siguientes datos en el pre y postoperatorio (24 horas a 3 meses): PIO, longitud axial medida con método de no contacto (IOLMaster 700, swept-source biometry, Carl Zeiss Meditec AG), equivalente esférico, y medidas de la curvatura anterior y posterior de la córnea con Pentacam HR (Oculus, Inc.) en los 8 mm centrales. Astigmatismo de cara anterior, ASA, eje plano de ASA, astigmatismo de cara posterior, PSA, eje plano PSA, astigmatismo corneal total, TCA, 4 mm, radio anterior de la córnea, plano, curvo y medio [Rf (A), Rs (A), Rm (A)], radio posterior de la córnea [Rf (B), Rs (B), Rm (B)], elevación corneal anterior (ACE max, ACE min, ACE central), elevación corneal posterior (PCE max, PCE min, PCE central) y profundidad de cámara anterior (ACD).

CAMBIOS ENDOTELIALES

Para el análisis de los cambios endoteliales se realizó un estudio prospectivo en el que se incluyeron 46 ojos de pacientes que íban a ser intervenidos de cirugía de glaucoma con el implante Preserflo en cámara anterior y MMC 0.2 mg/ml en el mismo centro (Unidad de Glaucoma del Hospital HLA Moncloa, Oftalvist Madrid).

Se incluyeron tanto pacientes de cirugía combinada como no combinada, pero en este segundo grupo se analizaron sólo pacientes pseudofáquicos.

En todos los pacientes se evaluó preoperatoriamente la paquimetría central media y la población endotelial mediante microscopía especular (Topcon specular microscope SP-1P, Topcon Corporation, Tokyo, Japan).

En cada una de las visitas postoperatorias (1, 3, y 6 meses, un año) se recogieron datos sobre la densidad de células endoteliales centrales (ECD). Asímismo, se utilizó AS-OCT para evaluar la distancia entre el tubo y el endotelio e iris (método publicado por Tan et al.[8] y la longitud total de mismo en cámara anterior. Mediante AS-OCT se evaluó la presencia de sinequias anteriores periféricas.

El análisis de datos se realizó mediante diagramas de puntos, diagramas de barra y de caja. La reducción media mensual de células endoteliales (MMR) se calculó dividiendo el cambio de ECD (preoperatorio – postoperatorio) entre el número de meses desde la cirugía. La relación entre la distancia tubo-endotelio y la pérdida endotelial se analizó mediante análisis de regresión con modelos mixtos y con el coeficiente de correlación de Spearman.

La muestra se dividió en varios grupos para análisis: dos grupos en función del tipo de cirugía (combinada faco-Preserflo-MMC 0.2 mg/ml frente a Preserflo – MMC 0.2 mg/ml) y tres grupos divididos por la distancia del tubo al endotelio (TE) a) TE < 200 µm b) TE 201-500 µm c) TE > 500 µm.

TÉCNICA QUIRÚRGICA

La técnica quirúrgica reportada por nuestro grupo está disponible en: https://journals.lww.com/glaucomajournal/Fulltext/2021/10000/Changes_to_Corneal_Topography_and_Biometrics_After.8.aspx.

Resultados

DINÁMICA DE FLUÍDOS

Las imágenes de microscopía electrónica de Preserflo mostraron diámetros luminales de 67,73 x 65,95 µm y 63,66 x 70,54 µm. El diámetro total fue 337,2 µm, y el ancho de la pared, 154 µm. El cálculo teórico de la resistencia al flujo (R_{THEO}) para una viscosidad del humor acuoso de 0,7185 centipoises (cP) fue de 1,3 mmHg/(µL/min). Por tanto, asumiendo un flujo medio y constante de humor acuoso de 2 µL/min, la presión diferencial a través del dispositivo (Δp) se estimó 2,6 mmHg. El experimento gravitacional de flujo permitió medir la resistencia experimental R_E=1.806 mmHg/(µL/min), que en condiciones fisiológicas teniendo en cuenta la viscosidad del humor acuoso fue R_{PHYS} = 1.301 mmHg/(µL/min), consistente con la resistencia teórica al flujo.

CLASIFICACIÓN CLÍNICA Y MORFOLÓGICA. GEOMETRÍA DE LA AMPOLLA.

La PIO preoperatoria mediana, 20,7 mmHg (rango, 12-30) disminuyó a 8,5 mmHg (rango, 4-17), 8,9 mmHg (rango, 5-17), 10,4 mmHg (rango, 8-16) y 10,9 mmHg (rango, 9-17) a las 24 horas, una semana, un mes y 3 meses, respectivamente (p<0,001). La morfología predominante de la ampolla de filtración fue multiforme con múltiples capas desde las primeras 24 horas a los 3 meses, mostrando una reducción progresiva de capas y microquistes a lo largo del seguimiento. El porcentaje de ampollas uniformes (ausencia de líquido entre los tejidos) en la primera semana fue bajo (3,5%). Los diámetros horizontal y vertical de la zona de filtración epiescleral aumentaron desde las primeras 24 horas hasta el tercer mes. La expansión horizontal (406 ± 127 µm a la semana, p = 0,04; 712 ± 211 µm al mes, p = 0,02 y 952 ± 218 µm a los 3 meses, p < 0,001), fue mayor que la expansión vertical (16±18 µm, p = 0,3 a las 24 horas; 63±27 µm, p = 0,02 al mes y 137 ± 34 µm, p < 0,001 a los 3 meses) sin hallar correlación con la PIO (r = -0,3, p = 0,2).

TOPOGRAFÍA Y BIOMETRÍA

La presión intraocular disminuyó significativamente desde un valor medio preoperatorio de 21,8 ± 5,2 y 16,5 ± 1,5 mmHg en los grupos de cirugía no combinada y combinada (respectivamente) a una media de 10,9±1,8 y 10,1±1,1 mmHg a los 3 meses de la cirugía (p<0,01). El astigmatismo corneal anterior, posterior y total (ASA, PSA, TCA) aumentó en cada grupo de la siguiente manera: ASA 0,4 ± 0,3/ 0,2 ± 1,0 D; PSA 0,08 ± 0,1/ 0,03 ± 0,1 D; TCA 0,4 ± 0,3/ 0,2 ± 0,9 D a los 3 meses. La elevación corneal anterior y posterior (ACE max, ACE min, PCE max) aumentó en la primera semana (p=0,01) sin cambios significativos a los 3 meses en el grupo de cirugía no combinada. Los cambios observados en el grupo de cirugía combinada no fueron significativos. La longitud axial disminuyó en 0,13±0,23 y 0,2±0,07 mm en cada grupo (p=0,01). Se observó una correlación significativa entre la PIO y la elevación máxima de la superficie posterior de la córnea en el exámen preoperatorio (r=0,93, p= 0,02).

CAMBIOS ENDOTELIALES

La densidad endotelial central disminuyó significativamente al año de la cirugía (7,4%, p=0,04), con una reducción media mensual de -14,6 ± 25 células/mm^2. La pérdida de células endoteliales parecía ser mayor cuanto menor era la distancia TE (distancia media TE = 482,9 ± 238 µm). Por un lado, se observó una reducción del 18% de ECD en el grupo < 200 µm frente al 1% en el grupo > 500 µm (p=0,08) al año de la cirugía. Por otro lado, el análisis de regresión lineal de modelos mixtos mostró esa misma tendencia (mayor pérdida cuanto menor distancia entre el tubo y el endotelio), pero la pérdida se reducía con el tiempo para una misma distancia. Por ejemplo, un tubo situado a 0 µm del endotelio perdería -174,8 ± 65,2 células/mm^2 al mes frente a 30,2 ± 11,3 células/mm^2 al año (p<0,01). A los 6 meses y al año, los tubos ubicados a > 600 µm del endotelio mostraron una disminución de densidad endotelial cercana a cero.

Discusión

Los diferentes estudios de esta tesis doctoral examinan el implante Preserflo desde el punto de vista de las ciencias básicas mediante el análisis teórico y experimental de la dinámica de fluídos, la morfología y geometría de la ampolla de filtración (consecuencia directa de la liberación de humor acuoso en el espacio subconjuntival), y desde el punto de vista clínico mediante el análisis de los cambios topográficos, biométricos y endoteliales.

En contra de lo que se ha afirmado en algunos estudios publicados al respecto, en esta tesis doctoral se demuestra que este dispositivo no fue diseñado específicamente para evitar la hipotonía, ya que la presión diferencial medida experimentalmente (2,6 mmHg) es inferior a 5 mmHg. Sin embargo, la resistencia al flujo asociada a su diámetro interno y longitud parecen reducir el riesgo de hipotonía respecto a la trabeculectomía. En comparación con otros métodos de restricción de flujo, XEN45 y Preserflo parecen ser comparables entre sí y probablemente más seguros que los dispositivos de drenaje tradicionales.

Mediante el análisis de la morfología de la ampolla de filtración se demuestra que la estandarización del flujo volumétrico proporcionado por Preserflo genera, en la mayoría de los casos, ampollas de filtración tempranas similares a las ampollas de trabeculectomía funcionantes (morfología multiforme con múltiples capas de tejido conectivo separadas por humor acuoso, líquido epiescleral cavidades y microquistes). La geometría de las cavidades de líquido epiescleral, difusas y posteriores en la mayoría de los pacientes, presentan diámetros verticales bajos, lo que podría reducir la tensión sobre la cápsula, la hipoxia, el depósito de colágeno y la fibrosis.

La ausencia de colgajo escleral, esclerectomía y suturas, así como la morfología posterior y difusa de la ampolla podrían explicar los cambios topográficos leves y transitorios observados a los 3 meses de la cirugía con este dispositivo. La inducción de astigmatismo a favor de la regla (0,4 D) fue inferior al reportado tras la trabeculectomía y comparable al de la esclerectomía profunda no perforante.

El efecto del implante Preserflo sobre la densidad de células endoteliales se asemeja a la pérdida endotelial asociada con los dispositivos de drenaje, una reducción contínua sostenida en el tiempo pero proporcionalmente inferior. Es probable que la reducción de las dimensiones del tubo y del flujo de humor acuoso justifiquen este hallazgo. Un año después de la cirugía, el porcentaje medio de pérdida endotelial (7,4 %) y la reducción media mensual de células endoteliales (-14,6 células/mm^2) parece comparable con la pérdida endotelial publicada con la válvula de Ahmed implantada en sulcus ciliar. Una menor distancia del extremo del implante Preserflo el endotelio parece causar una pérdida endotelial

más severa en cualquier momento del seguimiento, pero mayor en el postoperatorio temprano. Es decir, un mismo tubo situado siempre a la misma distancia induciría una mayor reducción endotelial al mes de la cirugía que al año. De acuerdo con los resultados de este estudio, aquellos tubos que se sitúen a más de 600 µm del endotelio inducirán pérdidas endoteliales cercanas a cero a partir de los 6 meses, lo que se podría interpretar como un posible factor de protección endotelial. La hipotonía y las sinequias anteriores periféricas son posibles factores de riesgo de pérdida endotelial en el postoperatorio inmediato.

Conclusiones

1. El implante Preserflo no fue diseñado específicamente para evitar la hipotonía, ya que la presión diferencial a través de este dispositivo es inferior a 5 mmHg y así ha sido demostrado experimentalmente en la presente tesis doctoral.
2. La mecánica de fluídos del implante Preserflo favorece la formación de ampollas multiformes y de diámetro vertical bajo en el postoperatorio temprano, factores que podrían contribuir a la reducción de la fibrosis durante el proceso de maduración de la ampolla de filtración.
3. La morfología y la geometría de la ampolla de filtración derivada de la restricción del flujo por el implante Preserflo de acuerdo con sus dimensiones (longitud y diámetro interno), parece mejorar el control de la presión intraocular reduciendo el porcentaje de hipotonía a corto y largo plazo respecto a la trabeculectomía.
4. La estandarización del flujo por este implante parece incrementar las probabilidades de obtener ampollas de filtración funcionantes a largo plazo de acuerdo con los resultados obtenidos en el postoperatorio temprano, lo que unido a una técnica quirúrgica más sencilla, podría facilitar la cirugía filtrante extendiendo su uso a un mayor número de oftalmólogos y pacientes.
5. La ausencia de colgajo escleral, esclerectomía y suturas, así como la morfología posterior y difusa de la ampolla podrían explicar los cambios topográficos leves y transitorios observados a los 3 meses de la cirugía con este dispositivo. La inducción de astigmatismo a favor de la regla parece significativamente inferior al inducido por la trabeculectomía y comparable al de la esclerectomía profunda no perforante. Una menor inducción de astigmatismo tras la cirugía podría suponer una ventaja a la hora de plantear el uso de lentes intraoculares tóricas o de diseños ópticos avanzados en los casos en los que así estuviera indicado.
6. La pérdida de células endoteliales relacionada con el implante Preserflo parece ser proporcionalmente inferior pero conceptualmente similar a la que se produce con los dispositivos de drenaje, una pérdida sostenida en el tiempo. Una menor distancia del tubo al endotelio, la hipotonía en el postoperatorio temprano y la presencia de sinequias anteriores periféricas parecen ser factores de riesgo de pérdida endotelial tras la cirugía.
7. De acuerdo con la evidencia científica publicada al respecto, la pérdida endotelial relacionada con el implante Preserflo parece ser comparable a la de su principal competidor, XEN45, comparable también a la de la válvula de Ahmed implantada en sulcus, e inferior (o al menos comparable) a la reportada tras la trabeculectomía

SUMMARY

Introduction

The reduction of intraocular pressure (IOP) has been proved the only valid treatment for glaucoma, which is the main cause for irreversible blindness worldwide[1]. In a recent study[2] carried out by the GBD 2019 Blindness and Vision Impairment Collaborators, among the global 33.6 million adults aged 50 years and older who were blind in 2020[3] the second leading cause of blindness was glaucoma (3.6 million cases, ranging from 2.8 to 4.4) after cataract.

The gold standard for glaucoma surgery is trabeculectomy, described and applied since the mid-1960s, but new surgical methods less invasive have been developed in order to reduce adverse events. One of the two micro-filtering glaucoma surgery devices available in the market is the Preserflo Microshunt (Santen Pharmaceutical Company Ltd, Osaka, Japan). This is an aqueous drainage shunt to be implanted ab externo to create a full-thickness fistula from the anterior chamber across to the subconjunctival space[4].

The flow through Preserflo has not yet been measured experimentally. The designers, based on the hypothesis that the Hagen-Poiseuille equation would "brake down" in small tube diameters and extremely hydrophobic materials such as poly(styrene-block-isobutylene-block-styrene), SIBS, did not measure the flow before the device was launched into the market. Despite the lack of knowledge about the flow properties, some studies have published that Preserflo was specifically designed to protect against hypotony. The effect that the resistance to flow through Preserflo may have on the bleb´s morphology, as well as the refractive, biometric and endothelial effects associated with this new glaucoma surgery technique are still unknown.

Objectives

1. To measure the dimensions of the Preserflo implant with field emission electron microscopy (FE-SEM) in order to confirm the internal diameter of the tube (70 µm), as stated by the manufacturer.
2. To calculate the theoretical resistance to flow (R_{THEO}) and pressure drop (Δp).
3. To experimentally measure the flow (Q) through Preserflo with a gravity-driven flow test where the hydrostatic pressure is the only motor behind the fluid flow.
4. To perform a clinical and morphological classification and a geometrical anaylisis of the filtering blebs associated with this device in the early postoperative period (24 hours to 3 months).
5. To analyze the effects that this surgical technique has on visual acuity, refraction, topography and biometry in the early postoperative period (1 week to 3 months).
6. To study the endothelial changes associated with the implantation of the Preserflo Microshunt from the first month to the first year after surgery.

Materials and methods

FLUID DYNAMICS

The luminal diameter of the tube was measured with a Quanta FEG 250 scanning electron microscope (SEM). Different measures were taken from the lumen, including edge to edge (vertically and horizontally), and edge to shadow.

The Hagen-Poiseuille equation[5] was used to calculate the theoretical resistance to flow (R_{THEO}, mmHg/(µL/min)) and the pressure differential or pressure drop (Δp= p1-p2, mmHg) through Preserflo for a known volumetric flow rate, the mean aqueous humor production in humans (Q, 2 µL/min)[6] and an aqueous humor viscosity of 0.7185 centipoises (cP) at 36 °C for primary open angle glaucoma (POAG)[7].

Experimental setup: a sample of Preserflo was inserted into a tube with a larger diameter (DT=1 mm), with both ends immersed in a physiological saline solution used to limit the presence of bubbles in the circuit, connecting two different containers located at different heights. The top container was sealed to prevent from bubble formation. Once the fluid had crossed the implant, it continued through the tube until it reached the bottom container, where the mass was measured with a precision balance (Mettler-Toledo, PB 3002-S, Columbus Ohio, USA). The difference in height between the top fluid reservoir and the bottom container induced the hydrostatic pressure P, being the only force behind the fluid flow in the circuit according to the Hagen-Poiseuille law. No surface tension mechanisms (such as droplet formation) should affect the fluid dynamics.

CLINICAL AND MORPHOLOGICAL CLASSIFICATION. BLEB GEOMETRY

To analyze the bleb features, the study was designed as a prospective, single-center case series of 28 eyes of 28 consecutive patients who underwent Preserflo glaucoma surgery with mitomycin C (MMC) at the Glaucoma Unit of the HLA Moncloa Hospital, Oftalvist Madrid. All patients were previously diagnosed with primary open-angle glaucoma (POAG).

Baseline preoperative characteristics were collected from all patients, including demographics, type of surgery (combined or stand-alone) ocular parameters and number of drugs.

During follow-up, IOP, best corrected visual acuity (BCVA) and anterior segment optical coherence tomography (AS-OCT) measurements from the tube and the size of the bleb were analyzed at 24 hours, one week, one month and three months. The number of patients with hypotony, the number of patients who required glaucoma medication, number of medications and reinterventions (needling or open revision) were also collected.

The bleb morphology was analyzed with slit lamp examination by the same examiner who performed the OCT analysis. Photographs were obtained at each visit to classify the blebs according to the Indiana Bleb Appearance Grading Scale (IBAGS).

TOPOGRAPHY AND BIOMETRY

This study was a prospective, single-center case series. 30 eyes of 29 patients were consecutively recruited at the Glaucoma Unit of the HLA Hospital Universitario Moncloa (Oftalvist Group, Madrid, Spain) and underwent glaucoma surgery with Preserflo.

Baseline preoperative characteristics were collected from all patients, including demographics, type of surgery (combined or stand-alone) and ocular parameters.

The following indices were collected, pre and postoperatively (1 day, 1 week, 1 month and 3 months): IOP, axial length (AL) using a noncontact method (IOLMaster 700, swept-source biometry, Carl Zeiss Meditec AG), spherical equivalent (SE), Pentacam HR (Oculus, Inc.) and measurements of the anterior and posterior corneal curvatures in the central 8-mm-diameter area, including anterior surface astigmatism (ASA), ASA axis (flat), posterior surface astigmatism (PSA), PSA axis (flat), total corneal astigmatism (TCA, 4 mm), anterior corneal radius flat, steep, and mean [Rf (A), Rs (A), Rm (A)], posterior corneal radius flat, steep, and mean [Rf (B), Rs (B), Rm (B)], anterior corneal elevation (ACE max, ACE min, ACE central), posterior corneal elevation (PCE max, PCE min, PCE central) and anterior chamber depth (ACD).

ENDOTHELIAL CHANGES

This study was an observational, prospective study that included 46 eyes that underwent PRESERFLO implantation in the upper-temporal or upper-nasal quadrant in the anterior chamber (AC) in the Glaucoma Department of the Oftalvist Clinic-Moncloa HLA Hospital (Madrid, Spain). Both "stand alone" and combined cataract phacoemulsification and Preserflo procedures were included, but only pseudophakic eyes were considered for "stand alone" Preserflo implantation.

All patients underwent preoperative evaluation of the central corneal thickness (CCT) and noncontact specular microscopy (Topcon specular microscope SP-1P, Topcon Corporation, Tokyo, Japan) for corneal endothelial evaluation prior to Preserflo implantation.

During postoperative visits, these measurements were repeated while accounting for the central endothelial cell density (ECD). During follow-up, AS-OCT was used to evaluate the distance of the tube from the endothelium and iris and its length in the anterior chamber. Measurements from the distal superior end of the beveled tip of the tube were performed perpendicular to the internal surface of the cornea (tube-endothelium, TE, distance), and from the distal inferior end of the tube to the iris plane (tube-iris distance, TI). These measurements were repeated at 1 week and at 1, 3, 6 and 12 months after surgery. The length of the tube in the anterior chamber (TL) was measured from the beveled tip to the angle at 3 months. Peripheral anterior synechiae were evaluated by AS-OCT.

Graphic analysis of the data distribution was performed with scatterplots, boxplots and bar graphs. The mean monthly reduction (MMR) was calculated by dividing the ECD change (preoperative -postoperative) by the number of months since surgery. Mixed model linear regression analysis and Spearman correlation index were used to analyze the relation between the tube-endothelium distance and the endothelial cell loss.

The sample was divided into different groups for analysis: Two groups divided by the type of surgery (combined phaco-Preserflo surgery vs. pseudophakic eyes who received the implant as a "solo" procedure). Three groups divided by the tube-endothelium (TE) distance: a) TE distance < 200 µm b) TE distance 201-500 µm c) TE distance > 500 µm.

SURGICAL TECHNIQUE

The surgical technique was reported by our group at: https://journals.lww.com/glaucomajournal/Fulltext/2021/10000/Changes_to_Corneal_Topography_and_Biometrics_After.8.aspx.

Results

FLUID DYNAMICS

SEM images of the Preserflo showed luminal diameters of 67.73 x 65,95 µm and 63.66 x 70.54 µm. The total diameter was 337.2 µm, and the wall was 154 µm wide. The theoretical calculation of the resistance to flow (R_{THEO}) for an aqueous humor (AH) viscosity of 0.7185 centipoises (cP) was 1.3 mmHg/(µL/min). Hence, assuming a constant AH flow of 2 µL/min, the pressure drop across the device (Δp) was estimated to be 2.6 mmHg. The gravity-flow driven experiment allowed us to measure the experimental resistance to flow R_E=1.806 mmHg/(µL/min), which in physiological conditions accounting for the aqueous humor viscosity, was R_{PHYS} = 1.301 mmHg/(µL/min), in agreement with the theoretical resistance to flow.

CLINICAL AND MORPHOLOGICAL CLASSIFICATION. BLEB GEOMETRY

The average median preoperative IOP of 20.7 (range, 12-30) mmHg decreased to 8.5 (range, 4-17), 8.9 (range, 5-17), 10.4 (range, 8-16) and 10.9 (range, 9-15) mmHg at 24 hours, one week, one month and 3 months, respectively (p<0.001). A multiform morphology on AS-OCT prevailed at all time points, with a 3.5% rate of a uniform bleb morphology at the first week. The horizontal and vertical diameters of the blebs increased from baseline to the third month. The horizontal expansion (406±127 µm on day 7, p = 0.04, 712±211 µm on day 30, p = 0.02 and 952±218 µm on day 90, p < 0.001) was greater than the vertical expansion (16±18 µm, p = 0.3 on day 1, 63±27 µm, p = 0.02 on day 30 and 137±34 µm, p < 0.001 on day 90) without a correlation with the IOP (r = -0.3, p = 0.2).

TOPOGRAPHY AND BIOMETRY

The IOP decreased from 21.8±5.2 and 16.5±1.5 mmHg at baseline to 10.9±1.8 and 10.1±1.1mmHg at 3 months in the non-combined and combined groups (p<0.01). The anterior, posterior and total corneal astigmatism (ASA, PSA, TCA) increased in each group 0.4±0.3/ 0.2±1.0 D, 0.08±0.1/ 0.03±0.1 D and 0.4±0.3/ 0.2±0.9 D respectively at 3 months. The anterior and posterior corneal elevation (ACE max, ACE min, PCE max) increased on the first week (p=0.01) with no significant changes at 3 months in the non-combined group. The changes observed in the combined group were not significant. The axial length (AL) decreased 0.13±0.23 and 0.2±0.07 mm in each group (p=0.01). There was a significant correlation between the IOP and the maximum elevation of the posterior surface of the cornea at the preoperative examination (r=0.93, p= 0.02).

ENDOTHELIAL CHANGES

Central ECD decreased significantly at 1 year (7.4%, p=0.04), with a MMR of -14.6 ± 25 cells/mm^2. A shorter distance from the tube to the endothelium showed a trend to increase the loss of endothelial cells. At one year, there was an 18% ECD reduction in the < 200 µm group vs. 1% in the > 500 µm group (p=0.08). Linear regression mixed model analysis showed that tubes located closer to the endothelium induced a higher mean monthly reduction of EC, but the loss decreased with time for the same tube position in the anterior chamber. A tube situated 0 µm from the endothelium would lose -174.8±65.2 cells/mm^2 at 1 month vs. 30.2±11.3 cells/mm^2 at 12 months, p<0.01. At 6 months and 1 year, tubes located > 600 µm from the endothelium showed EC loss close to zero.

DISCUSSION

The studies included in this dissertation examine the basic science and the clinical features of the Preserflo implant by means of the theoretical and experimental analysis of the fluid properties, and the study of the bleb morphology, visual acuity, topography, biometry and endothelial cell loss.

It has bee demonstrated that this device was not specifically designed to avoid hypotony, given that the experimental pressure drop (2.6 mmHg) is lower than 5 mmHg, against some publications about the matter. However, the resistance to flow offered by the luminal diameter and length of the tube seems to decrease the rate of hypotonic below trabeculectomy. Compared to other flow restrictive methods, XEN45 and Preserflo seem to reduce the risk of hypotony in comparison with glaucoma drainage devices.

The standardization of flow at the specific flow rate provided by Preserflo has been shown to generate, in the majority of the cases, early filtering blebs that resemble the functioning trabeculectomy blebs (multiform morphology with multiple layers of connective tissue separated by aqueous humor, episcleral fluid cavities and mycrocists). The geometry of the episcleral fluid cavities is diffuse and posterior, with low vertical diameters that would reduce the tension on the capsule, reducing hypoxia, collagen deposition and fibrosis.

The absence of scleral flap, sclerectomy and sutures, as well as the posterior and diffuse bleb morphology could explain the mild and transient topographic changes observed 3 months after surgery with this device. The WTR astigmatism induction (0.4 D) seems to be lower than trabeculectomy and comparable to deep sclerectomy.

The effect of the Preserflo Microshunt on ECD resembles the ECD loss associated with long-tube glaucoma drainage devices, an ongoing loss of endothelial cells that continues over time but at a lower rate with Preserflo. At one year, the mean percentage of ECD loss (7.4%) and the mean monthly reduction (-14.6 cells/mm^2), seem to be comparable to the ECD loss reported for the Ahmed valve located in the ciliary sulcus. A shorter distance from the tip of the tube to the endothelium appears to cause more severe ECD loss anytime during follow up, but seems to be higher in the early postoperative period. A constant position of the tube in the anterior chamber would induce a higher loss of endothelial cells at one month than at one year. According to the results of the mixed model linear regression analysis, from the 6th month, tubes located at a distance higher than 600 µm will show an endothelial loss close to zero, which could be a possible protective factor for the endothelium. Hypotony and peripheral anterior synechiae seem to be risk factors for higher endothelial cell loss in the immediate postoperative period.

CONCLUSIONS

1. The experimental measurement of the fluid dynamics of the Preserflo Microshunt has demonstrated that this device was not specifically designed to avoid hypotony, since the pressure drop across the tube is lower than 5 mmHg, and it was experimentally demonstrated in the present dissertation.
2. The fluid dynamics of the Preserflo implant favor the formation of multiform and low-vertical-diameter blebs in the early postoperative period, factors that could contribute to the reduction of fibrosis during the filtration bleb maturation process.
3. The morphology and geometry of the filtration bleb derived from the flow restriction associated with Preserflo improves intraocular pressure control with respect to trabeculectomy, the gold standard in glaucoma-filtering surgery.
4. According to the early bleb morphology, the standardization of flow through this implant could increase the rate of functioning filtering blebs in the long term. Along with an easier surgical technique with fewer complications, Preserflo could offer an advantage over trabeculectomy by the standardization of results.
5. Preserflo´s bleb morphology, along with the absence of a scleral flap, sutures and sclerectomy, induces mild and transient refractive changes. These findings could be considered an advantage over trabeculectomy by increasing the accuracy of IOL calculations and the possibility of using advanced intraocular lens optic designs.
6. Endothelial cell loss reported after surgery with Preserflo appears to be inferior or at least comparable to that reported after trabeculectomy and comparable to XEN45 and the Ahmed valve when it is placed in the sulcus. A smaller distance from the tube to the endothelium and hypotony are possible risk factors for endothelial loss with this implant.

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INTRODUCCIÓN

INTRODUCCIÓN

1.1. Ultraestructura y fluídica del implante Preserflo

Uno de los principales desafíos de la cirugía filtrante de glaucoma es el control del flujo de salida del humor acuoso para prevenir la hipotonía. En el caso de los dispositivos de drenaje no valvulados, en ausencia de un método de restricción del flujo la resistencia ofrecida por los tejidos que rodean el cuerpo valvular no es capaz de evitar la hipotonía hasta al menos aproximadamente seis semanas después de la cirugía[1]. En este periodo existe riesgo de hipotonía severa, hemorragia coroidea[2], aplanamiento de la cámara anterior y más adelante, aparición de lesiones foveales que pueden suponer una seria amenaza para la visión a largo plazo[3].

La búsqueda del control de la salida del humor acuoso ha evolucionado en los últimos años con el diseño de nuevos implantes basados en la fórmula de restricción de flujo de Hagen-Poiseuille[4]. De acuerdo con esta ecuación, que rige las propiedades de los fluídos Newtonianos no compresibles a través de un tubo cilíndrico, la resistencia al flujo (R) y la presión diferencial (Δp) se pueden modificar mediante la variación de la longitud y el diámetro. La resistencia al flujo de salida y por tanto la presión diferencial, aumentan linealmente en relación con la longitud del tubo y disminuyen a la cuarta potencia del diámetro interno. De esta forma, en los últimos años se han lanzado al mercado dos nuevos tubos de dimensiones muy reducidas, el implante de glaucoma XEN (XEN-GGM, Allergan Plc, Parsippany, NJ, EE. UU.) y el dispositivo Preserflo MicroShunt (Santen Pharmaceutical Co., Osaka, Japón) que actúan como restrictores de flujo por su longitud y sobre todo por su diámetro interno, sin la necesidad de un mecanismo valvular. Ambos están diseñados para conectar la cámara anterior con el espacio subconjuntival sin un plato ubicado en el extremo distal del tubo. La resistencia teórica al flujo y la presión diferencial, de acuerdo con la ecuación de Hagen Poiseuille, debería ser mayor a través de XEN45 (6 mm de largo, 45 µm de diámetro interno) que a través de Preserflo (8,5 mm de largo, 70 µm diámetro interno).

En el estudio publicado por Sheybani et al.[5] se demostró experimentalmente que el implante XEN45 podría mantener la presión por encima de los valores de hipotonía numérica, (5 mmHg según la Asociación Mundial del Glaucoma, WGA[6], y valor propuesto también en grandes ensayos clínicos como el límite por debajo del cual la presión intraocular no es deseable[7]) dado que la presión experimental en estado estable era 8,9 mmHg (presión diferencial) y el valor teórico, 10,98 mmHg, ambos por encima del valor considerado como hipotonía.

En el caso de Preserflo, hasta la publicación del primer artículo de la presente tesis doctoral, no parecían existir estudios experimentales publicados en la literatura científica sobre el flujo y la presión diferencial. En el estudio de laboratorio publicado por el fabricante para explicar el proceso de desarrollo de Preserflo[8], se exponían como fundamentos del diseño los estudios experimentales publicados por Arrieta et al.[9] sobre el funcionamiento del tubo en ratones, y la búsqueda de un diámetro interno que fuera superior al de una célula endotelial suelta (40-50 µm). Los autores probaron diferentes diámetros, observando que por un diámetro de 53 micras no se producía flujo (probablemente por el material hidrofóbico del que está compuesto Preserflo). De manera empírica fueron aumentando el diámetro y observando los resultados que obtenían en animales de experimentación, hasta llegar a las actuales 70 micras. Los autores argumentaron que la ecuación de Hagen Poiseuille no sería aplicable a este tubo de diámetro tan pequeño y material tan hidrofóbico (poly (styrene-block-isobutylene-block-styrene), SIBS, para explicar por qué no se midió experimentalmente el flujo antes de su lanzamiento.

La presión diferencial (Δp) a través de Preserflo se puede calcular de forma teórica con la fórmula de Hagen Poiseuille, teniendo en cuenta la viscosidad del humor acuoso, (0,7185 centipoise (cP)[10] en pacientes con glaucoma primario de ángulo abierto) y la producción media de humor acuoso en el ser humano (2 µL/min, rango de 1,8 a 4,3 µL/min [11]). El valor resultante del cálculo, Δp = 2,6 mmHg, implicaría que el implante Preserflo en teoría no sería capaz de evitar la hipotonía sólo por su capacidad de restringir el flujo.

$$P = Q \times R$$

$$P = \frac{128 \times \mu \times L \times Q}{\pi \times d^4}$$

$$R = \frac{128 \times \mu \times L}{\pi \times d^4}$$

P=Δp= p1-p2 (presión diferencial)

µ= viscosidad dinámica

L= longitud

Q= flujo volumétrico

R= resistencia al flujo

$$R = \frac{128 \times 0.7185 \times 1.25 \times 10^{-7} \times 8.5}{3.1416 \times 0.07^4}$$

$$R = 1.3 \text{ mmHg/µL/min}$$

$$P = 2 \times 1.3$$

$$\Delta p = 2.6 \text{ mmHg}$$

Por este motivo, y con el objetivo de comprobar experimentalmente los hallazgos teóricos, realizamos un estudio experimental del flujo por el implante publicado en la revista internacional ***"Translational Vision Science and Technology"***[12]. Inicialmente se analizó la ultraestructura del implante con microscopía electrónica de barrido de emisión de campo (FESEM) para confirmar que el diámetro interno fuera consistente con las especificaciones proporcionadas por el fabricante. Posteriormente se calculó la resistencia teórica al flujo (R_{THEO}) y la presión diferencial no sólo para Preserflo, si no también para otros implantes como XEN45, dispositivos de drenaje tradicionales (Válvula de Ahmed, AGV; New World Medical, Rancho Cucamonga, CA, Implante Baerveldt, BGI, Abbott Medical Optics, Santa Ana, California, USA), dispositivo Paul Glaucoma Implant (Advanced Ophthalmic Innovations, Singapore) e implante Express P200 (Alcon, Fort Worth, TX, EEUU). Finalmente, se midió experimentalmente el flujo a través de Preserflo mediante un método gravitacional basado en el experimento publicado por Estermann et al.[13] y simulando diferentes presiones intraoculares. Se halló una relación lineal entre la masa y el tiempo

y la presión intraocular y el flujo. La resistencia experimental al flujo (R_E) permitió calcular la resistencia al flujo en condiciones fisiológicas (R_{PHYS}) teniendo en cuenta la viscosidad del humor acuoso. Mediante la ecuación de Hagen Poiseuille, se calculó la presión diferencial a través de Preserflo para una producción de humor acuoso de 2 µL/min[11], confirmando Δp= 2,6 mmHg.

La confirmación experimental de los cálculos teóricos nos permitiría afirmar que este implante no fue diseñado específicamente para evitar la hipotonía.

1.2. Morfología de la ampolla de filtración tras la cirugía de glaucoma con el implante Preserflo

La estandarización del flujo a través de un implante de glaucoma pequeño y fácil de insertar, capaz de transportar un volúmen suficiente de humor acuoso como para producir ampollas funcionantes a largo plazo mientras controla la hipotonía gracias a sus reducidas dimensiones, sería muy probablemente la idea detrás del desarrollo de los nuevos implantes de microcirugía filtrante de glaucoma.

El factor determinante del éxito de la cirugía filtrante es la función a corto y a largo plazo de la ampolla de filtración. Diferentes estudios publicados acerca de la ampolla de trabeculectomía nos enseñan que el factor fundamental para el buen funcionamiento futuro de la ampolla es la presencia de acuoso entre los tejidos en el postoperatorio temprano, distribuído de manera difusa y capaz de separar el tejido en múltiples capas. Theelen et al[14] describe este fenómeno como "stripping" o "decapado", la presencia de múltiples capas paralelas e hiporreflectivas dentro de la cápsula de Tenon. Nakano et al.[15] con tomografía de coherencia óptica de segmento anterior (AS-OCT), a su vez nos muestra que la probabilidad de éxito de la ampolla de trabeculectomía es mayor a los 6 meses de la cirugía en aquellas ampollas cuyas paredes eran multilaminares a las 2 semanas de la intervención.

Conociendo la importancia de la presencia de acuoso entre los tejidos en el postoperatorio temprano de la trabeculectomía, sería razonable esperar que los nuevos microimplantes de glaucoma (XEN45, Preserflo) tuvieran la capacidad de estandarizar el flujo aportando un volúmen suficiente de acuoso desde el postoperatorio inmediato como para conseguir ampollas multiformes en el postoperatorio temprano y funcionantes a largo plazo en un porcentaje elevado de casos, simplificando la cirugía filtrante y haciéndola quizá más accesible a un mayor número de oftalmólogos y pacientes. El análisis morfológico de la ampolla de Preserflo con AS-OCT podría determinar si Preserflo tiene o no la capacidad de formar ampollas multiformes en el postoperatorio temprano, factor predictivo de éxito de las ampollas de trabeculectomía. Hasta la publicación del segundo artículo de la presente tesis doctoral, no existían estudios publicados al respecto en la literatura.

Analizando la evidencia científica sobre la morfología de la ampolla de filtración de su principal competidor, XEN 45, sobre el que sí existen publicaciones en la literatura científica, la morfología de la ampolla parece ser muy diferente dependiendo de la técnica quirúrgica (con o sin disección de la conjuntiva). Con la técnica de conjuntiva cerrada parece aumentar la probabilidad de obstrucción del tubo por la cápsula de Tenon, lo que disminuiría el flujo de acuoso y la presencia de líquido entre los tejidos. Teus[16] describe la ampolla de filtración 1 año después de la cirugía con XEN 45 y mitomicina C (MMC) 0.01% como "conjuntiva filtrante" con escasa presencia de cavidades de fluído, en lugar de una verdadera ampolla de filtración tipo trabeculectomía. Por su parte, Dangda[17] con la técnica de conjuntiva abierta, reporta ampollas comparables a las de la trabeculectomía con cavidades de fluído epiescleral en un alto porcentaje de pacientes tras la cirugía (realizada con el uso de MMC 0.02%, dosis total, 60 µg).

Con el objetivo de analizar la ampolla de filtración en el postoperatorio temprano del implante Preserflo y como parte de la presente tesis doctoral, se realizó y publicó un estudio en la revista internacional *"Acta Ophthalmologica"*[18], sobre la morfología y la geometría de la ampolla de filtración analizada mediante AS-OCT, y la posible correlación entre las dimensiones de la ampolla y la presión intraocular.

1.3. Efectos biométricos y topográficos de la cirugía con el implante Preserflo

La trabeculectomía y otros procedimientos formadores de ampolla como el implante Express y la esclerectomía profunda no perforante (EPNP), se han relacionado con cambios en la biometría ocular[19], la queratometría y la topografía corneal[20-26]. Los cambios en la curvatura corneal pueden a su vez variar la refracción, la agudeza visual e incluso influir sobre el cálculo de la lente intraocular en cirugía combinada catarata-glaucoma. Los posibles mecanismos incluyen la extracción de tejido tras la disección del tapete escleral profundo[21], la tensión en las suturas[24], las ampollas grandes o avasculares y la ptosis palpebral[27]. Hipotéticamente, un procedimiento que no precise suturas, disección de tapete escleral superficial ni profundo, y que produzca ampollas más posteriores y difusas gracias a su diseño[13,18], podría suponer una ventaja frente a la trabeculectomía al disminuir los cambios refractivos y biométricos.

Analizando la evidencia científica al respecto, no se encontraron estudios publicados sobre los efectos refractivos del implante Preserflo, motivo por el cual se planteó e inició el estudio posteriormente publicado en la revista *"Journal of Glaucoma"* como parte de esta tesis doctoral[28]. En el estudio se analizaron los cambios en la biometría y la topografía durante los 3 primeros meses del postoperatorio con este implante.

1.4. Efectos sobre la densidad de células endoteliales del implante Preserflo

La pérdida excesiva de células endoteliales y el riesgo de descompensación corneal son complicaciones temidas en cirugía de glaucoma. Existen múltiples estudios sobre los efectos que la cirugía de glaucoma tiene sobre la densidad de células endoteliales[29-33], pero en los últimos años se viene prestando una especial atención a las nuevas técnicas menos invasivas, como la cirugía microincisional de glaucoma (MIGS) con implantes trabeculares y supracoroideos y la microcirugía subconjuntival con los dispositivos XEN45 y Preserflo.

A pesar de que la fisiopatología de la pérdida de densidad endotelial no es bien conocida, al menos tres mecanismos parecen implicados: daño mecánico por proximidad del extremo del tubo al endotelio, alto flujo por el implante con pérdida de células en la zona de endotelio más próxima al extremo del tubo, e inflamación postoperatoria[29,30].

Respecto a la evidencia acerca de la influencia de la distancia tubo-endotelio sobre la densidad endotelial, la tasa de reducción media mensual de células endoteliales centrales parece ser significativamente inferior cuando el tubo de la válvula de Ahmed se coloca en sulcus ciliar (más lejos de endotelio) respecto a cámara anterior[31]. En este mismo sentido, en un estudio con 3 años de seguimiento sobre la influencia de la posición del tubo del implante Baerveldt (Abbott Medical Optics, Chicago, Illinois, USA) sobre la densidad de células endoteliales, una menor distancia entre el tubo y el endotelio parecía acelerar la pérdida celular[33].

El implante Preserflo es un tubo conceptualmente similar a los tubos de Ahmed y Baerveldt, diferenciándose de ellos por sus reducidas dimensiones. Parecería lógico pensar que un menor flujo de acuoso y un menor volúmen ocupado en cámara anterior pudieran reducir la pérdida endotelial, aunque todos ellos

siguieran un mismo patrón de reducción de densidad de células endoteliales sostenida en el tiempo. Por otro lado, si con los tubos de Ahmed y Baerveldt se ha demostrado que una menor distancia tubo-endotelio acelera la pérdida endotelial, quizá el mismo efecto se podría dar con Preserflo.

Revisando la literatura no se hallaron estudios sobre el patrón de pérdida de células endoteliales o la influencia de la posición del tubo en cámara anterior sobre la densidad endotelial, motivo del último estudio incluído en la presente tesis doctoral y publicado en la revista internacional ***"Opthalmology and Therapy"***[34] acerca de la relación Preserflo-endotelio desde el primer mes hasta el primer año tras la cirugía.

OBJETIVOS / HIPÓTESIS

Objetivos/Hipótesis

2.1. Fluídica del implante Preserflo

1. Medir con Microscopía Electrónica de Emisión de Campo (FESEM) las dimensiones del implante Preserflo para confirmar que el diámetro interno del tubo mide 70 µm, de acuerdo con las dimensiones especificadas por el fabricante.
2. Una vez confirmado el diámetro interno (70 µm) con microscopía electrónica., calcular la resistencia al flujo (R_{THEO}) y la presión diferencial (Δp_{THEO}) teóricas por el implante Preserflo mediante la Ley de Hagen Poiseuille para una producción media de humor acuoso en el ser humano de 2 µL/min.
3. Medir experimentalmente el flujo por el implante Preserflo mediante un estudio gravitacional donde la única fuerza que impulse el flujo (Q) por el implante sea la presión hidrostática, y en el que se calcule la masa de suero salino que atraviesa el tubo en función del tiempo y el flujo en función de la presión intraocular simulada variando la presión hidrostática a diferentes alturas. El cálculo matemático se realizaría con MATLAB (versión R2018a).
4. Obtener el valor de resistencia experimental al flujo (R_E) y calcular la resistencia al flujo en condiciones fisiológicas (R_{PHYS}) teniendo en cuenta la viscosidad del humor acuoso en pacientes con glaucoma.
5. Calcular la presión diferencial experimental en condiciones fisiológicas (Δp_{PHYS}) mediante la ecuación de Hagen Poiseuille teniendo en cuenta R_{PHYS} y una producción de humor acuoso de 2 µL/min.
6. Comparar la presión diferencial teórica (Δp_{THEO}) y experimental en condiciones fisiológicas (Δp_{PHYS}). Si ambas coincidieran se validarían los resultados del estudio experimental y si siendo coincidentes fueran inferiores a 5 mmHg, se podría confirmar que el implante Preserflo no fue diseñado específicamente para evitar la hipotonía.
7. Si el experimento demostrara una relación lineal entre la masa y el tiempo y entre el flujo y diferentes presiones hidrostáticas, operando en números de Reynolds por debajo de la transición al régimen turbulento, se demostraría que el flujo por este implante es laminar y cumple con la Ley de Hagen Poiseuille en contra de lo publicado acerca de este implante por los autores implicados en su diseño.

2.2. Morfología y geometría de la ampolla de filtración tras la cirugía con el implante Preserflo

1. Clasificación clínica de las ampollas de filtración asociadas con el implante Preserflo en el postoperatorio temprano (24 horas a 3 meses) mediante la clasificación "Indiana Bleb Grading Appearance Scale" (IBAGS).
2. Clasificación morfológica de las ampollas de filtración mediante AS-OCT. Las ampollas se clasifican en dos grupos, uniformes y multiformes, y dentro del grupo de ampollas multiformes en multicapa, microquistes, cavidad de fluído epiescleral y sus grados (leve, moderado, alto), en cada visita desde las primeras 24 horas hasta los 3 meses.
3. Análisis geométrico de la ampolla de filtración mediante la medida de los diámetros horizontal y vertical de la cavidad de fluído epiescleral en cada visita desde las primeras 24 horas hasta los 3 meses.

4. Análisis de la posición del tubo en cámara anterior mediante AS-OCT, midiendo la posición del extremo del tubo respecto a iris y endotelio para comprobar su estabilidad entre las primeras 24 horas y los 3 meses.
5. Análisis del efecto de la cirugía con el implante Preserflo sobre la presión intraocular desde las primeras 24 horas hasta los 3 meses.
6. Describir el porcentaje de pacientes que presentaron hipotonía, requirieron reformación de cámara anterior con viscoelástico o presentaron fibrosis de la ampolla o encapsulamiento, necesitando reintervención mediante "needling" o revisión en quirófano.
7. Se plantean varias hipótesis:

    - El flujo de humor acuoso por el implante Preserflo en el postoperatorio temprano bajo la cápsula de Tenon favorece la formación, en la mayoría de los casos, de ampollas multiformes con múltiples capas (como prueba de la presencia de humor acuoso) y la formación de cavidades de fluído epiescleral, características que serían similares a las de las ampollas tempranas de trabeculectomía con más probabilidades de ser funcionantes a largo plazo.
    - La restricción del flujo asociado a las dimensiones del implante Preserflo favorece la formación de ampollas de baja presión, en las que el diámetro vertical es menor que el horizontal, y que se caracterizan por inducir una menor fibrosis de la pared de la ampolla.
    - La distribución del humor acuoso en el espacio subtenoniano en el postoperatorio temprano se asocia con un tipo de morfología y geometría de ampolla capaz de evitar la hipotonía clínica en un porcentaje mayor del esperado de acuerdo con su presión diferencial, demostrada experimentalmente.
    - La posición del tubo en cámara anterior permanece constante salvo en los casos que presenten hipotonía, en los que la posición relativa del implante respecto a endotelio e iris disminuirá proporcionalmente a la disminución de profundidad de la cámara anterior.
    - Un mayor diámetro horizontal de la ampolla se correlaciona con una mayor disminución de la presión intraocular.

2.3. Efectos biométricos y topográficos del implante Preserflo

1. Analizar, desde la primera semana hasta el tercer mes, los siguientes cambios en dos grupos de pacientes (cirugía combinada y cirugía no combinada):

    - Disminución de presión intraocular.
    - Cambios visuales y refractivos (agudeza visual mejor corregida, equivalente esférico, esfera y astigmatismo refractivos).
    - Cambios biométricos (profundidad de cámara anterior y longitud axial).
    - Cambios observados en el astigmatismo y eje del astigmatismo (cara anterior, cara posterior y astigmatismo total).
    - Cambios observados en la queratometría.
    - Cambios observados en la elevación de la cara anterior y posterior de la córnea, máxima, mínima y central.
    - Realizar una revisión bibliográfica extensa de los cambios refractivos asociados con otros tipos de cirugía de glaucoma (trabeculectomía, implante Express, EPNP y dispositivos de drenaje) para comparación con el implante Preserflo.

2. La hipótesis principal del estudio es que los cambios visuales, refractivos y biométricos asociados con este implante son leves y transitorios, recuperando valores preoperatorios a los 3 meses de la cirugía.

3. Los cambios leves y transitorios se asociarían a una técnica quirúrgica menos invasiva y a una morfología de ampolla más difusa y posterior que la de la trabeculectomía.

2.4. Efectos sobre la densidad de células endoteliales del implante Preserflo

1. Objetivo principal: Analizar el efecto de la cirugía de glaucoma con el implante Preserflo sobre la densidad de células endoteliales (ECD) durante el primer año desde la cirugía.
2. Objetivos secundarios:

    - Análisis del cambio en la densidad de células endoteliales (ECD postoperatoria-ECD preoperatoria) frente al tiempo desde la cirugía (número de días desde la intervención).
    - Análisis de la reducción mensual (MMR) de ECD de la población total del estudio (MMR= ECD preoperatoria - ECD postoperatoria / número de meses desde la cirugía).
    - Análisis del porcentaje de pérdida de ECD central (MMR /ECD preoperatoria)
    - Análisis de la influencia de la distancia entre el extremo distal del tubo y el endotelio e iris medido con AS-OCT sobre el cambio de ECD central mediante regresión lineal de modelos mixtos y análisis de correlación de Pearson.
    - Análisis de todas las variables anteriores por grupos, en función del tipo de cirugía (cirugía combinada frente a cirugía en solitario en pacientes previamente pseudofáquicos) y por la distancia entre el tubo y el endotelio (<200 µm, 201-500 µm, >500 µm).
    - Análisis de otros factores de riesgo de pérdida endotelial (edad, hipotonía, sinequias anteriores periféricas y profundidad de cámara anterior).

3. Hipótesis:

    - La hipótesis principal del estudio es que una menor distancia entre el extremo distal del tubo y el endotelio acelera la pérdida de células endoteliales.
    - Las hipótesis secundarias son las siguientes:

        - Siendo conceptualmente comparable al tubo de un dispositivo de drenaje, el patrón de pérdida de células endoteliales debe ser similar (patrón continuado en el tiempo superior al porcentaje de pérdida fisiológica asociada a la edad).
        - La pérdida de células endoteliales debe ser menor en comparación con los tubos de drenaje tradicionales debido a sus reducidas dimensiones, volumen en cámara anterior y reducción en el flujo de humor acuoso.
        - La hipotonía, la presencia de sinequias anteriores periféricas y una menor profundidad de la cámara anterior, pueden estar relacionadas con una mayor pérdida endotelial.

DISCUSIÓN

Discusión

3.1. Fluídica del implante Preserflo y su relación con la morfología y geometría de la ampolla de filtración

El primer bloque de estudios de la presente tesis doctoral por publicaciones se inicia con el estudio de la mecánica de fluídos por el implante Preserflo. En primer lugar, se analizó su ultraestructura mediante microscopía electrónica de barrido (FESEM, Field Emission Scanning Electron Microscope) en el Laboratorio de Microscopía Avanzada de la Universidad de Zaragoza. En segundo lugar, se analizó la mecánica de fluídos de este implante mediante un estudio experimental gravitacional realizado en el Departamento de Físicas y Matemática Aplicada de la Universidad de Navarra.

Se consideró necesario confirmar el diámetro interno del implante Preserflo sobre la premisa de que la medida del implante no siempre coincide con lo reportado por el fabricante. En el estudio publicado por Samsudin[35], los autores analizaron con microscopía electrónica el diámetro interno de los modelos P50 y P200 del implante Express. De acuerdo con Alcon, las medidas debían ser 50 y 200 micras respectívamente, pero las dimensiones reales eran 205 y 206 micras. En el estudio se midió experimentalmente la resistencia al flujo por ambos modelos, encontrando una diferencia significativa (P200, 0.01 mmHg/(µL/min); P50, 0.05 mmHg/(µL/min) entre ambos que no se justificaba por diferencias en el diámetro puesto que ambos medían lo mismo. Conversaciones con la casa comercial aclararon que el modelo P50 había sido modificado mediante la inserción de una barra metálica de 150 micras dentro del tubo, lo que justificaba por un lado el aumento de resistencia al flujo, pero por otro impedía medirla experimentalmente puesto que ya no se cumpliría en él la Ley de Hagen-Poiseuille. En el caso de Preserflo, las medidas observadas con FESEM (diámetro externo 337.2 µm e interno 67.73 x 65,95 µm y 63.66 x 70.54 µm), teniendo en cuenta que las imágenes tenían una ligera inclinación, parecían comparables a las aportadas por el fabricante (350 µm diámetro externo, 70 µm diámetro interno), lo que permitió validar el cálculo teórico de la resistencia al flujo y su posterior comparación con el resultado experimental del estudio gravitacional.

El estudio experimental gravitacional realizado para medir el flujo por el implante Preserflo[12] se basó en la Ley de Hagen-Poiseuille[4], según la cual una diferencia de presión constante induce un flujo constante de fluído Newtoniano no-compresible por un tubo cilíndrico. El flujo se puede medir en función del crecimiento lineal de la masa que pasa a través de un tubo a lo largo del tiempo. En el Experimento 1, utilizando una altura de botella de 80,1 cm, suero salino como fluído y asumiendo una densidad igual a la del agua, la presión hidrostática fue 58,92 mmHg. El flujo volumétrico experimental fue Q=50,72 g/24 h o Q= 35,22 µL/min, ya que la relación entre la masa y el tiempo observada era lineal y se regía por la ecuación $w(t)= 50,72\ t+w_0$ (R^2 =0,9988). En el Experimento 2 se analizó el flujo en función de la presión hidrostática a cinco alturas (simulando diferentes presiones intraoculares), estimando una resistencia experimental al flujo R_E=1,806 mmHg/(µL/min), que en condiciones fisiológicas teniendo en cuenta la viscosidad del humor acuoso, se calculó 1,301 mmHg/(µL/min) mediante la ecuación $R_{PHYS}= R_E \times (\mu_{AQHUM}/\mu_{WATER})$, en excelente acuerdo con el valor teórico R_{THEO}=1.3 mmHg/(µL/min). Los resultados experimentales permitirían a su vez calcular el flujo por el implante para cualquier presión intraocular, lo que podría ser útil en futuros estudios acerca de la función de la pared de la ampolla de filtración. Por ejemplo, para una presión intraocular de 15 mmHg, el flujo (Q), sería 8,9 µL/min.

La presión diferencial (Δp) a través de Preserflo, calculada con la Ley de Hagen Poiseuille teniendo en cuenta R_{PHYS} = 1,301 mmHg /(µL/ min), y un flujo medio de producción de humor acuoso en el ser hu-

mano de 2 µL/min[11] fue Δp=2,6 mmHg, comparable a la presión diferencial teórica (Δp_{THEO}=2,6 mmHg). Por último, el experimento operó en números de Reynolds en el rango 25-60, lejos del flujo turbulento, lo que significa que el flujo era laminar y la Ley de Hagen-Poiseuille era por tanto válida.

A la luz de los resultados del estudio experimental, que vienen a confirmar que la presión diferencial experimental es igual a la teórica, $\Delta p_{THEO} = \Delta p_E$ = 2,6 mmHg, se puede afirmar que el implante Preserflo no fue específicamente diseñado para evitar la hipotonía. Además, en contra de algunos estudios publicados por los autores implicados en el diseño[8], es posible medir el flujo de manera experimental a través de este dispositivo, ya que a pesar de sus pequeñas dimensiones y material hidrofóbico (SIBS), el flujo es laminar y la Ley de Hagen Poiseuille, válida.

Una vez conocida la presión diferencial por el implante Preserflo, se analizaron los resultados clínicos publicados al respecto para contrastar el teórico riesgo de hipotonía con la incidencia real de la misma, comprobando que en realidad es similar a la del implante XEN45[36,37] (a pesar de tener una resistencia 3,4 veces inferior al flujo), ligeramente inferior al de la Válvula de Ahmed[38] y el dispositivo Paul GI[39] y muy inferior al de la trabeculectomía[40], tanto a corto como a largo plazo. En un estudio con 300 pacientes operados de trabeculectomía publicado por Abbas et al.[40], el porcentaje de hipotonía ascendía hasta el 47% en cualquier momento del seguimiento, mientras en el estudio multicéntrico de dos años de seguimiento sobre la eficacia y seguridad de Preserflo con dos concentraciones diferentes de MMC (0.02% vs. 0.04%)[41], el porcentaje de hipotonía era de 16.3% en el primer mes en el grupo MMC 0.04% frente a 0% en el grupo 0.02%. A largo plazo, el porcentaje de hipotonía era nulo, 0% en ambos grupos. En nuestro estudio de morfología de la ampolla de filtración[42], el porcentaje de hipotonía observado en el primer mes del postoperatorio con MMC 0.02% fue del 11%.

La incidencia de hipotonía clínica con el implante Preserflo parece indicar que probablemente sean la distribución del humor acuoso en el espacio sub-Tenoniano junto con la resistencia al flujo ejercida por el diseño del implante, los mecanismos implicados en el control de la presión intraocular en el postoperatorio temprano. A largo plazo, como consecuencia del proceso de maduración de la ampolla de filtración, la pared de la ampolla considerada funcionante ejerza una función reguladora del flujo de humor acuoso capaz de mantener estable la presión intraocular en cámara anterior, evitando la hipotonía. La morfología de la ampolla de filtración sería por tanto un factor determinante no sólo de la eficacia hipotensora, si no también del control de la presión intraocular. La investigación sobre la morfología de la distribución del humor acuoso tras la cirugía con el implante Preserflo podría revelar su capacidad formadora de ampollas funcionantes a corto y a largo plazo.

Como consecuencia de los hallazgos acerca de la fluídica del implante Preserflo y su inesperada capacidad para evitar la hipotonía, se planteó la necesidad de investigar la morfología y geometría de la distribución del humor acuoso en el espacio subtenoniano en el postoperatorio temprano. La investigación sobre el tema se desarrolló como parte de la presente tesis doctoral y fue publicada en la revista ***"Acta Ophthalmologica"***[42]. El estudio, realizado en 28 ojos de 28 pacientes desde las primeras 24 horas hasta los 3 meses, tenía como objetivo analizar la evolución morfológica de las ampollas de filtración clínicamente mediante la clasificación IBAGS "Indiana Bleb Apperance Grading Scale"[43] y morfológicamente mediante AS-OCT. Se recogieron tomografías de todos los pacientes en las diferentes visitas, midiendo tanto el tubo en cámara anterior respecto a endotelio e iris, como los diámetros horizontal y vertical de la cavidad de fluído epiescleral (análisis geométrico), realizando a su vez una clasificación morfológica de acuerdo con las características de la ampolla (uniforme vs. multiforme, y dentro de multiforme, presencia de multicapa, microquistes, zona de filtración de humor acuoso y sus grados, leve, moderado o alto).

El tipo más frecuente de ampolla hallada en estos pacientes se situaba bajo la cápsula de Tenon en una

localización posterior al limbo esclerocorneal, con una zona de drenaje de humor acuoso formada alrededor del tubo de expansión posterior. A las 24 horas, en un 28% de los casos la extensión horizontal era superior a los límites medibles, mientras a los 3 meses este tipo de ampolla se observó en un 68% de los casos. El diámetro horizontal y el vertical aumentaron significativamente desde el postoperatorio inmediato hasta el tercer mes, siendo en la mayoría de los casos el diámetro horizontal muy superior al vertical, lo que explicaría la apariencia clínica de estas ampollas (difusas y posteriores). El tejido conectivo situado por encima de la zona de filtración mostraba en la mayoría de los casos un aspecto de multicapa, tipo "hojaldre" con capas de tejido separadas por espacios de líquido y microquistes subepiteliales, muy similar al fenómeno descrito como "stripping phenomena" por Theelen[14]. La morfología uniforme (ausencia de espacios líquidos con estroma hiperreflectivo) fue poco frecuente en esta serie (3.5% en la primera semana), en contraste con los altos porcentajes de uniformidad publicados con trabeculectomía, 20.8%[15] y XEN, 48%[15,44], dos semanas después de la cirugía. La comparación directa con los resultados de uniformidad de trabeculectomía publicados por Nakano et al[15] (20,8%) fue en realidad difícil, ya que el método de clasificación utilizado en nuestro estudio siguió el método publicado por Lenzhofer et al.[44] para el análisis de la morfología de la ampolla de XEN45 en lugar del método utilizado por Nakano. En su estudio, Nakano consideraba uniforme la ausencia de líquido en la pared de la ampolla, aún cuando existiera cavidad de flúído epiescleral, mientras en el nuestro se consideró uniforme la ausencia total de líquido.

Probablemente, un hipotético mismo flujo de acuoso por debajo del tapete escleral de una trabeculectomía y por el implante Preserflo generase un porcentaje muy similar de uniformidad y multiformidad de la ampolla, pero en condiciones reales, la estandarización del flujo podría suponer una ventaja frente a la trabeculectomía, restando importancia a la habilidad y experiencia del cirujano a la hora de controlar el flujo mediante las suturas del tapete escleral. Una técnica quirúrgica más sencilla junto con la estandarización del flujo, siempre y cuando éste fuera suficiente para separar los tejidos evitando la adherencia entre ellos y favoreciendo la formación de ampollas funcionantes, podría extender el uso de este tipo de implantes a un mayor número de oftalmólogos y pacientes.

En comparación con el porcentaje de ampollas uniformes en el postoperatorio temprano reportadas con el dispositivo XEN45 cuando éste es implantado sin abrir conjuntiva y Tenon[44], (3,5% Preserflo vs. 48% XEN45), un menor volúmen de acuoso asociado a una mayor resistencia al flujo por el propio implante (3,4 veces superior a Preserflo) y una mayor resistencia al flujo de salida reportado con ésta técnica[45] podrían explicar la importante diferencia morfológica observada entre ambos dispositivos.

El riesgo de fibrosis disminuye cuando las ampollas de filtración son multiformes en el postoperatorio temprano[15,46]. Además, la morfología multiforme de la ampolla de filtración parece ser un factor predictivo de éxito en cirugía filtrante de glaucoma[15]. Un año después de la trabeculectomía, la tasas de éxito y el control de la presión intraocular eran superiores en pacientes que en el postoperatorio temprano habían mostrado una morfología multiforme de la ampolla de filtración, con microquistes y canales de fluído multicapa paralelos y persistentes[46], características muy similares a las de la ampolla de filtración Preserflo[42] en el postoperatorio temprano y muy diferentes de la "conjuntiva filtrante" descrita 1 año después de que el implante XEN 45 fuera implantado ab-interno y sin disección de conjuntiva y Tenon[16].

En los estudios publicados por Nakano y Narita[15,46], la concentración de MMC (0.04%) fue superior a la utilizada en nuestro estudio (0.02%). En el caso del estudio de Nakano[15], a los 6 meses de la cirugía el porcentaje de ampollas que mostraban una morfología favorable era el 58%, con un 42,5% de suturolísis y un 16% de needling. En nuestro estudio, un 93% de las ampollas presentaban cavidad de fluído epiescleral moderada o difusa con un 9% de revisiones en quirófano por fibrosis de la ampolla. Dado que el tiempo de seguimiento en nuestro estudio es menor, a más largo plazo los porcentajes de ampollas funcionantes

podrían en realidad ser similares entre ambas técnicas, una vez iniciado el proceso de maduración de la ampolla de Preserflo a partir del tercer mes desde la cirugía[47]. Si no tuviéramos en cuenta la diferencia en el tiempo de seguimiento, podríamos deducir que trabeculectomía y Preserflo favorecen la formación de un tipo de ampolla de morfología similar, pero con una menor concentración de MMC en el caso de Preserflo, lo que podría suponer una ventaja al disminuir el riesgo de efectos adversos relacionados con el uso de mayores concentraciones de MMC descrita con este implante[41].

Con respecto a XEN45, de acuerdo con los estudios de morfología de ampolla de filtración analizada con AS-OCT publicados por Teus[16] y Lenzhofer[44], el abordaje ab-interno sin disección conjuntival e inyección de MMC 0.01% parece aumentar el porcentaje de fibrosis (48% en las dos primeras semanas)[44] respecto a Preserflo y producir ampollas más planas y con menos cavidades de fluído respecto a la trabeculectomía[16]. Sin embargo, cuando este dispositivo se coloca bajo la cápsula de Tenon mediante una técnica abierta (Dangda et al.[17]) disminuye el riesgo de obstrucción del tubo, formándose verdaderas ampollas de filtración con los mismos rasgos morfológicos que los de las ampollas funcionantes de la trabeculectomía[15,46] y los de las ampollas formadas con Preserflo[42], siempre y cuando se utilice una dosis más alta de MMC (inyección subconjuntival de 60 µg en 0,15 ml de MMC 0,2 mg/ml a 10 mm del limbo) que la utilizada en nuestro estudio[42] (11,9-17 µg, MMC 0.2 mg/ml con esponjas durante 2 minutos[47]). Los resultados reportados por Dangda con conjuntiva abierta y una dosis significativamente mayor de MMC son sobresalientes, ya que 22 de 24 pacientes presentaron ampollas funcionantes a los 12 meses. Según los autores, la disección amplia del complejo conjuntiva-Tenon y la dosis alta de MMC podría justificar la alta tasa de éxito quirúrgico y morfológico.

Por tanto, comparando los resultados morfológicos de la ampolla Preserflo frente a la ampolla XEN45, cuando XEN45 se implanta sin disección de la conjuntiva, el riesgo de fibrosis parece ser muy superior al de Preserflo (implantado siempre con disección conjuntival) y su capacidad de formar ampollas multiformes, muy inferior, lo que reduciría la probabilidad de obtener a largo plazo ampollas funcionantes sin realizar maniobras de needling, revisión de ampolla o inyección de MMC para reducir la fibrosis asociada al menor flujo de acuoso. Por otro lado, cuando ambos implantes se colocan mediante la técnica de conjuntiva abierta, ambos parecen capaces de formar ampollas tipo trabeculectomía, con cavidades de fluído epiescleral en la mayoría de los casos, siempre y cuando se utilice una dosis mayor de MMC con XEN45 para compensar la mayor resistencia al flujo. En este caso, al igual que sucede al comparar la morfología de la ampolla Preserflo respecto a trabeculectomía, una menor dosis de MMC para alcanzar un mismo resultado morfológico podría suponer una ventaja de Preserflo respecto a XEN45 cuando ambos se implantan con apertura conjuntival.

Si queremos analizar los resultados clínicos, no ya morfológicos, en función de la técnica quirúrgica (conjuntiva abierta frente a cerrada) con un mismo implante, sólo podemos referirnos al implante XEN45. En un estudio comparativo XEN45 conjuntiva abierta frente a conjuntiva cerrada[48], con una misma dosis de MMC (0,4 mg/ml, dosis media de 54 µg en el grupo cerrado y 49,1 µg en el grupo abierto mediante inyección subconjuntival) se encontró una mayor tasa de éxito completo y una menor tasa de needling en el grupo de conjuntiva abierta. A los 12 meses, el porcentaje de reducción de la PIO fue significativamente mayor en el grupo abierto (43,1% grupo abierta, 24,8% grupo cerrada) y la tasa de needling, menor (11,8% grupo abierta vs 36,1% grupo cerrada). Por tanto, con la misma dosis de MMC, el éxito quirúrgico parece ser mayor cuando XEN 45 se implanta disecando conjuntiva y Tenon que cuando se implanta con la técnica de conjuntiva cerrada.

Si el factor a analizar es el efecto que sobre los resultados clínicos tiene el diámetro del tubo, compararemos estudios donde la técnica quirúrgica sea común (conjuntiva abierta) pero los implantes sean diferen-

tes (XEN45, Preserflo). Conocemos que la morfología de la ampolla es similar entre Preserflo y XEN45 si la dosis de MMC es más alta con XEN45[17], pero si se utiliza la misma dosis y concentración (0.02% aplicada en esponjas 2 minutos, dosis total de MMC 11,9 a 17 µg[47]), y ambos implantes se colocan tras disecar la conjuntiva, ¿cuáles son los resultados clínicos? En nuestro estudio de 28 pacientes[42], un 9% de ellos requirieron revisión en quirófano debido a fallo de la ampolla durante los primeros 3 meses, frente a un 32% de needling con XEN45 publicado por Grover et al[49].

Tanto los resultados de los estudios sobre morfología de la ampolla como los resultados de los estudios clínicos de eficacia parecen coincidir en que el porcentaje de éxito es mayor con la técnica de conjuntiva abierta frente a conjuntiva cerrada para ambos microimplantes. La dosis de MMC se deberá incrementar según disminuye el calibre del tubo para compensar la fibrosis de los tejidos asociada al aumento de resistencia al flujo y disminución del volumen de acuoso. Un aumento de 3,4 veces en la resistencia al flujo parece traducirse en un aumento similar (3-4 veces, 9% frente a 32%) del riesgo de fibrosis.

La comparación entre XEN45 y Preserflo en términos de eficacia y seguridad de la cirugía dependiendo de la técnica quirúrgica y concentración de MMC, parece indicar que la inyección de MMC con conjuntiva cerrada, utilizada exclusivamente con XEN45, incrementa el riesgo de complicaciones en comparación con la aplicación de MMC tras disección conjuntival y lavado con suero salino, técnica común a ambos dispositivos. Con la técnica de inyección de MMC-conjuntiva cerrada no parece que el aumento de concentración se traduzca en un aumento de eficacia[47]. De acuerdo con los estudios publicados al respecto, las dosis de MMC más comúnmente inyectadas durante la cirugía XEN45-conjuntiva cerrada no aumentan la eficacia (10 µg[50], 20 µg[51,52] y 40 µg[52]), y a pesar de ser más bajas que las utilizadas por Dangda[17]en su estudio XEN45-disección conjuntival (60 µg), parecen aumentar las complicaciones (9% de ampollas avasculares y 1% de erosiones conjuntivales[53] con XEN45-conjuntiva cerrada frente a 0% con XEN45-conjuntiva abierta-60 µg de MMC 0.02%[17] o 0% con Preserflo-conjuntiva abierta-MMC 0.02%[41,42] o 0.04%[41]).

En cuanto a la seguridad del uso de MMC a mayores concentraciones con el implante Preserflo, tan sólo parece existir evidencia clínica sobre la eficacia y seguridad[41], pero no sobre la influencia que una mayor concentración de MMC pudiera tener sobre la morfología de la ampolla de filtración. El uso de MMC 0.04% parece inducir una mayor disminución de presión intraocular en el sexto mes del postoperatorio con un incremento del número de pacientes libres de medicación a los dos años (90,3%) frente a sólo la mitad en el grupo MMC 0.02%. A cambio, el riesgo general de complicaciones es mayor (55,8% en el grupo 0.04% frente a 15,6% en el grupo 0.02%), incluyendo un 7% de fugas de humor acuoso en el postoperatorio temprano en el grupo 0.04% frente a 0% en el grupo 0.02%. La investigación sobre el efecto que la MMC 0.04% pudiera tener sobre la morfología de la ampolla de filtración de Preserflo podría complementar la escasa evidencia clínica sobre el uso de una u otra concentración con este dispositivo.

De acuerdo con los hallazgos acerca de la seguridad y eficacia en el uso de MMC con los nuevos microimplantes, parece razonable pensar que el riesgo de complicaciones asociadas con el uso de MMC disminuye al utilizar la técnica de conjuntiva abierta independientemente del implante, probablemente por la posibilidad de lavar la MMC con suero salino cuando se realiza disección conjuntival. A pesar de haber sido demostrado que la incidencia de efectos secundarios, especialmente la hipotonía, es superior con mayores concentraciones de MMC, pero no tan claramente con mayor tiempo de exposición[47], en el caso de la técnica de conjuntiva cerrada, aunque la concentración fuera más baja la exposición sería crónica, lo que podría justificar el mayor riesgo de complicaciones. Por otro lado, el incremento de la dosis de MMC no parece incrementar la eficacia cuando se utiliza la técnica de conjuntiva cerrada, pro-

bablemente por el alto riesgo de obstrucción del implante, mientras se incrementa el riesgo de producir ampollas avasculares y erosiones conjuntivales. La técnica de disección conjuntival parece incrementar la eficacia y seguridad tanto de XEN45 como de Preserflo.

El análisis de la geometría de la cavidad de fluído epiescleral de la ampolla, frecuentemente observada al menos hasta los 3 meses tras la cirugía con Preserflo[42], muestra una reducción del diámetro vertical respecto al horizontal. La distribución de fuerzas dentro de la cavidad, de acuerdo con su geometría, va a determinar el proceso de maduración de la pared hacia la formación de una membrana filtrante cuya función es la regulación de la presión intraocular. La reducción del diámetro vertical, de acuerdo con la Ley de Laplace[54], disminuye la presión en el interior de la cavidad de fluído reduciendo la tensión en la pared (especialmente en el techo según el modelo computacional de Gardiner[55]), disminuyendo la hipoxia tisular, el depósito de colágeno y aumentando la porosidad y por tanto la conductividad hidráulica.

El proceso de formación de la cavidad de fluído epiescleral comprende una secuencia de acontecimientos que se inician con el paso del humor acuoso desde la cámara anterior hacia el espacio subtenoniano. Según la Ley de Hagen Poiseuille[4], la presión intraocular (presión en cámara anterior) genera un flujo de acuoso a través del implante hacia la ampolla hasta que se alcanza el equilibrio entre la presión en cámara anterior y la presión dentro de la ampolla[55]. Una vez alcanzado el equilibrio de presiones, la resistencia al flujo por el tubo sería muy inferior a la resistencia por la malla trabecular, por lo que el tubo no ofrecería una resistencia adicional al circuito[55] en contra de la teoría de "resistencia en serie" propuesta por Lim et al[56] para los tubos de pequeño diámetro. Por tanto, en primer lugar, parece físicamente posible reducir las dimensiones de los tubos de cirugía filtrante de glaucoma sin disminuir la eficacia hipotensora. En segundo lugar, continuando con el modelo de Gardiner[55] según el cual una presión en la ampolla de 17 mmHg no induce la formación de tejido cicatricial pero por encima de 34 mmHg sí lo hace, si atendemos a los valores medios de presión intraocular desde las primeras 24 horas hasta los 3 meses reportados en nuestro estudio de morfología de ampolla[42] (10.9 ± 1.8 mmHg a los 3 meses) y asumiendo que la presión transcorneal es igual a la de la ampolla, el estímulo cicatricial a bajas presiones sería muy bajo en las ampollas Preserflo, lo que reduciría la cicatrización de la pared y favorecería la porosidad.

El efecto de la presión intra-ampolla sobre la maduración de la pared se puede explicar desde el punto de vista anatomo-patológico, matemático-físico y físico-bioquímico:

1. Punto de vista anatomo-patológico: De acuerdo con el estudio publicado por Molteno[1] acerca de la correlación entre los factores de control de la fibrosis de la cápsula y los cambios histopatológicos, la presión intra-ampolla desencadena diferentes respuestas en los tejidos. En caso de no haber flujo, (presión cero en la ampolla), no se estimula ninguna respuesta y los tejidos se adhieren entre si. Cuando la presión es moderada (6-12 mmHg) e inferior a la presión de cierre de los capilares, se inicia una respuesta fibroproliferativa que conduce a la formación de una cápsula filtrante y se inhibe la respuesta fibrodegenerativa que conllevaría el depósito excesivo de colágeno hacia la formación de cápsulas gruesas e impermeables. Cuando la presión en la ampolla es más alta (25 a 35 mmHg) y superior a la presión media de cierre de los capilares, se retrasa la respuesta fibroproliferativa y se acelera la fibrodegenerativa, con un depósito excesivo de colágeno. En este aspecto, el estado previo de la conjuntiva también parece jugar un papel en la permeabilidad final de la pared de la ampolla. Mastropasqua et al[58], analizando el grosor de la conjuntiva como biomarcador predictivo de la función, muestran que una conjuntiva fina e hiperreflectiva en el preoperatorio (analizada con AS-OCT) afecta negativamente al flujo de humor acuoso a través de la pared de la ampolla.
2. Punto de vista matemático-físico: Según el estudio publicado por Wilcox[54], en las ampollas de

menor diámetro vertical la presión sobre la pared es menor (de acuerdo con la Ley de Laplace), lo que reduce la tensión sobre la pared y el depósito excesivo de colágeno como consecuencia de la hipoxia tisular. Este tipo de ampollas presentan una conductividad hidráulica más alta asociada a una superficie filtrante total menor (la conductividad hidráulica es directamente proporcional al flujo a través del implante y producción de humor acuoso, e inversamente proporcional a la superficie filtrante y presión intraocular). La presión de cierre de los capilares del tejido subconjuntival estimada por Gardiner[55] es 20 mmHg, por tanto, en las ampollas que superen dicha presión, se observará isquemia, palidez y el inicio del proceso de fibrosis.

3. Punto de vista físico-bioquímico: De acuerdo con el modelo de fibrosis conjuntiva-Tenon propuesto por Gater et al[57]el flujo constante de acuoso tras la cirugía de glaucoma induce fibrosis vía presión de cizallamiento sobre la pared de la ampolla, promoviendo la sobre-expresión de citoquinas, (TGF-b, TNF-a, VEGF e IL-1), que incrementan a proliferación de los fibroblastos de la cápsula de Tenon y de la conjuntiva.

Por tanto, aunque existen otros muchos factores implicados en el proceso de maduración de la ampolla de filtración hacia el éxito en forma de ampolla con cavidad fluídica y pared filtrante, o el fracaso en forma de fibrosis (concentración y tiempo de aplicación de la MMC, glaucoma uveítico y neovascular, traumatismo conjuntival, queratoplastia penetrante, cirugía previa de glaucoma, efecto de los colirios hipotensores sobre la conjuntiva, edad, raza…), la geometría de la cavidad ejerce por sí misma un efecto crucial en el proceso madurativo, y ésta depende asímismo del flujo y volúmen de acuoso transportado por el tubo. En caso de obstrucción del tubo y ausencia de fluído, los tejidos se adherirán y fibrosarán[1]. Por el contrario, si el volúmen de fluído es excesivo, aumentará el diámetro vertical, la presión dentro de la ampolla superará la presión de cierre de los capilares del tejido subconjuntival favoreciendo la isquemia de los tejidos, la presión de cizallamiento sobre la pared inducirá la liberación de citoquinas proinflamatorias, y como consecuencia se producirá un desproporcionado depósito de matriz extracelular (proteína mayoritaria, colágeno) en la pared de la ampolla lo que disminuirá la permeabilidad, el grosor y la conductividad hidráulica, encapsulando la ampolla.

De acuerdo con los resultados obtenidos acerca de la presión intraocular en el postoperatorio temprano junto con los resultados geométricos de la cavidad de fluído[42], en la mayoría de los casos el implante Preserflo parecería capaz de transportar un volúmen moderado de acuoso a presiones bajas (inferiores a la presión de cierre de los capilares), distribuído en diámetros verticales bajos y horizontales amplios. Una presión baja dentro de la ampolla reduciría la isquemia del tejido subconjuntival y la presión de cizallamiento sobre la pared, frenando el incremento de citoquinas asociado a la propia cirugía de glaucoma y el estímulo fibrodegenerativo descrito por Molteno[1], favoreciendo la porosidad de la pared y reduciendo el riesgo de fibrosis.

Las ampollas que en su evolución presenten una mayor superficie filtrante deberán tener una menor conductividad hidráulica de la pared para evitar la hipotonía (pared más fibrosa, hiperreflectiva y quizá más fina), y las ampollas con una menor superficie filtrante deberán tener paredes más conductivas para mantener baja la presión intraocular. Dentro de estos parámetros, la ampolla tendrá diferentes morfologías pero una misma capacidad reguladora del flujo y la presión intraocular, salvo en casos extremos de encapsulamiento o fibrosis. La investigación sobre la morfología de la ampolla Preserflo a más largo plazo, determinará si las leyes físicas que rigen la presión intra-ampolla y sus efectos bioquímicos y anatomopatológicos, confirman finalmente esta hipótesis.

En conclusión, el implante Preserflo muestra una alta capacidad formadora de ampollas multiformes desde el postoperatorio temprano hasta los 3 meses, factor predictivo de éxito de la trabeculectomía.

Las ampollas asociadas con Preserflo presentan además cavidades de fluído epiesclerales en un porcentaje alto de pacientes, lo que también se ha descrito como una característica de la ampolla de filtración funcionante. En relación con su principal competidor, la evidencia científica más reciente muestra que el implante XEN45 también tiene capacidad de formar ampollas funcionantes como las de trabeculectomía y Preserflo, siempre y cuando la conjuntiva y la cápsula de Tenon se abran para asegurar que el extremo distal no queda obstruído, y que la dosis de MMC utilizada para evitar la fibrosis sea mayor ya que el flujo por el implante va a ser considerablemente menor que por Preserflo.

La estandarización del flujo en cirugía filtrante de glaucoma gracias al desarrollo del implante Preserflo podría suponer una ventaja frente a la trabeculectomía, cirugía de referencia de glaucoma descrita y aplicada desde los años 60, por su capacidad para transportar un volúmen de acuoso sujeto a escasas variaciones hacia el espacio subtenoniano, suficiente para formar ampollas multiformes en el postoperatorio temprano, y evitar la hipotonía en la mayoría de los casos. Una técnica quirúrgica más simple, unida a un flujo controlado que evite las complicaciones más frecuentes (hipotonía por exceso de flujo y fibrosis de la ampolla por defecto), podría ampliar el uso de esta técnica de cirugía de glaucoma.

3.2. Efecto del implante Preserflo sobre la visión, topografía, refracción, biometría y densidad de células endoteliales

En el segundo bloque de estudios de la presente tesis doctoral por artículos se analizan los efectos que el implante Preserflo presenta sobre la visión, refracción, topografía, biometría y densidad de células endoteliales. Dado que la morfología de la ampolla de filtración es posterior al limbo y difusa y para implantarlo no es necesaria la disección de un tapete escleral ni suturas, se plantea la hipótesis de que los efectos sobre la curvatura de la córnea (caras anterior y posterior) son inferiores a los observados con la trabeculectomía, técnica de referencia en cirugía de glaucoma. Por otro lado, un menor flujo de humor acuoso y un menor volúmen ocupado por el tubo en cámara anterior, podrían favorecer una menor pérdida de células endoteliales con este implante respecto a los tubos de drenaje tradicionales, siendo una menor distancia entre el tubo y el endotelio un posible factor de riesgo de pérdida endotelial.

En el artículo publicado en la revista ***"Journal of Glaucoma"***[28] como parte de esta tesis doctoral, se analizan los cambios en la queratometría, astigmatismo y elevación de la córnea, así como los cambios refractivos, la longitud axial y la profundidad y volúmen de la cámara anterior en el postoperatorio temprano (24 horas a 3 meses) en 30 ojos de 29 pacientes operados con el implante Preserflo. Los pacientes fueron analizados en dos grupos, cirugía no combinada y cirugía combinada (facoemulsificación del cristalino con implante de lente intraocular e implante Preserflo con aplicación de mitomicina C 0.2 mg/ml durante 2 minutos). El análisis de los cambios corneales se llevó a cabo con un sistema de tomografía que utiliza una cámara de Scheimplflug (Pentacam HR, Oculus, Inc.) para la obtención de imágenes en sección transversal del segmento anterior (50 imágenes en 2 segundos con 138.000 puntos de evaluación). Una semana después de la cirugía, el incremento medio en el astigmatismo de la cara anterior (en inglés "ASA") en el grupo de cirugía no combinada fue de 0,7 dioptrías (D) y de 0,8 D en el astigmatismo total (en inglés, TCA). En el grupo de cirugía combinada, los cambios fueron ligeramente superiores, con un incremento medio de 1 D en el astigmatismo de la cara anterior y 1,2 D en el astigmatismo total. En ambos grupos, el incremento observado en la primera semana fue disminuyendo hacia el tercer mes, mostrando una diferencia residual respecto al astigmatismo preoperatorio de 0,4D de ASA y TCA en el grupo de cirugía no combinada y 0,2 D en el de cirugía combinada. Los cambios observados en el astigmatismo de la cara posterior fueron muy leves (0,1 D) y similares en ambos grupos en la primera semana y no significativos en el tercer mes (0,08 y 0,03 D). La elevación de la cara anterior y posterior de la córnea mostró tendencia a aumentar durante la primera semana, recuperando valores preoperatorios al tercer mes. Es probable que la caída brusca

de presión intraocular en el postoperatorio inmediato induzca un aumento de la curvatura corneal con el consecuente incremento del astigmatismo y elevación de la córnea.

Existen numerosos estudios sobre el efecto de la cirugía de glaucoma sobre el astigmatismo corneal para poder comparar con los hallados tras la cirugía con el implante Preserflo. Respecto a la trabeculectomía, existen al menos 8 estudios publicados con seguimientos entre 3 meses y un año en los que el hallazgo común es la inducción de astigmatismo a favor de la regla. El primer estudio sobre el tema fue publicado por Hugkulstone[20], quien observó mediante queratometría una disminución estadísticamente significativa en el radio vertical de la córnea de 7,71mm (valor preoperatorio) a 7,36 mm desde el primer día del postoperatorio y durante los siguientes 28 días. En el caso del implante Preserflo, hallamos cambios similares a los descritos por Hugkulstone[20] y Cunliffe[21], con una disminución en el radio más curvo de 7,6 mm a 7,5 mm en la primera semana junto con un cambio en el eje más plano del astigmatismo de 105° a 135° (eje más curvo de 15 a 45°), lo que sugiere que tanto la trabeculectomía como el implante Preserflo inducen un aumento de la curvatura vertical de la córnea en el postoperatorio inmediato. En estudios posteriores que utilizaban métodos de medida del astigmatismo algo más avanzados (topografía, vídeoqueratografía computerizada,), Rosen et al.[23] observó un incremento del astigmatismo a favor de la regla de 1,5 a 2,5 D a las 12 semanas de la cirugía y Dietze et al.[24] de 1,4 D una semana tras la cirugía y de 1 D a las 12 semanas. En nuestro estudio, el astigmatismo total inducido por Preserflo a las 12 semanas de la cirugía (0,4 D) fue menor al hallado tras la trabeculectomía y clínicamente no relevante. Es poco probable que las diferencias halladas entre trabeculectomía y Preserflo se deban a una infraestimación del astigmatismo, ya que el método de medida utilizado en nuestro estudio (Pentacam HR), ha demostrado su superioridad frente a la topografía de discos de Plácido al ser capaz de medir no sólo la cara anterior si no la posterior con alta precisión[59], por lo que consideramos fiables los resultados de nuestro estudio.

Más allá de la trabeculectomía, existen varios estudios acerca de los cambios corneales asociados con otras técnicas de cirugía filtrante de glaucoma. Hammel et al.[25] en un estudio de 19 ojos operados mediante la colocación del implante Express analizó con Pentacam los cambios que se producían en el astigmatismo corneal. El implante Express, al contrario que el implante Preserflo, requiere el tallado de un tapete escleral y la colocación de suturas cuya tensión controle el flujo de salida del humor acuoso, ya que la resistencia al flujo es muy baja y muy inferior a la de Preserflo (Ex-Press P200 R=0.01 mmHg/(µL/min); P50 0,05 mmHg/(µL/min)[35], Preserflo 1.301 mmHg/(µL/min)[12]. Con un seguimiento de 3 meses, el incremento en el astigmatismo tanto de la cara anterior (2,6 a 4,7 D) como de la cara posterior (0.4 a 0.9 D) de la córnea fueron claramente superiores a los encontrados con Preserflo (0,4 D de incremento en el astigmatismo total a los 3 meses). En el caso de la esclerectomía profunda no perforante, existen estudios comparativos con trabeculectomía donde se observa una menor inducción de astigmatismo. Egrilmez et al.[26] reporta un cambio de 0,62 D con EPNP frente a 1,06 D con trabeculectomía en el postoperatorio temprano de EPNP con implante no reabsorbible T-Flux, y una menor inducción de astigmatismo en contra de la regla a los 6 meses (0,62 D vs. 1,24 D). Otros estudios comparativos entre EPNP y trabeculectomía encuentran resultados similares[60]. En resumen, los estudios publicados sugieren que la trabeculectomía y el implante Express inducen un mayor astigmatismo que la esclerectomía profunda no penetrante y el implante Preserflo.

Existen relativamente pocos estudios publicados sobre los cambios en el astigmatismo y la refracción con las técnicas de cirugía de glaucoma mínimamente invasivas (en inglés, MIGS) y con los dispositivos de drenaje de tubo largo (en inglés, GDD). Ioannidis et al.[61], analizando los cambios refractivos asociados a la cirugía combinada de femto-facoemulsificación del cristalino con implante de dos iStent inject (Glaukos, San Clemente, CA, Estados Unidos) en la malla trabecular, reportaron una disminución significativa del as-

tigmatismo preoperatorio (0.91 a 0.41 D a las 4 semanas) probablemente asociada a la cirugía de catarata con un concomitante tratamiento del astigmatismo corneal, no con los implantes de glaucoma que por su localización profunda y su mínimo tamaño es poco probable que induzcan cambios corneales. En cuanto a los efectos refractivos de los dispositivos de drenaje, en el momento actual no tenemos conocimiento de publicaciones al respecto, ni tampoco en relación con el implante XEN45. Tan sólo en el estudio realizado por Tzu et al.[62] se compararon los resultados refractivos entre cirugía de catarata, cirugía combinada con trabeculectomía y cirugía combinada con un implante de Ahmed o Baerveldt, observando un mayor cambio en el cilindro corneal en cirugía combinada frente a no combinada, pero sin distinguir entre trabeculectomía y dispositivos de drenaje.

Entre los mecanismos propuestos para explicar los cambios corneales asociados a la cirugía de glaucoma (en especial la trabeculectomía), la retracción del borde de la córnea asociada a la disección del tapete escleral profundo[21], la cauterización del lecho escleral[23] y la excesiva tensión de las suturas del tapete escleral[24], parecen ser los responsables del aumento de curvatura del diámetro vertical de la córnea. Otros factores, como las ampollas de filtración grandes, la ptosis palpebral y la hipotonía, también han sido descritos como posibles causas de inducción de astigmatismo[27]. Por su parte, el uso de mitomicina C intraoperatoria parece estar asociado con una mayor duración de los cambios corneales[63]. En nuestra serie, donde se utilizó mitomicina C 0,2 mg/ml durante 2 minutos en todos los casos, los cambios en el astigmatismo en el postoperatorio temprano parecen ser al menos comparables con los de la trabeculectomía con la misma concentración de MMC. Un seguimiento a más largo plazo de estos pacientes podría dilucidar si existen o no cambios posteriores, aunque a la luz de los resultados obtenidos en nuestro estudio la tendencia parece ser la de recuperar valores preoperatorios a los 3 meses de la cirugía.

En el caso de la cirugía con el implante Preserflo, son varios los factores que podrían explicar los cambios leves y transitorios observados en la curvatura corneal. Por un lado, no es necesaria la creación de un tapete escleral con suturas que puedan generar tensión sobre la córnea, ni tampoco una esclerectomía profunda, ambos descritos como posibles causas de astigmatismo. Por otro, el tipo de morfología de ampolla más frecuente en los 3 primeros meses tras la cirugía es difusa (diámetro vertical menor que horizontal), posterior respecto al limbo gracias a la longitud del tubo (8,5 mm) y formada por múltiples capas paralelas separadas por fluído[42] lo que podría a su vez explicar la escasez de efectos sobre la córnea. En nuestra serie[42] y en otros estudios de eficacia y seguridad[41] no se reportaron casos de ampollas avasculares, relacionadas también con cambios corneales. Por último, a pesar de ser un procedimiento penetrante, la ausencia de esclerectomía profunda y el pequeño diámetro externo del tubo (350 µm) probablemente produzcan pocos cambios en la morfología del ángulo, lo que explicaría los cambios leves en el astigmatismo y similares a los asociados a la EPNP[60].

Durante el análisis de los resultados del estudio de cambios corneales y biométricos tras la cirugía con Preserflo, se encontró una correlación positiva entre la presión intraocular basal sin medicación y la elevación de la cara posterior de la córnea, hallazgo previamente descrito por Arranz-Márquez et al[64]. Es posible que la disminución de la histéresis corneal en pacientes con glaucoma[65] favorezca una córnea más deformable en la que aumente la curvatura de la cara posterior en respuesta al aumento de presión intraocular. La histéresis corneal baja y la paquimetría fina son factores de riesgo independientes de progresión del glaucoma[66], quizá el aumento de la elevación de la cara posterior lo sea también, lo que aún está por investigar.

3.3 Cambios observados en la densidad de células endoteliales tras la cirugía con el implante Preserflo

En el artículo publicado en la revista *"Ophthalmology and Therapy"*[34] como último estudio de la presente tesis doctoral y segunda parte del bloque dedicado a los cambios corneales, se analizan los cambios sobre la cara posterior de la córnea, concretamente la capa de células endoteliales. El estudio incluyó 46 ojos a los que se realizó microscopía especular (Topcon SP-1P specular microscope, Topcon Corp. Tokyo, Japan) para analizar los cambios en la densidad de células endoteliales centrales (células/mm^2) entre el preoperatorio y el postoperatorio (1,3,6 meses y un año). En todas las visitas se realizó un exámen de la posición del tubo en la cámara anterior mediante la herramienta de medida disponible en el software del tomógrafo de coherencia óptica Optovue Avanti Widefield AS-OCT. Las medidas se realizaron siguiendo el método publicado por Tan et al[33], trazando una línea perpendicular a la cara interna de la córnea desde el extremo del Preserflo para medir la distancia tubo-endotelio (TE), y perpendicular al iris para medir la distancia tubo-iris (TI). Asímismo, se midió la longitud total del tubo en cámara anterior. Para el análisis de resultados se utilizaron varios sistemas gráficos de distribución de datos (diagrama de puntos, gráficos de caja y gráficos de barra) de la densidad de células endoteliales (en inglés, ECD) total, el cambio en la ECD (ECD postoperatoria-ECD preoperatoria) frente a tiempo desde la cirugía y la reducción media mensual (en inglés, MMR) de ECD. La MMR se calculó dividiendo el cambio en ECD (preoperatoria-postoperatoria) entre el número de meses desde la cirugía. También se calculó el porcentaje de pérdida central de ECD dividiendo el cambio mensual en ECD entre el ECD preoperatorio. Para analizar la influencia de la posición del tubo respecto a endotelio e iris en la pérdida de ECD se utilizaron modelos mixtos lineales y el índice de correlación de Pearson. La muestra fue dividida en diferentes grupos para un mejor análisis, dos grupos en función del tipo de cirugía (combinada faco-Preserflo vs. implante Preserflo sin cirugía de catarata en pacientes pseudofáquicos) y tres grupos en función de la distancia tubo-endotelio (<200 µm, 201-500 µm, > 500 µm).

El análisis de los resultados mostró que el perfil de pérdida de células endoteliales es característico de este implante, se relaciona con la posición del tubo en cámara anterior respecto a endotelio e iris, y parece ser independiente de la longitud total del tubo en cámara anterior. El porcentaje medio de disminución de ECD observado en las diferentes visitas se incrementaba en el tiempo (0.04% al mes, 2,3% a los 3 meses, 5,1% a los 6 meses y 7,4% al año), sugiriendo que el patrón de pérdida de células endoteliales es continúa en el tiempo y es similar al descrito tras la cirugía con dispositivos de drenaje[29], pero proporcionalmente inferior (AGV 10,5% y BGI 13,1% al año[31,33]).

La pérdida endotelial relacionada con la trabeculectomía y la facoemulsificación es diferente a la de los dispositivos de drenaje y el implante Preserflo. En estos casos, la disminución de densidad endotelial se relaciona con la cirugía, detectándose desde el postoperatorio temprano y estabilizándose al cabo de unos meses. En el caso de la trabeculectomía con MMC, el porcentaje de pérdida es similar a los 3 meses, 9,5%, y al año, 10%[67], y con respecto a Preserflo, el porcentaje y pérdida de células endoteliales (13,9%) y la reducción media (-265 células/mm^2) a los 3 meses[69] parecen ser superiores a los resultados encontrados en nuestra serie (2,3% y -150 células/mm^2).

El análisis de la reducción media mensual central de densidad y células endoteliales tras la cirugía con Preserflo mostró una disminución mensual mayor en el primer mes, progresivamente inferior hasta el primer año (1 mes, -125,8 ± 160 células/mm^2; 1 año -14,6 ± 25 células/mm^2). Probablemente la mayor pérdida inicial se justifique por los casos de cirugía combinada, casos de hipotonía, y por la pérdida asociada a los tubos situados muy próximos al endotelio.

En el grupo de cirugía combinada, el porcentaje medio de disminución de ECD fue superior en el primer mes (7,7% combinada frente a 0% no combinada), mientras que al año la pérdida fue comparable e incluso ligeramente superior en el grupo de cirugía no combinada, lo que sugiere que una parte de la pérdida endotelial en el postoperatorio temprano se debe a la facoemulsificación, mientras a largo plazo otros factores (probablemente relacionados con la presencia del tubo en cámara anterior y el flujo de humor acuoso) se asocian con la pérdida continuada de células endoteliales, superior a la pérdida fisiológica (0,6% al año)[70] pero inferior a la publicada con otros implantes (AGV, BGI). La pérdida endotelial en el grupo cirugía combinada fue comparable a la publicada por otros autores tras la facoemulsificación (7,6 a 9,5% a las dos semanas, 7,3% al año[70,71,72]), lo que aporta consistencia a los resultados de nuestro estudio.

El análisis por grupos de distancia tubo-endotelio sugirió que una menor distancia tubo-endotelio aceleraba la pérdida endotelial. El porcentaje de disminución endotelial fue superior en el grupo <200 µm (18%), que en los grupos 201-500 µm (11%) y >500 µm (1%). Por otro lado, la reducción media mensual en el primer mes fue superior en el grupo <200 µm (-149 ± 76 células/mm^2), frente al grupo 201-500 µm (-129 ± 195 células/mm^2) y el grupo > 501 ± (-71 ± 79 células/mm^2) y al año de la cirugía, superior en los dos grupos por debajo de 500 µm (-11 ± 9 células/mm^2, -20 ±29 células/mm^2) frente al grupo >500 µm (-3 ± 14 células/mm^2).

Un análisis más profundo de los datos mediante regresión lineal de modelos mixtos mostró una relación lineal inversa entre la reducción media mensual de ECD y la distancia tubo-endotelio en todos los momentos del seguimiento. De acuerdo con este modelo, en el primer mes los tubos situados a 0 µm del endotelio perdían -174,8 ± 65,2 células/mm^2 ($p < 0.01$) pero perdían menos células a los 3 meses (-62,2 ± 27,8 células/mm^2, p=0,02), a los 6 meses (-33,9 ± 12,8 células/mm^2, p<0,01) y al año (-30,2 ± 11,3 células/mm^2, p<0,01). A los 6 meses y al año, los tubos situados a más de 600 µm mostraron pérdidas endoteliales cercanas a cero. El análisis mediante el índice de correlación de Pearson al año de la cirugía vino a confirmar la relación inversa entre la distancia TE y la ECD (r=-0,5, p<0,01) y reveló una relación directa (r=0,38, p=0,05) entre la distancia tubo-iris y la densidad de células endoteliales, es decir, una mayor densidad de células endoteliales 1 año después de la cirugía se relacionaba con una mayor distancia del tubo al endotelio y una menor distancia del tubo al iris.

A la vista de los resultados de este estudio, se deduce que la proximidad del tubo al endotelio incrementa la pérdida de células endoteliales con una mayor influencia en el postoperatorio temprano, y que una distancia tubo-endotelio igual o superior a 600 µm puede ser un factor de protección, ya que los tubos situados a esa distancia no muestran pérdida endotelial a partir de los 6 meses. De manera similar, en el estudio publicado por Zhang et al.[32] donde se compara la pérdida de células endoteliales entre la válvula de Ahmed situada en sulcus o en cámara anterior, la pérdida mensual de ECD es mayor en el grupo con el tubo situado en cámara anterior (-29,3 células/mm^2) frente al tubo situado en sulcus (-15,3 células/mm^2). En nuestra serie, la reducción media mensual de ECD al año de la cirugía (-14,6 células/mm^2), parece comparable a la reducción media mensual de ECD con la válvula de Ahmed implantada en sulcus. En el caso del dispositivo Baerveldt, Tan[33], utilizando un modelo mixto lineal reporta una mayor pérdida de células endoteliales cuanto más próximo el tubo del endotelio. La comparación directa entre su estudio y el nuestro no parece sin embargo posible, ya que aunque ambos tubos se implantaran con el mismo ángulo, la diferente longitud en cámara anterior produciría diferencias en la distancia del tubo al endotelio siguiendo el mismo método de trazado de línea perpendicular desde el extremo del tubo.

En nuestro estudio se analizan otros posibles factores de riesgo de pérdida endotelial independientes de la posición del tubo en cámara anterior. Una menor profundidad de cámara anterior y la presencia de sinequias posteriores periféricas, parecen estar asociadas con una mayor pérdida de células endoteliales.

3.4 Fisiopatología de la pérdida endotelial con el implante Preserflo

Existen diferentes mecanismos implicados en la pérdida de células endoteliales asociada con la presencia de un tubo en cámara anterior. Por un lado, un cierto grado de inflamación crónica podría comprometer el endotelio e incrementar el riesgo de descompensación corneal[70], lo que parecería un mecanismo común para cualquier tipo de tubo, incluyendo Preserflo. Por otro lado, un posible desplazamiento del tubo, como ocurre con el tubo de Baerveldt cuando se implanta "libre" en cámara anterior podría ser un factor de riesgo de pérdida endotelial[33]. Tan[33] reporta una mayor pérdida endotelial cuando los tubos se sitúan "libres" en cámara anterior frente a los tubos transiridianos (más estables), proponiendo el movimiento del tubo como posible factor causal. La posición del tubo de Preserflo analizada en nuestro estudio sobre ampollas de filtración[42] no mostró diferencias significativas en su posición respecto a endotelio e iris desde las primeras 24 horas hasta los 3 meses, salvo en aquellos casos que presentaron hipotonía en el postoperatorio inmediato. Por tanto, en el caso de Preserflo, no parece ser el movimiento sino la distancia entre el tubo y el endotelio, la causa de la pérdida endotelial. La exploración dinámica mediante AS-OCT de la posición del tubo respecto al endotelio durante el frotamiento de la córnea podría ayudar a aclarar este punto.

En conclusión, la pérdida de células endoteliales asociada con el implante Preserflo recuerda al patrón de pérdida asociado con los dispositivos de drenaje tradicionales (pérdida sostenida en el tiempo), siendo de menor magnitud pero superior a la pérdida fisiológica anual en el ser humano. Una menor distancia entre el tubo y el endotelio parece acelerar la pérdida endotelial, siendo una distancia superior a 600 µm un posible factor de protección. Al año de la cirugía, el porcentaje de pérdida endotelial central (7,4%) y la reducción media mensual de densidad de células endoteliales (-14.6 células/mm^2) parecen comparables a la pérdida reportada con la Válvula de Ahmed colocada en sulcus. La hipotonía y la presencia de sinequias anteriores periféricas parecen ser factores de riesgo de pérdida endotelial con este implante.

CONCLUSIONES

Conclusiones

4.1. Análisis con microscopía electrónica de la ultraestructura del implante Preserflo y estudio experimental gravitacional de la mecánica de fluídos

1. Los diámetros interno y externo del implante Preserflo analizados con microscopía electrónica de emisión de campo (FESEM) son comparables con las medidas aportadas por el fabricante, lo que permite validar los cálculos teóricos de resistencia al flujo (R_{THEO}) y presión diferencial (Δp_{THEO}) y los resultados experimentales de mecánica de fluídos.
2. La resistencia al flujo teórica por el implante Preserflo, calculada mediante la Ley de Hagen Poiseuille para un flujo medio de producción de humor acuoso de 2 µL/min, es R_{THEO} = 1,3 mmHg/(µL/min) y la presión diferencial, Δp_{THEO} = 2,6 mmHg.
3. Es posible medir de manera experimental el flujo por el implante Preserflo, ya que a pesar de sus reducidas dimensiones y material hidrofóbico el flujo a través del tubo es laminar, operando en números de Reynolds por debajo de la transición al flujo turbulento, lo que valida la Ley de Hagen Poiseuille para este implante.
4. La relación entre la masa en función del tiempo y el flujo en función de la presión es lineal y ha sido demostrada con los dos experimentos gravitacionales de fluídica a través de Preserflo. El flujo volumétrico experimental hallado fue Q= 35,22 (µL/min) y la resistencia experimental al flujo R_E=1,806 mmHg/(µL/min).
5. Mediante regresión lineal, hallada experimentalmente entre la presión y el flujo, es posible calcular el flujo a través del implante Preserflo para cualquier presión intraocular, lo que podría permitir el desarrollo de futuras investigaciones acerca del funcionamiento de este y otros dispositivos de drenaje en cirugía de glaucoma.
6. La resistencia al flujo en condiciones fisiológicas (teniendo en cuenta la viscosidad del humor acuoso en pacientes con glaucoma) calculada a partir de la resistencia hallada experimentalmente es R_{PHYS}= 1.3 mmHg/µL/min, muestra un excelente acuerdo con la resistencia al flujo teórica R_{THEO}= 1.3 mmHg/µL/min, lo que valida el método experimental utilizado en este estudio y sus resultados.
7. La presión diferencial experimental, calculada mediante la ley de Hagen Poiseuille contando con la resistencia al flujo en condiciones fisiológicas (R_{PHYS}= 1.3 mmHg/µL/min) y el flujo medio de humor acuoso en el ser humano (2 µL/min), Δp_E = 2,6 mmHg, es comparable a la presión diferencial teórica (Δp_{THEO}= 2,6 mmHg).
8. La resistencia al flujo a través de Preserflo (R_{PHYS}= 1.3 mmHg/µL/min), no debería resultar redundante para la circulación de humor acuoso entre la cámara anterior y la ampolla una vez alcanzado el equilibrio de presiones, ya que se ha demostrado en el estudio de fluídica experimental que el flujo es laminar y de acuerdo a los estudios publicados al respecto por otros autores, la resistencia al flujo por diámetros tan reducidos como el de Preserflo ofrecen menos resistencia al flujo que la malla trabecular, lo que avalaría la reducción del calibre de los tubos en cirugía filtrante de glaucoma sin disminuir la eficacia hipotensora.
9. La presión diferencial teórica (Δp_{THEO}) y la experimental en condiciones fisiológicas Δp_{PHYS}, son comparables (Δp_E= Δp_{PHYS} = 2,6 mmHg) e inferiores a 5 mmHg, valor de presión intraocular considerado como no deseable en grandes ensayos clínicos e "hipotonía numérica" de acuerdo con The World Glaucoma Association.
10. Dado que la presión diferencial a través del implante Preserflo es inferior a 5 mmHg, se puede afirmar que este implante no fue diseñado específicamente para evitar la hipotonía por sus pro-

piedades físicas, sino de acuerdo con los resultados obtenidos en animales de experimentación.

11. A pesar de su teórica inhabilidad para evitar la hipotonía de acuerdo con su diseño, la tasa de hipotonía clínica es comparable a la de su principal competidor XEN45, inferior a la de los dispositivos de drenaje tradicionales (Válvula de Ahmed e implante Baerveldt) y el dispositivo Paul Glaucoma Implant y muy inferior a la de la trabeculectomía, a corto y especialmente a largo plazo.

4.2. Morfología y geometría de la ampolla de filtración en el postoperatorio temprano del implante Preserflo

1. La clasificación clínica IBAGS de las ampollas Preserflo en el postoperatorio temprano muestra que la mayoría de ellas tienen una altura baja o moderada, una extensión horizontal de unas dos horas de reloj, una vascularización media y una incidencia del 0% de ampollas avasculares, y 0% en el test de Seidel.
2. El tipo morfológico más frecuente de ampolla de filtración analizado con AS-OCT en el postoperatorio temprano (24 horas a 3 meses) tras la cirugía con el implante Preserflo junto con MMC 0.2 mg/ml, aplicada con esponjas durante 2 minutos (dosis 11,9 a 17 µg), es la ampolla multiforme con múltiples capas paralelas de tejido separadas por zonas hiporreflectivas (presencia de humor acuoso) y cavidades de fluído epiescleral en un alto porcentaje de pacientes. Los microquistes subepiteliales son más frecuentes en el postoperatorio inmediato y tienden a disminuir con el tiempo. La morfología multicapa y la presencia de cavidades de fluído epiescleral son factores predictivos positivos de la función de la ampolla de trabeculectomía a largo plazo.
3. El tipo más frecuente de ampolla de filtración observada en el postoperatorio temprano del implante Preserflo con el uso de MMC 0.02% parece comparable morfológicamente a la ampolla de trabeculectomía con MMC 0.04% publicada en la literatura científica al respecto.
4. Respecto a su principal competidor, XEN45, ambos implantes parecen capaces de formar ampollas de filtración multiformes con cavidades de fluído epiescleral comparables a la trabeculectomía, siempre y cuando se utilice en ambos casos la técnica de disección conjuntiva-cápsula de Tenon para evitar la obstrucción del tubo por esta última y que la dosis de MMC aplicada en el caso de XEN45 sea superior para compensar la mayor resistencia al flujo y menor volúmen de acuoso transportado para la misma presión intraocular.
5. Tanto los resultados de los estudios sobre morfología de la ampolla de filtración como los resultados de los estudios clínicos de eficacia parecen coincidir en que el porcentaje de éxito de la cirugía filtrante es mayor con la técnica de disección conjuntival frente a conjuntiva cerrada cuando se implantan este tipo de tubos no valvulados que restringen el flujo por la reducción del diámetro interno. Cuando se utiliza la técnica de disección conjuntival, la dosis de MMC se debe incrementar según disminuye el calibre del tubo, ya que al aumentar la resistencia al flujo disminuirá el volúmen de acuoso transportado hacia el espacio subtenoniano, favoreciendo la fibrosis. Un aumento de 3,4 veces en la resistencia al flujo parece traducirse en un aumento similar (3-4 veces superior, 9% frente a 32%) del riesgo de fibrosis con el implante XEN45 frente al implante Preserflo.
6. El análisis geométrico de la ampolla de filtración de Preserflo en el postoperatorio temprano muestra un incremento significativo de los diámetros horizontal y vertical de la cavidad de fluído intra-ampolla desde las primeras 24 horas hasta los 3 meses. El diámetro vertical medio de las ampollas es inferior al diámetro horizontal, lo que justificaría los hallazgos clínicos de la clasificación IBAGS obtenidos en este estudio (ampollas difusas poco elevadas).
7. Las ampollas Preserflo presentan diámetros verticales inferiores a los horizontales, y presiones

intra-ampolla bajas a los 3 meses de acuerdo con los resultados de presión intraocular media reportados (10.9 ± 1.8 mmHg). Según la Ley de Laplace, la reducción del diámetro vertical junto con bajas presiones intra-ampolla, favorecen la reducción de la tensión en la pared (tensión de cizallamiento) reduciendo la incidencia de hipoxia al no superar la presión de cierre de los capilares de la conjuntiva (20 mmHg) y el depósito excesivo de matriz extracelular, cuya principal proteína es el colágeno. Las presiones bajas favorecen además el proceso fibroproliferativo en la pared de la ampolla en detrimento del proceso fibrodegenerativo, lo que conduce a la formación de ampollas más porosas y permeables. La disminución de la tensión de cizallamiento en la pared de la ampolla reduce además la sobre-expresión de citoquinas proinflamatorias (TGF-b), relacionado con la disección de los tejidos en cirugía filtrante de glaucoma, lo que podría favorecer la formación a largo plazo de paredes filtrantes y ampollas de buen funcionamiento gracias a la particular restricción del flujo asociada con las dimensiones del implante Preserflo.

8. La posición del tubo en cámara anterior respecto a endotelio e iris se mantuvo estable sin diferencias significativas entre las primeras 24 horas y los 3 meses, salvo en los casos de hipotonía donde las distancias se redujeron de manera transitoria hasta la resolución del cuadro clínico. La exploración dinámica de la posición del tubo en cámara anterior mediante AS-OCT durante el frotamiento de la córnea, podría arrojar luz sobre la estabilidad real del tubo en condiciones fisiológicas.
9. El implante Preserflo reduce de manera significativa la presión intraocular durante los 3 primeros meses del postoperatorio. El porcentaje de needling reportado fue 3,5% y el porcentaje de revisiones en quirófano, 9%. El porcentaje de hipotonía durante el primer mes fue 11%.

4.3. Efecto del implante Preserflo sobre la córnea: topografía, refracción y biometría

1. La disminución de la presión intraocular por grupos de cirugía combinada y no combinada fue significativa en todas las visitas postoperatorias hasta el tercer mes ($p<0,01$).
2. La agudeza visual mejor corregida disminuyó significativamente en ambos grupos en la primera semana tras la cirugía, recuperando valores preoperatorios a los 3 meses.
3. La profundidad de la cámara anterior se incrementó significativamente en el grupo de cirugía combinada como consecuencia de la facoemulsificación e implante de lente intraocular pero permaneció estable en el grupo de cirugía no combinada al tercer mes del postoperatorio. La longitud axial disminuyó significativamente en ambos grupos.
4. El astigmatismo se incrementó en ambos grupos (cirugía no combinada y cirugía combinada) en la primera semana, mostrando una reducción progresiva hasta el tercer mes. El incremento del astigmatismo de la cara anterior fue superior en la primera semana en el grupo de cirugía combinada (1D vs. 0,7D), así como el astigmatismo total (1,2D vs 0,8D). A los 3 meses, el cambio en el astigmatismo corneal total fue de 0,4D en el grupo de cirugía no combinada, y 0,2D en el grupo de cirugía combinada.
5. La elevación de la cara anterior y posterior de la córnea se incrementó en la primera semana en ambos grupos, recuperando valores preoperatorios a los 3 meses.
6. El implante Preserflo y la trabeculectomía parecen inducir un aumento de la curvatura vertical de la córnea en el postoperatorio temprano, pero en el caso de Preserflo el cambio en el astigmatismo total observado a los 3 meses de la cirugía parece ser inferior al reportado con trabeculectomía.
7. Con respecto a otras técnicas de cirugía filtrante de glaucoma, de acuerdo con los resultados de este estudio y los estudios publicados por otros autores en la literatura científica, la trabeculectomía y el implante Express inducirían un cambio en el astigmatismo superior al de la esclerectomía

profunda no perforante y el implante Preserflo, comparables entre si.

8. No existen estudios publicados sobre el efecto refractivo del implante XEN45 para comparación con Preserflo, y la literatura sobre los dispositivos de drenaje sobre el tema es muy escasa y no ofrece resultados comparables a los obtenidos estudiando el implante Preserflo.
9. Los cambios leves y transitorios observados tras la cirugía de glaucoma con el implante Preserflo podrían estar relacionados con la ausencia de tapete escleral, esclerectomía profunda y suturas, así como con la morfología de la ampolla de filtración, alejada del limbo, difusa y con ausencia de ampollas avasculares según los resultados reportados en el estudio de morfología clínica y con AS-OCT de las ampollas de filtración en este mismo periodo postoperatorio.
10. Como hallazgo adicional, durante el análisis de datos, se reportó una correlación positiva significativa entre la elevación de la cara posterior de la córnea y la presión intraocular no medicada, hallazgo descrito previamente en la literatura y que podría ser un nuevo biomarcador de las modificaciones biomecánicas de la córnea en pacientes con glaucoma.

4.4. Efecto del implante Preserflo sobre la córnea: densidad de células endoteliales

1. El porcentaje de disminución endotelial en la población total del estudio (cirugía combinada y cirugía no combinada en pacientes pseudofáquicos) un año después de la cirugía fue de 7,4% y la reducción media mensual de densidad de células endoteliales, -14,6 células/mm^2.
2. La pérdida de células endoteliales comienza en el postoperatorio inmediato y continúa en el tiempo, de manera similar a la descrita tras la cirugía con los dispositivos de drenaje tradicionales, una pérdida sostenida en el tiempo superior a la pérdida fisiológica asociada al envejecimiento en el ser humano. Respecto a la trabeculectomía y la facoemulsificación, donde la pérdida se relaciona con la cirugía y posteriormente se estabiliza, la pérdida endotelial con Preserflo parece seguir un patrón diferente y más parecido al de los tubos de drenaje.
3. La comparación entre la pérdida de células endoteliales a los 3 meses de la cirugía entre trabeculectomía y Preserflo, ambos con el uso de MMC 0.2 mg/ml, muestra, sin embargo, que la reducción endotelial es mayor que la observada con Preserflo (trabeculectomía 13,9% y reducción media mensual -265 células/mm^2, Preserflo 2,3% y -150 células/mm^2).
4. Con respecto a su principal competidor, XEN45, la pérdida endotelial parece ser comparable a los 3 meses de la cirugía (2,1% XEN45, 2,3% Preserflo), y en ambos casos inferior a la reportada tras la trabeculectomía (10%). Conociendo la pérdida endotelial anual asociada con Preserflo, y siendo la de XEN45 a los dos años de la cirugía 15,4%,la pérdida endotelial parece comparable entre XEN45 y Preserflo a largo plazo.
5. La comparación entre grupos de cirugía combinada y no combinada sugiere que la pérdida endotelial en el primer mes observada en el grupo de cirugía combinada se debe a la facoemulsificación, comparable a la pérdida endotelial publicada con esta técnica en la literatura, y superior a la reducción media observada en el grupo de cirugía no combinada (0%). A más largo plazo, la pérdida endotelial se iguala entre los dos grupos, y probablemente se asocia con la presencia del tubo en cámara anterior y el flujo de humor acuoso.
6. Con respecto a los dispositivos de drenaje tradicionales implantados en cámara anterior, el porcentaje de pérdida al año de la cirugía parece ser superior al reportado en el presente estudio con Preserflo (Válvula de Ahmed 10,5% a 15,3%, Preserflo 7,4%).
7. La proximidad entre el extremo del implante Preserflo y el endotelio parece incrementar la magnitud de la pérdida endotelial, igual que sucede con los implantes Ahmed y Baerveldt de acuerdo con estudios publicados al respecto.
8. Respecto a la válvula de Ahmed, la pérdida endotelial observada con Preserflo parece ser comparable a la reportada con la Válvula de Ahmed cuando el tubo se implanta en sulcus (Preserflo -14,6

células/mm^2, Ahmed -15,3 células/mm^2).

9. Mediante análisis de regresión lineal de modelos mixtos entre la distancia del extremo del implante Preserflo y el endotelio y la reducción de densidad endotelial, una menor distancia tubo-endotelio se relaciona con una mayor pérdida endotelial, mayor en el postoperatorio inmediato y menor a más largo plazo. Se deduce que la inserción del tubo en cámara anterior en cualquier posición produce una pérdida endotelial inicial que se va reduciendo en el tiempo, pero de mayor magnitud cuanto menor es la distancia entre el tubo y el endotelio.
10. El análisis por grupos de distancia tubo-endotelio viene a confirmar los hallazgos de regresión lineal de modelos mixtos, ya que la pérdida endotelial al año de la cirugía es superior porcentualmente en los tubos situados a menos de 200 µm (17,9%) frente a los situados a más de 500 µm (1,1%).
11. Una distancia entre el extremo del implante Preserflo y el endotelio superior a 600 µm parece ser un factor de protección, ya que los tubos situados a más de esa distancia mostraron pérdida endotelial cercana a cero a los 6 y 12 meses de la cirugía.
12. Una menor profundidad de cámara anterior y la presencia de sinequias anteriores periféricas como consecuencia de la hipotonía en el postoperatorio temprano se relacionan con una mayor pérdida de células endoteliales.

Conclusiones Generales

1. El implante Preserflo no fue diseñado específicamente para evitar la hipotonía, ya que la presión diferencial a través de este dispositivo es inferior a 5 mmHg y así ha sido demostrado experimentalmente en la presente tesis doctoral.
2. La mecánica de fluídos del implante Preserflo favorece la formación de ampollas multiformes y de diámetro vertical bajo en el postoperatorio temprano, factores que podrían contribuir a la reducción de la fibrosis durante el proceso de maduración de la ampolla de filtración.
3. La morfología y la geometría de la ampolla de filtración derivada de la restricción del flujo por el implante Preserflo de acuerdo con sus dimensiones (longitud y diámetro interno), mejora el control de la presión intraocular reduciendo el porcentaje de hipotonía a corto y largo plazo respecto a la trabeculectomía.
4. La estandarización del flujo por este implante parece incrementar las probabilidades de obtener ampollas de filtración funcionantes a largo plazo de acuerdo con los resultados obtenidos en el postoperatorio temprano, lo que, unido a una técnica quirúrgica más sencilla, podría facilitar la cirugía filtrante extendiendo su uso a un mayor número de oftalmólogos y pacientes.
5. La morfología de la ampolla de filtración, la ausencia de tapete escleral, suturas y esclerectomía profunda, parecen reducir los cambios refractivos asociados con este implante, reportados como leves y transitorios, lo que supondría una ventaja a la hora de plantear el uso de lentes intraoculares tóricas o de diseños ópticos avanzados en los casos en los que así estuviera indicado.
6. La pérdida de células endoteliales relacionada con el implante Preserflo parece comparable a la de su principal competidor, el implante XEN45, inferior a la trabeculectomía, y comparable a la de la Válvula de Ahmed cuando el tubo se coloca en sulcus. Una menor distancia del tubo al endotelio y la hipotonía son factores de riesgo de pérdida endotelial con este implante.

SUMMARY

Introduction

5.1 Preserflo ultrastructure and fluid dynamics

One of the main challenges of glaucoma filtering surgery is the control of aqueous humor (AH) outflow to prevent hypotony. In the case of nonvalved glaucoma drainage devices, in the absence of a flow-restrictive method, the resistance to flow offered by the tissues that surround the valve plate cannot prevent hypotony until at least approximately six weeks after surgery[1]. In this period, there is a risk of severe hypotony, choroidal hemorrhage[2], flattening of the anterior chamber and, subsequently, hypotony-associated foveal lesions that lead to long-lasting visual acuity loss[3].

The search for the control of aqueous humor outflow has evolved in recent years with the design of new implants based on the Hagen-Poiseuille[4] flow restriction formula. Resistance to flow (R) and pressure drop (Δp) can be modified by means of the length and width of a tube according to this equation, which governs the properties of noncompressible Newtonian fluids through cylindrical pipes. The resistance to flow is directly proportional to the pressure drop, and both values increasing linearly in relation to the length of the tube and decreasing to the fourth power of the internal diameter. Thus, in recent years, two new "miniaturized" tubes have been launched, the XEN glaucoma implant (XEN-GGM, Allergan Plc, Parsippany, NJ, USA) and the Preserflo Microshunt (Santen Pharmaceutical Co., Osaka, Japan), to act as flow restrictors just by their dimensions, without the need for a valved mechanism. Both devices were designed to connect the anterior chamber with the subconjunctival space without a plate located at the end of the tube. The theoretical resistance to flow and pressure drop, according to the Hagen Poiseuille equation, should be greater for XEN 45 (6 mm long, 45 µm internal diameter) than for Preserflo (8.5 mm long, 70 µm internal diameter).

In the study published by Sheybani et al.[5], it was experimentally demonstrated that the XEN 45 implant could maintain the back pressure above the values of numerical hypotony (5 mmHg according to the World Glaucoma Association and large clinical trials[6,7]). The authors reported that the experimental steady-state pressure through XEN 45 was 8.9 mmHg and the theoretical value was 10.98 mmHg.

Regarding Preserflo´s flow properties, to the best of our knowledge, there have been no studies on the experimental measurement of the flow through the implant. The laboratory study carried out by the manufacturer to explain the development of this device[8] showed that the tube dimensions had been based on the empirical data generated by the rabbit studies carried out by Arrieta et al.[9], who searched for a luminal diameter that was at least greater than that of a sloughed endothelial cell (40-50 µm). The authors tried different luminal diameters, noting that there was no flow when the internal diameter was as low as 53 µm (probably due to the hydrophobic properties of the chemical composition of Preserflo). The researchers progressively increased the size and empirically reached the actual diameter (70 µm) based on the results obtained in test animals. They did not experimentally measure the flow through the implant, based on the hypothesis that the Hagen-Poiseuille equation would "brake down" in small tube diameters and extremely hydrophobic materials such as poly(styrene-block-isobutylene-block-styrene), SIBS.

The pressure drop (Δp) through Preserflo, calculated with the Hagen Poiseuille formula (Δp=2.6 mmHg),

for an aqueous humor viscosity of 0.7185 centipoise (cP)[10] in open angle glaucoma and a mean aqueous humor production of 2 µL/min in humans[11], would theoretically make Preserflo unable to avoid hypotony just by means of its capacity to restrict the flow.

$$P = Q \times R$$

$$P = \frac{128 \times \mu \times L \times Q}{\pi \times d^4}$$

$$R = \frac{128 \times \mu \times L}{\pi \times d^4}$$

P=Δp= p1-p2 (pressure drop)

µ= dynamic viscosity

L= length

Q= volumetric flow

R= resistance to flow

$$R = \frac{128 \times 0.7185 \times 1.25 \times 10^{-7} \times 8.5}{3.1416 \times 0.07^4}$$

$$R = 1.3 \text{ mmHg/µL/min}$$

$$P = 2 \times 1.3$$

$$\Delta p = 2.6 \text{ mmHg}$$

For this reason, we conducted a study that aimed to analyze the ultrastructural and flow properties of the Preserflo device to confirm the theoretical findings that were finally published in the international journal "***Translational Vision Science and Technology***"[12]. Initially, the ultrastructure was analyzed with a field emission scanning electron microscope (FESEM) to confirm that the luminal diameter was consistent with the specifications provided by the manufacturer. In addition, the theoretical resistance to flow and pressure drop were calculated, not only through Preserflo, but also through XEN 45 and other glaucoma drainage devices for comparison (Ahmed Glaucoma Valve [AGV; New World Medical, Rancho Cucamonga, CA], Baerveldt Glaucoma Implant [BGI, Abbott Medical Optics, Santa Ana, California, USA], Paul Glaucoma Implant [Advanced Ophthalmic Innovations, Singapore] and Express P200 [Alcon, Fort Worth, TX, EEUU]). Finally, an experimental fluid-flow test using a gravity-flow setup based on the same principles as those of the experiment published by Estermann[13] was performed, simulating different intraocular pressures (IOPs). A linear relation was found between the collected mass as a function of time and the flow as a function of the simulated IOP. The experimental resistance to flow that was found with this method (R_E) was

extrapolated to more physiological conditions (R_{PHYS}) accounting for the viscosity of the aqueous humor in open angle glaucoma, and the Hagen Poiseuille formula was used to calculate the pressure drop with R_{PHYS} and the mean aqueous humor production of 2 µL/min in humans[11].

The experimental validation of the theoretical calculations confirmed that the Preserflo implant was not specifically designed to avoid hypotony.

5.2 Bleb morphology after glaucoma surgery with the Preserflo Microshunt

The standardization of flow through a small glaucoma tube that was easy to implant, capable of transporting a sufficient volume of aqueous humor to produce functioning filtering blebs in the long term while controlling hypotony due to its reduced dimensions, would probably be the idea behind the development of new implants in microfiltering glaucoma surgery.

The primary factor involved in the success of glaucoma filtering surgery is that the bleb becomes and remains functional. The presence of aqueous humor, capable of separating the tissue into multiple layers in the early postoperative period, seems to be crucial to the development of a successful filtering bleb in the long term. The presence of aqueous humor between the tissues was described by Theelen et al.[14] as "stripping phenomena", multiple parallel layers and hyperreflectivity inside Tenon´s capsule. According to Nakano´s findings using anterior segment optical coherence tomography (AS-OCT) to analyze trabeculectomy blebs[15], the blebs that showed a multilayered wall 2 weeks after surgery were more likely to show good bleb function at 6 months.

Based on the concept that the separation of the subconjunctival tissue by the aqueous humor in the early postoperative period increases the probability of bleb success, it would be reasonable to expect that the new microfiltering implants (XEN45, Preserflo) had been designed to carry a sufficient volume of aqueous from the anterior chamber to the subconjunctival space enough to reduce fibrosis and increase long-term bleb function. Along with the easy surgical technique, a higher rate of predictable results could be an advantage for these new implants. In this sense, the morphological analysis of the Preserflo´s filtering bleb with AS-OCT could determine whether this implant has the capacity to form multiform blebs in the early postoperative period, which is a predictive factor of success in trabeculectomy. Until the publication of the second study of this dissertation, there were no studies published about this subject in the literature.

Analysis of the published studies about the bleb morphology after surgery with the XEN45 implant (Preserflo´s main competitor) revealed the important morphological differences between the filtering blebs depending on whether the conjunctiva was dissected. With the closed conjunctiva approach, the probability of tube obstruction by Tenon is higher, decreasing the flow of aqueous and the presence of fluid between the tissues. Teus et al.[16] described the blebs formed 1 year after XEN45 implanted with a closed conjunctiva and MMC 0.01% as a "filtering conjunctiva" with scarce presence of fluid, instead of a real trabeculectomy bleb. Dangda et al.[17], who used the open conjunctiva technique and MMC 0.02% (total dose, 60 µg), reported blebs that were morphologically comparable to those formed by trabeculectomy, with episcleral fluid cavities in the majority of the patients.

To the best of our knowledge, the bleb morphology explored by AS-OCT in the early postoperative period of the Preserflo Microshunt had not been investigated until the publication of our study in the journal "***Acta Ophthalmologica***"[16]. In the study, AngioVue OCT software (Optovue, Inc., Fremont, CA, USA) was used to analyze the bleb morphology in the early postoperative period (24 hours to 3 months) and the possible correlation between the bleb geometry and the IOP.

5.3 Effect of the Preserflo Microshunt on corneal topography and ocular biometry

Glaucoma filtering surgery, like trabeculectomy and other bleb-forming procedures such as the Ex-Press glaucoma implant and nonpenetrating deep sclerectomy (NPDS), have been related to changes in ocular biometry[19], keratometry and corneal topography[20-26]. Changes to the corneal curvature may have an impact on refraction, visual acuity and, thus, IOL power calculation. The cause of these changes may be related to the removal of tissue under the scleral flap in trabeculectomy[21], tight sutures[24], large drainage or avascular blebs and ptosis[27]. Hypothetically, a sutureless and nonscleral flap-dependent technique, as well as deeper and more posterior filtering blebs[13,18], could provide advantages over trabeculectomy by reducing the amount of induced corneal and refractive changes.

The lack of data on the refractive changes induced by this implant prompted us to evaluate the biometric and topographic changes that occurred during the first 3 months of the postoperative period. The study was published in the ***"Journal of Glaucoma"***[28] as part of this dissertation.

5.4 Effect of the Preserflo Microshunt on endothelial cell density

Endothelial cell loss (ECL) leading to corneal decompensation is one of the major concerns regarding glaucoma surgical procedures. Many studies have reported the effects of glaucoma surgery on corneal endothelial cells (CECs)[29-33], but in recent years, attention has been focused on the effects of relatively new, less invasive techniques, such as microincisional glaucoma surgery (MIGS) and microfiltering surgery with new tube shunts (XEN 45, Preserflo).

Although the pathophysiology of endothelial cell loss is not well understood, it has been proposed to involve at least three mechanisms: first, mechanical damage derived from the proximity of the implant to the endothelium; second, the high fluid flow of aqueous humor through the tube, inducing ECL proximal to the tube entry site; and third, postoperative inflammation[29,30].

Based on the evidence published about the influence of the position of the tube of the Ahmed glaucoma valve with respect to the endothelium, the rate of mean monthly reduction of central endothelial cells has recently been reported to be significantly higher when the tube is located in the anterior chamber than when it is located in the ciliary sulcus[31]. Similarly, a three-year follow-up study about the position of the tube of the Baerveldt Glaucoma Implant as a risk factor for endothelial cell loss showed that a shorter tube-endothelium distance accelerated the loss of endothelial cells[33].

The Preserflo device is conceptually comparable to the Ahmed and Baerveldt implants, all of which are tubes that differ in their dimensions. Theoretically, a lower rate of aqueous flow through a smaller luminal diameter and a lower volume occupied in the anterior chamber could reduce the rate of ECL with Preserflo compared to the larger tubes. Moreover, if a shorter tube-endothelium distance has been shown to increase ECL with AGV and BVI, the same effect can probably be found after Preserflo implantation.

However, the effects of the location of Preserflo in the anterior chamber (distance from the endothelium and the iris and the total length of the tube) on ECD have not yet been analyzed. The last study included in this dissertation aimed to evaluate the effect of tube location on corneal endothelial cell density after implantation of the Preserflo Microshunt and was published in the international Journal ***"Ophthalmology and Therapy"***[34]

OBJECTIVES / HYPOTHESIS

Objectives/Hypothesis

6.1 Fluid dynamics of the Preserflo Microshunt

1. To measure the dimensions of the Preserflo implant with field emission electron microscopy (FE-SEM) in order to confirm the internal diameter of the tube (70 µm), as stated by the manufacturer.
2. Once the internal diameter (70 µm) has been confirmed by FESEM, to calculate the theoretical resistance to flow (R_{THEO}) and pressure drop (Δp_{THEO}) through Preserflo using the Hagen Poiseuille equation accounting for a mean aqueous humor production of 2 µL/min in humans.
3. To experimentally measure the flow (Q) through Preserflo with a gravity-driven flow test where the hydrostatic pressure is the only motor behind the fluid flow. The flow will be measured as the collected mass as a function of time, and the fluid flow will be measured as a function of the intraocular pressure, simulated by varying the hydrostatic pressure at different heights. The mathematical calculations will be obtained with MATLAB (version R2018a).
4. To experimentally obtain the resistance to flow (R_E) and to calculate the resistance to flow in physiological conditions (R_{PHYS}) accounting for the viscosity of the aqueous humor in glaucoma patients.
5. To calculate the experimental pressure drop in physiological conditions (Δp_{PHYS}) using the Hagen Poiseuille equation accounting for R_{PHYS} and an aqueous humor production of 2 µL/min.
6. To compare the theoretical pressure drop (Δp_{THEO}) with the experimental pressure drop under physiological conditions (Δp_{PHYS}). If they were comparable and lower than 5 mmHg, it could be confirmed that the Preserflo implant is not specifically designed to avoid hypotony.
7. If the experiment showed a linear relation between the collected mass and the time and the flow at different hydrostatic pressures while operating at Reynolds numbers below the transition to the turbulent regime, it would be demonstrated that the flow through Preserflo is laminar and ruled by the Hagen Poiseuille Law. These findings would prove that the statement made by the authors involved in the design of this implant (the Hagen Poiseuille Law "broke down" with Preserflo and was not applicable) is wrong.

6.2 Bleb´s morphology and geometry in the early postoperative period after surgery with Preserflo

1. Clinical classification of the filtering blebs associated with the Preserflo implant in the early postoperative period (24 hours to 3 months) with the IBAGS classification (Indiana Bleb Grading Appearance Scale).
2. Morphological classification of the filtering blebs with AS-OCT. The blebs will be classified into two groups, uniform and multiform, and in the multiform group, different features will be described (multilayered stroma, microcysts, episcleral drainage pathway or episcleral fluid cavity) at three levels (low, moderate or high) at each visit from 24 hours to 3 months.
3. Geometrical analysis of the filtering bleb measuring the horizontal and vertical diameters of the fluid cavity at each visit from 24 hours to 3 months.
4. An analysis of the tube´s position in the anterior chamber with AS-OCT, measuring the distance from the distal end of the tube to the endothelium and iris to prove the tube´s position stability from 24 hours to 3 months.
5. Analysis of the effect of the Preserflo device on the IOP from 24 hours to 3 months.

6. To describe the percentage of patients with hypotony during follow-up, accounting for those who required anterior chamber reformation, and the number of patients who developed bleb fibrosis and required needling or open surgical revision.
7. Several hypotheses were raised:

    - The flow of aqueous through Preserflo in the early postoperative period below Tenon´s capsule favors the formation of multiform blebs with multilayered stroma and episcleral fluid cavities in the majority of patients. These features have been associated with a higher rate of success of the filtering bleb after trabeculectomy.
    - The restriction of flow associated with the dimensions of the tube favors the formation of low-pressure blebs due to a low vertical diameter. This type of bleb geometry has been associated with decreased fibrosis of the bleb wall.
    - The distribution of the aqueous humor in the sub-Tenon space in the early postoperative period is associated with a particular morphological and geometrical distribution of fluid, where the force exerted by the tissues is capable of avoiding hypotony in a higher percentage of patients than expected by the theoretical and experimental pressure drop.
    - The position of the tube in the anterior chamber remains stable from 24 hours to 3 months except in the cases of hypotony, where the distance from the tube to the endothelium and iris decreases proportionally to the decrease in the anterior chamber depth.
    - A higher horizontal diameter of the episcleral fluid cavity induces a larger decrease in the intraocular pressure.

6.3 Effect of the Preserflo Microshunt on topography and biometry

1. To analyze, from the first week to the third month, the following changes in two groups of patients (combined phaco-Preserflo surgery and Preserflo standalone surgery):

    - Intraocular pressure decrease.
    - Visual and refractive changes (best corrected visual acuity, spherical equivalent, refractive sphere, refractive astigmatism).
    - Biometrical changes (anterior chamber depth and axial length).
    - Changes observed in the amount and axis of astigmatism (anterior, posterior and total astigmatism).
    - Changes observed in keratometry.
    - Changes observed in the anterior and posterior corneal elevation (maximum, minimum and central).
    - To perform an extensive review of the evidence concerning refractive changes associated with other types of glaucoma surgery (trabeculectomy, Express implant, NPDS and long-tube drainage devices) for comparison with Preserflo.

2. The main hypothesis of this study was that the visual, refractive and biometric changes associated with this implant are mild and transient, recovering preoperative values at 3 months.
3. Mild and transient refractive changes would be associated with a less invasive technique and a lower, diffuse and more posterior bleb morphology compared to that formed by trabeculectomy.

6.4 Effect of the Preserflo Microshunt on endothelial cell density:

1. Main objective: To analyze the effect of glaucoma surgery with the Preserflo implant on endothelial cell density (ECD) during the first year after surgery.
2. Secondary objectives:

    - Analysis of the change in ECD (postoperative ECD-preoperative ECD) vs. time from surgery (number of days since surgery).
    - Analysis of the mean monthly reduction (MMR) in ECD in the total population of the study (MMR = preoperative ECD – postoperative ECD/number of months since surgery).
    - Analysis of the percentage of central ECD loss (MMR/preoperative ECD).
    - Analysis of the influence that the distance from the distal end of the tube to the endothelium and iris, measured with AS-OCT, may have on the change in central ECD, with linear regression mixed models and Pearson correlation index.
    - Analysis of data by groups divided by the type of surgery (combined phaco-Preserflo vs. Preserflo standalone in pseudophakic patients) and by the tube-endothelium distance (<200 µm, 201-500 µm, >500 µm).
    - Analysis of other risk factors for endothelial cell loss (age, hypotony, peripheral anterior synechiae (PAS) and anterior chamber depth).

3. Hypothesis:

    - The main hypothesis of the study was that a lower distance from the distal end of the tube and the endothelium accelerates endothelial cell loss.
    - The secondary hypotheses were the following:

        - Being conceptually comparable to the tube of a traditional drainage device, the pattern of endothelial cell loss should be similar (continued loss over time, higher than the physiological loss of endothelial cell density associated with age).
        - The loss of endothelial cells should be inferior in comparison with traditional drainage devices due to the Preserflo´s reduced dimensions, volume in the anterior chamber and the reduction of the aqueous humor outflow in comparison to wider tubes.
        - Hypotony, PAS and a lower anterior chamber depth might be associated with a greater decrease in endothelial cells.

DISCUSSION

Discussion

7.1 Preserflo´s fluid dynamics and its relationship with bleb morphology and geometry

The first section of the studies of the present dissertation began with an investigation of the physical fluid properties of the Preserflo implant. First, the ultrastructure of the implant was analyzed by means of scanning electron microscopy with an FESEM microscope at the Advanced Microscopy Laboratory of the University of Zaragoza. Second, the fluid dynamics of the implant were analyzed by means of a gravitational experimental study carried out at the Department of Physics and Applied Mathematics at the University of Navarra.

The objective of analyzing the ultrastructure was to validate the internal and external dimensions to contrast with those provided by the manufacturer, so that the theoretical calculation was adjusted to the real measurements of the device and the comparison with the experimental measurement was reliable. Previous discordance between the experimental flow properties and the device dimension was published by Samsudin et al.[35] with the Express implant. The authors found a significantly higher resistance to flow through the P50 model than through the P200 model (SEM dimensions 205 and 206 µm for P200 and P50, respectively). In that study, the resistance to flow through both designs was measured experimentally, and a significant difference was found between them (P200, 0.01 mmHg/(µL/min); P50, 0.05 mmHg/(µL/min), which could not be explained by different internal diameters. The explanation was provided by Alcon; the P50 model had a 150 µm metal bar inside the lumen to restrict the flow. In the case of Preserflo, the measurements observed with FESEM (external diameter of 337.2 µm, internal diameters of 67.73 x 65.95 µm and 63.66 x 70.54 µm) were comparable with the measurements provided by the manufacturer (350 µm external diameter, 70 µm internal diameter), accounting for a slight tilt of the images. Therefore, the comparison between the experimental results and the theoretical calculations was reliable.

The gravity-driven experimental study carried out to measure the flow through the Preserflo Microshunt[12] was based on the Hagen-Poiseuille formula. According to this equation, a constant hydrostatic pressure induces a constant flow through a cylindrical pipe for noncompressible Newtonian fluids[4]. The flow can be measured as a function of the linear growth of the mass that passes through the tube over time. In Experiment 1, using a bottle height of 80.1 cm and saline solution as the fluid and assuming a density equal to that of water, the calculated pressure drop was 58.92 mmHg. The experimental volumetric flow found was Q = 50.72 g/(24 h) or Q = 35.22 (µL/min) because the relation between mass and time was linear and governed by the equation $w(t)= 50.72\ t+w_0$ (R^2 = 0.9988). In Experiment 2, the flow was analyzed as a function of the differential pressure at five heights, estimating an experimental resistance to flow R_E = 1,806 mmHg/(µL/min), which under physiological conditions, taking into account the viscosity of the aqueous humor, and using the equation $RE_{PHYS} = R_E \times (\mu_{AQHUM}/\mu_{WATER})$, was R_{PHYS} =1,301 mmHg/(µL/min), in excellent agreement with the theoretical value R_{THEO} = 1.3 mmHg/(µL/min). The experimental results (linear relation between IOP and flow) would allow us to calculate the flow for any IOP, which could be useful for future investigations with this device. In real conditions, for example, for an IOP of 15 mmHg, the flow (Q) would be 8.9 µL/min.

With the Hagen Poiseuille formula, it was estimated that Δp = 2.6 mmHg assuming aqueous humor production in humans, 2 µL/min[11] and R_{PHYS} = 1,301 mmHg/(µL/min), which was comparable to the theoretical pressure drop (Δp_{THEO}=2.6 mmHg). Furthermore, the experiment operated with Reynolds numbers in the range between 25 and 60, far below the turbulent flow, meaning that the flow was

laminar and the Hagen-Poiseuille Law was valid.

Considering that $\Delta p_{THEO} = \Delta p_{PHYS}$ = 2,6 mmHg, it is possible to affirm that the Preserflo implant cannot by itself avoid hypotony, if by hypotony we understand the value of 5 mmHg. Our findings clearly contradict the theory that the Hagen Poiseuille Law is not valid for Preserflo due to its hydrophobic material (SIBS) and small diameter and that this implant was designed specifically to hold the pressure in the anterior chamber[8].

Once the pressure drop through Preserflo was validated, the clinical results published in this regard were analyzed to contrast the theoretical risk of hypotony with its actual incidence. It was verified that the hypotony rates were similar to those published for XEN 45[36,37] (despite a resistance to flow 3.4-fold higher through XEN45), which were inferior to those published for Ahmed's Valve and the Paul Glaucoma Implant[39] and far below those published for trabeculectomy[40] in the short and long term. In a 300-patient study published by Abbas et al.[40], the percentage of hypotony after trabeculectomy at any time during follow-up was 47%, while in the multicentric study published about Preserflo´s safety and efficacy with two different concentrations of MMC (0.02% vs. 0.04%)[41], the percentage of hypotony was 16.3% in the first month in the MMC 0.04% group vs. 0% in the 0.02% group. In the long term up to the second year, the percentage of hypotony was 0% in both groups. In our study on bleb morphology[42], the percentage of hypotony reported in the first month with MMC 0.02% was 11%.

The scientific evidence regarding the incidence of clinical hypotony with Preserflo suggests that the distribution of the aqueous humor in the sub-Tenon space, together with the resistance to flow offered by the tissues, exerts a back pressure capable of avoiding hypotony in the early postoperative period in most patients. In the long term, the maturation process of the bleb wall may lead to the formation of a filtering membrane that regulates the IOP thanks to a modulation of the filtering capacity, avoiding hypotony. Preserflo´s bleb morphology would, therefore, be a determining factor not only for hypotensive efficacy but also for the regulation of intraocular pressure. The investigation of the morphology of the Preserflo´s bleb could reveal the bleb-forming capacity of this implant in the short and long term.

As a consequence of the findings about the fluid dynamics of Preserflo and its unexpected capacity to avoid hypotony, the need arises to investigate the ultrastructure of Preserflo´s bleb. The analysis of the morphology and geometry of the distribution of aqueous in the sub-Tenon´s space during the first 3 months after the implantation of Preserflo and its correlation with the intraocular pressure was analyzed in this dissertation with the publication of a study in the scientific journal "***Acta Ophthalmologica***"[42]. The objective of the study, carried out in 28 eyes of 28 patients from 24 hours to 3 months, was to analyze the bleb features clinically by following the IBAGS classification "Indiana Bleb Appearance Grading Scale"[43] and morphologically by using AS-OCT. With this tool, images were taken from the location of the tube in the anterior chamber (analysis of the stability of the tube in the anterior chamber), the horizontal and vertical diameters (geometrical analysis) of the episcleral fluid cavities of the blebs, and morphological classification (uniform vs. multiform, and within multiform, presence of multilayer, microcysts and posterior episcleral fluid cavity or "drainage pathway" and its degrees [mild, moderate, or high]) in all visits.

The most common type of bleb found in these patients was multiform and multilayered, located beneath Tenon's and posterior from the limbus, showing an episcleral aqueous humor cavity formed around the tube that spread posteriorly. At 24 hours, 28% of the cases showed a horizontal diameter of the fluid cavity higher than the measurable limits, while at 3 months, this type of bleb was observed in 68% of the cases. The horizontal and vertical diameters increased significantly from the immediate postopera-

tive period to the third month, although the horizontal diameter was higher than the vertical diameter, explaining the broad, posterior and diffuse clinical morphology of these blebs at least up to the third month. The connective tissue located above the filtration zone showed in most cases a multilayered morphology with layers of tissue separated by spaces of liquid and subepithelial microcysts similar to the "stripping phenomena" described by Theelen et al.[12]. Uniform morphology (absence of fluid spaces with hyperreflective stroma in our study) was rare in this series (3.5% in the first week), in contrast to the high percentages of uniformity published with trabeculectomy (Nakano, 20.8%[15]) and XEN45 (Lenzhofer, 48%[44]), two weeks after surgery. Direct comparison between our results and those reported by Nakano with trabeculectomy might not be reliable, since the methodology used in our study to classify the blebs followed the method published by Lenzhofer[44] to analyze the bleb with XEN45 instead of Nakano´s protocol. By "uniform", they meant the absence of fluid in the bleb wall, whether there was an episcleral fluid cavity or not. In our study, "uniform" was considered the total absence of fluid.

Most likely, a hypothetical equal flow rate of aqueous through trabeculectomy and Preserflo would produce a comparable percentage of multiform and uniform blebs, but in real conditions, the standardization of flow through Preserflo could provide an advantage over trabeculectomy by reducing the flow variability associated with the surgeon´s skills to control flow through the tension on the sutures. A simpler surgical technique along with a standardized but sufficient flow of aqueous provided by this implant, enough to separate the tissues from the early postoperative period and increasing bleb function in the long term, could extend the use of this type of glaucoma surgery to a higher number of ophthalmologists and patients.

Comparing the percentage of uniform blebs in the early postoperative period reported for XEN45 implanted with a closed conjunctiva approach[44], (3,5% Preserflo vs. 48% XEN45), a lower volume of aqueous associated with a higher resistance to flow (3.4-fold higher through XEN45) and a higher resistance offered by the tissues with the closed technique[45] could explain the significant morphological differences observed between Preserflo and XEN45 blebs.

The risk of fibrosis is lower when the blebs are multiform in the early postoperative period[15,46]. In addition, multiformity is a predictive factor for the success of filtering glaucoma surgery[15]. At 1-year post-trabeculectomy, higher success rates and better IOP control were noted in patients who showed an initial multiform bleb morphology with microcysts and persistent parallel multilayer channels[46], characteristics that are very similar to the bleb morphology encountered after the implantation of Preserflo[42] and quite different from the "filtering conjunctiva" described 1 year after XEN 45 placed ab interno with a closed conjunctiva[16].

In the studies published by Nakano and Narita[15,46], the concentration of MMC (0.04%) was higher than the concentration used in our study with Preserflo (0.02%). Nakano reported 58% of morphological functioning blebs at 6 months with 42.5% suturolisis and 16% needling. In our study, 93% of the blebs showed moderate or diffuse episcleral fluid cavities at 3 months with 9% of them receiving surgical revision due to bleb fibrosis. The difference in the follow-up time could underestimate the effect of the bleb maturation process that is initiated 3 months after surgery[47], and with a longer follow-up, the percentage of functioning blebs could be comparable, with lower concentrations of MMC in the case of Preserflo probably due to the standardization of flow. Lower concentrations of MMC have been shown to reduce the rate of adverse events with Preserflo[41].

With regard to XEN45, according to the studies published by Teus[16] and Lenzhofer[44], the ab interno approach with this device without conjunctival dissection and using injected MMC 0.01% seems to

increase the percentage of fibrosis (48% in the first 2 weeks)[44] compared to that after Preserflo and to form flatter blebs containing less fluid compared to those formed by trabeculectomy[16]. Nevertheless, when XEN45 is implanted underneath Tenon´s using an open conjunctiva approach (Dangda et al.[17]), the risk of tube obstruction by Tenon decreases, and the outflow of aqueous creates "true blebs" that resemble those of trabeculectomy[15,46] and Preserflo[42], as long as a higher dose of MMC is used (subconjunctival injection of 60 µg in 0,15 ml of MMC 0,2 mg/ml, 10 mm away from limbus) than the dose used in our study[42] (11,9-17 µg, MMC 0.2 mg/ml applied with sponges for 2 minutes[47]). Dangda[17] reported outstanding results with this technique, with 22 out of 24 patients showing functioning blebs at 12 months. According to the authors, the wide dissection of the complex conjunctiva-Tenon and the high dose of MMC could justify the results observed with XEN45.

Therefore, comparing the morphological results of the Preserflo´s bleb with XEN45, when XEN45 is implanted without conjunctival dissection the risk of fibrosis is higher than that reported with Preserflo, and its bleb-forming capacity, lower, reducing the chance of obtaining functioning blebs in the long term without needling, surgical revision or MMC injection to reduce fibrosis (associated with a lower flow of aqueous). On the other hand, when both tubes are implanted with an open conjunctiva, both seem capable of forming trabeculectomy-like blebs, with episcleral fluid cavities in the majority of the cases as long as a higher dose of MMC is used with XEN45 to compensate for the higher resistance to flow. In this case, just as occurs with trabeculectomy vs. Preserflo, a lower dose of MMC to achieve the same morphological result could be an advantage of Preserflo over XEN45.

To analyze the influence that the surgical technique (open vs. closed conjunctiva) has on the clinical results, not just on the bleb morphology, we can only refer to XEN45. In a comparative study between XEN45 open vs. closed conjunctivas[48], applying the same dose of MMC (0,4 mg/ml, mean dose 54 µg in the closed group and 49,1 µg in the open group, both injected), the complete success rate was higher and the needling rate lower with an open conjunctiva. At 12 months, the percentage of IOP reduction was significantly higher in the open group (24.8% closed, 43.1% open), and the rate of needling was lower (11.8% open vs. 36.1% closed). Therefore, with the same MMC dose, surgical success seems to be higher when XEN45 is implanted with dissection of the conjunctiva and Tenon.

Analyzing the effect of the tube diameter on clinical results, we need to compare studies with open conjunctiva approaches and different implants (Preserflo vs. XEN45). We already know that the bleb morphology is comparable if a higher dose of MMC is used with XEN45[17]; however, if the dose and concentration of MMC is the same (0.02% sponges for 2 minutes, total dose of MMC: 11,9 to 17 µg[47]) and both are implanted below Tenon´s with conjunctival dissection, what are the clinical outcomes? In our 28-patient study[42], 9% of subjects required open surgical revision due to bleb fibrosis up to the third month, while 32% required needling with XEN45 (Grover et al.[49]).

Both morphological and clinical studies support the finding that the rate of success is higher with the open conjunctiva technique. With this approach, the dose of MMC should be increased proportionally to the decrease in the luminal diameter of the tube, the increase in the resistance to flow and the reduction in aqueous humor volume in the sub-Tenon´s space that will increase fibrosis. A 3.4-fold increase in flow resistance seems to triple fibrosis as well (9% for Preserflo vs. 32% for XEN45).

The comparison between Preserflo and XEN45 in terms of safety and efficacy, depending on the surgical technique and MMC concentration, seems to indicate that the injection of MMC with a closed conjunctiva (used exclusively with XEN45), increases the risk of complications in comparison with the open technique washing out the MMC. In addition, with the injected MMC-closed conjunctiva technique

the increase of the MMC concentration doesn´t seem to improve efficacy[47]. The most common doses used with XEN45 closed-conjunctiva (10 µg[50], 20 µg[51,52] y 40 µg[52]) have been reported not to improve efficacy while increasing complications (9% avascular blebs, 1% conjunctival erosions)[53] despite a lower dose than the dose used by Dangda with XEN45-open conjunctiva (60 µg)[17]. Compared to Preserflo (0% avascular blebs and conjunctival erosions with MMC 0.02%[41,42] and MMC 0.04%[41]), complications associated with XEN45 and injection of MMC with closed conjunctiva seem to be higher.

Regarding the use of higher concentrations of MMC with the Preserflo device, there seems to be only clinical[41] but not morphological evidence regarding the effects of an increased concentration. The use of MMC 0.04% shows a trend to achieve lower IOPs at 6 months, increasing the proportion of patients free of medication (90.3%) at 2 years vs. only 50% with MMC 0.02%[41]. In return, the risk of complications is higher (55.8% with MMC 0.04% vs. 15.6% with MMC 0.02%), including 7% of bleb leaks in the early postoperative period with a higher concentration. The investigation of the hypothetical effect that a higher dose of MMC might have on bleb morphology could help clarify the scarce clinical evidence about the superiority of MMC 0.04%.

According to the evidence about the use of MMC with the new implants, it seems reasonable to think that the risk of complications associated with the use of MMC increases with the closed conjunctiva technique regardless of the implant, probably due to the possibility of washing out the MMC through an open conjunctiva. Despite having shown that the incidence of side effects, especially hypotony, is higher with higher concentrations of MMC but not clearly so with longer exposure times[47], in the case of the closed conjunctiva technique, the exposure would be chronic, increasing the risk of adverse events. On the other hand, the increase of the concentration of MMC with the closed-conjunctiva technique does not seem to increase efficacy, probably due to the increased risk of tube´s obstruction. The dissection of the conjunctiva and Tenon seems to increase the efficacy of both Preserflo and XEN45.

The geometrical analysis of the episcleral fluid cavity inside the bleb, frequently observed for at least 3 months after surgery with Preserflo[42], shows a reduced vertical diameter relative to a larger horizontal diameter. The distribution of forces within the cavity, according to its geometry, will determine the maturation process of the wall toward the formation of a filtering membrane whose function is the regulation of intraocular pressure. The reduction of the vertical diameter, according to Laplace's Law[54], decreases the pressure inside the fluid cavity, reducing the tension in the wall (especially in the superior cap according to Gardiner's computational model[55]), reducing tissue hypoxia and collagen deposition and increasing porosity and therefore hydraulic conductivity.

The formation of the episcleral fluid cavity comprises a sequence of events that begin with the passage of aqueous humor from the anterior chamber to the subtenon space. According to the Hagen-Poiseuille Law[4], the intraocular pressure (pressure in the anterior chamber) generates a flow of aqueous through the implant toward the bleb until the pressure balance is reached. Once the pressure is equal between the AC and the bleb, the resistance to flow through the tube would be much lower than the resistance through the trabecular meshwork, so the tube would not offer any additional resistance to flow[55], in clear contradiction to the "resistance in series" theory proposed by Lim et al.[56] for small tube diameters. Therefore, it is physically possible to reduce the dimensions of the glaucoma filtering surgery tubes without reducing the hypotensive efficacy. Furthermore, if the mean values of intraocular pressure from the first 24 hours up to 3 months are low and equal to the pressure inside the bleb (10.9 ± 1.8 mmHg at 3 months)[42] and we assume that Gardiner´s model with a bleb pressure of 17 mmHg does not induce scar formation but above 34 mmHg it does, the cicatricial stimulus at low pressures would be very low in the Preserflo´s blebs, reducing scar formation and increasing porosity.

The effect of intrableb pressure on the bleb maturation process can be explained from the histopathological, mathematical-physical and physical-biochemical points of view:

1. Histopathological: According to Molteno´s findings[1] among the factors that control capsule fibrosis and histopathological changes, the intrableb pressure triggers different tissue responses. In the case of no flow (zero pressure inside the bleb), no response is stimulated, and the tissues adhere to each other. When the pressure is moderate (6-12 mmHg) and lower than the closing pressure of the capillaries, a fibroproliferative response that leads to the formation of a filtering capsule is initiated, but the fibrodegenerative response that would lead to excessive deposition of collagen toward the formation of thick, impermeable capsules does not begin. When the pressure inside the bleb is higher (25 to 35 mmHg) and greater than the mean closing pressure of the capillaries, the fibroproliferative response is delayed, and the fibrodegenerative response is accelerated, with excessive deposition of collagen. In this regard, the previous state of the conjunctiva also seems to play a role in the final permeability of the bleb wall. Mastropasqua et al.[58], analyzing the thickness of the conjunctiva as a predictive biomarker of function, showed that a thin and hyperreflective conjunctiva in the preoperative period (as analyzed with AS-OCT) negatively affected the flow of aqueous humor through the bleb wall.

2. Mathematical-physical: According to the study published by Wilcox[54], blebs with smaller vertical diameters experience less pressure on the bleb wall (Laplace's Law), decreasing the excessive collagen deposition related to hypoxia. This type of bleb (formed around cylindrical tubes, lower vertical diameter) has an increased hydraulic conductivity associated with a smaller total filtering surface (hydraulic conductivity is directly proportional to the flow through the implant and production of aqueous humor and inversely proportional to the filtering surface and intraocular pressure). Hypoxia of the tissues will occur when the pressure is higher than 20 mmHg (closing pressure of the capillaries of the subconjunctival tissue estimated by Gardiner[55]). Blebs with higher pressures will show ischemia, pallor, and subsequent fibrosis.

3. Physical-biochemical: According to the conjunctival-Tenon fibrosis model proposed by Gater[57], the constant flow of aqueous after glaucoma surgery induces fibrosis via shear stress on the bleb wall, promoting overexpression of cytokines (TGF-b, TNF-a, VEGF and IL-1), which increases the proliferation of Tenon's capsule and fibroblasts of the conjunctiva.

Therefore, although there are many other factors involved in the maturation process of the filtration bleb toward success with a fluidic cavity and filtering wall or fibrosis (concentration and application time of the MMC, uveitic and neovascular glaucoma, conjunctival trauma, penetrating keratoplasty, previous glaucoma surgery, effect of hypotensive eye drops on the conjunctiva, age, race, etc.), the geometry of the cavity itself exerts a crucial effect on the bleb maturation process. In case of obstruction of the tube and absence of fluid, the tissues will adhere and fibrose[1]. If the volume of fluid is excessive, the vertical diameter will increase, the pressure inside the bleb will exceed the closing pressure of the capillaries of the subconjunctival tissue with subsequent ischemia, the shear-stress on the wall will induce the release of proinflammatory cytokines, and as a consequence of all of these events, the extracellular matrix (major protein, collagen) deposit in the bleb wall will be disproportionate, decreasing the permeability, thickness of the wall and hydraulic conductivity. The bleb will become encapsulated with the formation of a Tenon cyst. However, according to the early postoperative intraocular pressure results together with the geometric distribution of fluid in the early postoperative period[42], in most cases, the flow restriction provided by Preserflo delivers the aqueous humor at low pressures (lower than the closing

pressure of the capillaries), promoting low-height, low-pressure blebs. As we have already learned, the low pressure of the Preserflo bleb could decrease ischemia, shear stress, cytokine overexpression, and the fibrodegenerative response of the tissues, reducing the risk of fibrosis.

Blebs that develop larger filtering surfaces will probably exhibit lower hydraulic conductivity of the wall to regulate the intraocular pressure and avoid hypotony (hyperreflective, thinner walls?), and lower filtering surfaces will need higher hydraulic conductivities (hypo-reflective, thicker walls?) to avoid IOP spikes. Within these parameters, the functioning Preserflo blebs will probably show different morphologies but a constant capacity to regulate flow and intraocular pressure, except in extreme cases of encapsulation or fibrosis. Research on the morphology of the Preserflo bleb in the long term will determine whether the physical laws that govern intrableb pressure and its biochemical and pathological effects finally confirm this hypothesis.

In conclusion, the Preserflo implant shows a high capacity to form multiform blebs from the early postoperative period up to the third month, a predictive factor of success with trabeculectomy. Preserflo blebs exhibit episcleral fluid cavities in a high percentage of patients, another feature of the functioning filtering bleb. Compared to its main competitor, the latest scientific evidence clearly shows that XEN45 has the ability to form functioning blebs that resemble trabeculectomy and Preserflo, provided that the conjunctiva and Tenon's capsule are opened to ensure that the tip of the distal end is not obstructed, and the dose of MMC used to prevent fibrosis is considerably higher to compensate for the higher resistance to flow.

The standardization of flow in filtering glaucoma surgery, thanks to the development of the Preserflo implant, could represent an advantage over trabeculectomy, the reference glaucoma surgery described and applied since the 1960s, due to its ability to deliver a constant volume of aqueous, enough to form multiform blebs in the early postoperative period and to avoid hypotony in most cases. A simpler and more reproducible surgical technique, together with the standardization of flow and a lower rate of complications, could spread the use of these new implants for glaucoma surgery.

7.2 Effects of the Preserflo Microshunt on topography, refraction, biometry and endothelial cell density

In the second part of this dissertation, the visual, refractive and biometric effects of the Preserflo Microshunt are investigated, as well as the effect on endothelial cells. Since bleb morphology has been reported to be low, posterior and diffuse and the surgery does not require scleral flap dissection or sutures, it is hypothesized that the effects on corneal curvature (anterior and posterior) are lower than those observed after trabeculectomy, the gold-standard technique in glaucoma surgery. On the other hand, a lower flow of aqueous humor and a lower volume occupied by the tube in the anterior chamber could favor a lower loss of endothelial cells compared to traditional drainage tubes, with the smaller distance between the tube and the endothelium being a possible risk factor for endothelial loss.

In the article published in the ***"Journal of Glaucoma"***[28] as part of the present dissertation, changes observed in keratometry, astigmatism and corneal elevation were analyzed, as well as the refractive changes, the axial length and the anterior chamber depth and volume. The study was carried out in 30 eyes of 29 patients who underwent surgery with the Preserflo Microshunt and were followed for 3 months. The data were analyzed by group, that is, noncombined surgery versus combined surgery (phacoemulsification with PRESERFLO and adjunctive MMC 0.2 mg/ml for 2 minutes vs. Preserflo standalone).

The analysis of the corneal changes was carried out with a tomography system that uses a Scheimplflug camera (Pentacam HR, Oculus, Inc.) to obtain cross-sectional images of the anterior segment (50 images in 2 seconds with 138,000 points of evaluation). One week after surgery, the mean increase in anterior surface astigmatism (ASA) in the noncombined group was 0.7 D and 0.8 D in total astigmatism (TCA). In the combined group, the changes were slightly higher, with a mean increase of 1 D in ASA and 1.2 D in TCA. In both groups, the increase observed in the first week decreased toward the third month, showing a slight residual difference compared to preoperative values of 0.4 D of ASA and TCA in the noncombined group and 0.2 D in the combined group. The changes observed in posterior astigmatism were very slight (0.1 D) and similar in both groups in the first week and nonsignificant in the third month (0.08 and 0.03 D). The elevation of the anterior and posterior surface of the cornea showed a tendency to increase during the first week, recovering preoperative values at the third month. It is likely that the sudden drop in the intraocular pressure in the immediate postoperative period induces an increase in corneal curvature with a consequent increase in astigmatism and elevation of the cornea.

There is wide evidence on the effect of glaucoma surgery on corneal astigmatism compared with that of Preserflo. Regarding trabeculectomy, there are at least 8 published studies with follow-up times between 3 months and one year in which the common finding is the induction of with-the-rule (WTR) astigmatism. The first study on the subject was published by Hugkulstone[20], who observed by means of keratometry a statistically significant decrease in the vertical radius of the cornea from 7.71 mm (preoperative value) to 7.36 mm from the first postoperative day and during the following 28 days. In the case of the Preserflo implant, we found changes similar to those described by Hugkulstone[20] and Cunliffe[21], with a decrease in the steepest radius from 7.6 mm to 7.5 mm in the first week together with a change in the flattest axis of the astigmatism from 105° to 135° (steepest axis from 15 to 45°), suggesting that both trabeculectomy and Preserflo induce an increase in the vertical curvature of the cornea in the immediate postoperative period. In later studies that used somewhat more advanced astigmatism measurement methods (topography, computerized videokeratography), Rosen[23] observed an increase of 1.5 to 2.5 D in WTR astigmatism 12 weeks after surgery, and Dietze[24], 1.4 D one week after surgery and 1 D at 12 weeks. In our study, the total astigmatism induced by Preserflo 12 weeks after surgery (0.4 D) was less than that found after trabeculectomy and was deemed not clinically relevant. It is unlikely that the differences found between trabeculectomy and Preserflo are due to an underestimation of the astigmatism, since the measurement method used in our study (Pentacam HR) has shown its superiority over Placido disc topography by being able to measure not only the anterior but also the posterior face with high precision[59], which is why we consider our results reliable.

Several studies have reported corneal changes associated with other filtering glaucoma surgery techniques beyond trabeculectomy. Hammel et al.[25], in a study of 19 eyes that underwent surgery with the Express implant, analyzed the changes in corneal astigmatism with Pentacam. The Express implant, unlike Preserflo, requires the dissection of a scleral flap and sutures to control the outflow. resistance to flow is much lower than that of Preserflo (Express P200 R=0.01 mmHg/(µL/min); P50 0.05 mmHg/(µL/min)[35], Preserflo 1,301 mmHg/(µL/min)[12]. Over a 3-month follow-up, the increase in anterior and posterior astigmatism (2.6 to 4.7 D; 0.4 to 0.9 D) was clearly superior to that with Preserflo (0.4 D increased TCA at 3 months). Regarding nonperforating deep sclerectomy, there are studies that have compared NPDS with trabeculectomy, finding a lower induction of astigmatism with NPDS. Egrilmez[26] reported a change of 0.62 D after NPDS compared to 1.06 D after trabeculectomy in the early postoperative period and less induction of WTR astigmatism at 6 months (0.62 D vs. 1.24 D). Other studies have found similar results between deep sclerectomy and trabeculectomy [60]. In summary, the scientific evidence suggests that trabeculectomy and Express induce greater astigmatism than nonpenetrating

deep sclerectomy and Preserflo.

There are relatively few studies that report changes in astigmatism and refraction with minimally invasive glaucoma surgery (MIGS) and long-tube drainage device (GDD) techniques. Ioannidis et al.[61], analyzing the refractive changes associated with combined femto-phacoemulsification surgery with implantation of two iStent injections (Glaukos, San Clemente, CA, United States) in the trabecular meshwork, reported a significant decrease in preoperative astigmatism (0.91 to 0.41 D at 4 weeks) probably associated with cataract surgery associated with the concomitant treatment of corneal astigmatism. The iStents were less likely to be responsible for the refractive changes due to their deep location in the trabecular meshwork and minimal size. Regarding the refractive effects of long-tube drainage devices, to the best of our knowledge, there are no studies that investigate corneal and refractive changes. The study conducted by Tzu et al.[62] compared the refractive results between cataract surgery, surgery combined with trabeculectomy, and surgery combined with the Ahmed or Baerveldt devices, reporting a greater change in the corneal cylinder in the combined vs. the not combined group, but without a separate analysis between trabeculectomy and GDD.

The possible mechanisms involved in the corneal changes associated with glaucoma surgery (especially trabeculectomy) include retraction of the edge of the cornea associated with dissection of the deep scleral flap[21], cauterization of the scleral bed[23], and excessive tension on the sutures[24]. Other factors, such as large filtration blebs, ptosis and hypotony, have also been described as possible causes of astigmatic shift[27]. The use of intraoperative mitomycin C seems to be associated with longer lasting corneal changes. In our series, using MMC 0.2 mg/ml for 2 minutes in all cases, the astigmatic change was at least comparable or even lower than trabeculectomy with the same concentration of MMC. A longer-term follow-up could elucidate whether there are further refractive changes, although the trend seems to be to recover preoperative values 3 months after surgery.

In the case of Preserflo, several factors could explain the slight and transient changes observed in corneal curvature. On the one hand, there is no scleral flap, no sutures and no deep sclerectomy. On the other hand, the bleb is usually low, posterior to the limbus and diffuse according to the IBAGS and AS-OCT classifications[42]. In our series[42] as well as other studies[41], we did not report avascular blebs, another mechanism related to corneal changes. Although Preserflo surgery is a penetrating procedure, the absence of deep sclerectomy and the slight angle changes due to the reduced external diameter (350 µm) probably explain the slight corneal changes, comparable to NPDS[60].

Considering incidental findings, a positive correlation was found between the nonmedicated intraocular pressure and the elevation of the posterior surface of the cornea, a finding previously described by Arranz-Márquez[64]. Most likely, lower corneal hysteresis in patients with glaucoma[65] favors a more deformable cornea in which the curvature of the posterior face increases in response to the increase in intraocular pressure. Low corneal hysteresis and thin pachymetry are well-known independent risk factors for glaucoma progression[66]. Perhaps the increased elevation of the posterior surface is a new biomarker of POAG, but this remains to be investigated.

7.3 Changes observed in endothelial cell density after surgery with the Preserflo Microshunt

In the article published in the journal *"Ophthalmology and Therapy"*[34] as the last study of

this dissertation, the changes observed in the endothelial cell layer over the first year after surgery with Preserflo were analyzed. The study included [46] eyes that underwent specular microscopy (Topcon SP-1P specular microscope, Topcon Corp. Tokyo, Japan) to analyze changes in the central endothelial cell density (cells/mm2) from baseline to 1 month, 3 months, 6 months and one year after surgery. At all visits, examination of the position of the tube in the anterior chamber was performed using the measurement tool available in the Optovue Avanti Widefield AS-OCT optical coherence tomography software. Measurements were made following the method published by Tan et al.[33] by drawing a line perpendicular to the inner face of the cornea from the distal end of the Preserflo, (tube-endothelium distance, TE) and perpendicular to the iris (tube-iris distance, TI). Likewise, the length of the tube in the anterior chamber was measured. Various graphical data distribution systems (scatterplots, box plots, and bar graphs) of the total endothelial cell density (ECD), change in ECD (postoperative ECD–preoperative ECD) vs. time since surgery and mean monthly reduction (MMR) in ECD. MMR was calculated by dividing the change in ECD (preoperative-postoperative) by the number of months since surgery. The percentage central loss of ECD was also calculated by dividing the monthly change in ECD by the preoperative ECD. To analyze the influence of the position of the tube with respect to the endothelium and iris on the loss of ECD, linear mixed models and the Pearson correlation index were used. The sample was divided into different groups for a better analysis: two groups based on the type of surgery (combined phaco-Preserflo vs. Preserflo implantation without cataract surgery in pseudophakic patients) and three groups based on the tube-endothelium distance (< 200 µm, 201-500 µm, > 500 µm).

The results showed that the profile of endothelial cell loss is characteristic of this implant, related to the position of the tube in the anterior chamber with respect to the endothelium and iris, and independent of the total length of the tube in the anterior chamber. The percentage of mean ECD decrease observed in the different visits increased over time (0.04% per month, 2.3% at 3 months, 5.1% at 6 months and 7.4% per year), suggesting that the pattern of endothelial cell loss was a continuous loss over time, similar to that described after surgery with drainage devices[29] but to a lesser extent (AGV 10.5% and BGI 13.1% per year[31,33]).

Endothelial loss associated with trabeculectomy and phacoemulsification is different from that associated with drainage devices and Preserflo. Endothelial loss in these cases is related to surgery and is detected in the early postoperative period, stabilizing after a few months. In the case of trabeculectomy with MMC, the percentage of loss is similar at 3 months (9.5%) and one year (10%)[67], and compared to the corresponding results of Preserflo, the percentage of endothelial cell loss (13.9%) and mean reduction (-265 cells/mm2) at 3 months reported with trabeculectomy69 seem to be higher than the results found in our series (2.3% and -150 cells/mm^2).

The central mean monthly reduction in ECD after Preserflo implantation was observed to be higher in the first month, showing a progressive decrease until one year (1 month, -125.8 ± 160 cells/mm2; 1 year -14.6 ± 25 cells/mm^2). The greater initial loss could be justified by the group receiving combined surgery, the cases that developed hypotony, and by the loss associated with the tubes located very close to the endothelium.

In the combined surgery group, the mean decrease in ECD was higher in the first month (7.7% combined vs. 0% standalone), while at one year, the loss was comparable and even slightly higher in the group receiving noncombined surgery, which suggests that part of the endothelial loss in the early postoperative period is due to phacoemulsification, while in the long term, other factors (probably related to the presence of the tube in the anterior chamber and the flow of aqueous humor) are associated with

a continued loss of endothelial cells, higher than the physiological loss (0.6% per year)[70], but lower than that published with other implants (AGV, BGI). The endothelial loss in the combined surgery group was comparable to that published by other authors (7.6 to 9.5% at two weeks, 7.3% at one year[70,71,72]), which provides consistency to the results of our study.

The analysis of tube-endothelium distance by group suggested that a smaller distance accelerated endothelial loss. The percentage of endothelial decrease was higher in the <200 µm (18%) group than in the 201-500 µm (11%) and >500 µm (1%) groups. On the other hand, the mean monthly reduction observed in the first month was greater in the <200 µm (-149 ± 76 cells/mm^2) group than in the 201-500 µm (-129 ± 195 cells/mm^2) and > 501 ± (-71 ± 79 cells/mm^2) groups. One year after surgery, the MMR was higher in the groups below 500 µm (-11 ± 9 cells/mm^2, -20 ± 29 cells/mm^2) than in the group above 500 µm (-3 ± 14 cells/mm^2).

Further analysis of data using mixed model linear regression showed an inverse linear relationship between mean monthly ECD reduction and TE distance at all follow-up times. According to this model, in the first month, the tubes located 0 µm from the endothelium lost -174.8 ± 65.2 cells/mm2 ($p < 0.01$) but lost fewer cells at 3 months (-62.2 ± 27.8 cells/mm^2, $p=0.02$), at 6 months (-33.9 ± 12.8 cells/mm^2, $p<0.01$) and at one year (-30.2 ± 11.3 cells/mm^2, $p<0.01$). At 6 months and one year, the tubes located at more than 600 µm showed close to zero endothelial losses. Analysis using the Pearson correlation index one year after surgery confirmed the inverse relationship between TE distance and ECD ($r=-0.5$, $p<0.01$) and revealed a direct relationship ($r=0.38$, $p=0.05$) between the tube-iris distance and the density of endothelial cells; that is, a higher density of endothelial cells 1 year after surgery was related to a greater distance from the tube to the endothelium and a lower distance from the tube to the iris.

In light of the results of this study, it follows that the proximity of the tube to the endothelium increases the loss of endothelial cells with a greater influence in the early postoperative period and that a tube-endothelium distance equal to or greater than 600 µm may be a protective factor considering that tubes located further did not show endothelial loss after 6 months. Similarly, in the study published by Zhang[32] (where the loss of endothelial cells is compared between the Ahmed valve located in the sulcus or in the anterior chamber), the monthly loss of ECD was greater in the group with the tube located in the anterior chamber (29.3 cells/mm^2) vs. sulcus (15.3 cells/mm^2). In our series, the mean monthly reduction in ECD per year (14.6 cells/mm^2) seems to be comparable to the mean monthly reduction in ECD with the Ahmed valve implanted in the sulcus. In the case of the Baerveldt device, Tan[33], using a linear mixed model, reported that the closer the tube was to the endothelium, the greater the loss of endothelial cells. However, a direct comparison between the two studies does not seem to be possible, considering that the length of the tube is usually higher and variable with GDDs, changing the total length to the endothelium for tubes implanted on the same angle.

In this study, other possible risk factors for endothelial cell loss independent of the position of the tube in the anterior chamber were analyzed. A shallower anterior chamber depth and the presence of peripheral posterior synechiae seem to be associated with a greater loss of endothelial cells.

7.4 Physiopathology of the endotelial cell loss with Preserflo

There are different mechanisms involved in the loss of endothelial cells associated with the presence of tubes in the anterior chamber. On the one hand, a certain degree of chronic inflammation could compromise the endothelium and increase the risk of corneal decompensation[70], a common mechanism for any type of tube, including Preserflo. On the other hand, a possible displacement of the tube, published at least with the Baerveldt tube when it is implanted "free" in the anterior chamber, could be a risk factor for endothelial loss, according to Tan[33]. The authors observed that tubes located "free" showed higher ECD loss than transiridial (more stable) tubes. They proposed that the movement of the tube increased the loss of endothelial cells. The position of the distal end of Preserflo was reported to be stable in the AC in our study regarding AS-OCT bleb morphology[42], except for the cases of early hypotony. Therefore, it is less likely that the movement of Preserflo was the cause for the loss of ECD.

In conclusion, the loss of endothelial cells associated with the Preserflo implant is reminiscent of the loss associated with traditional drainage devices (sustained loss over time) but of lesser magnitude than these and greater than the yearly physiological loss in humans. A smaller distance between the tube and the endothelium seems to accelerate endothelial loss, with a distance greater than 600 µm being a possible protective factor. One year after surgery, the percentage of central endothelial loss (7.4%) and the mean monthly reduction in endothelial cell density (-14.6 cells/mm^2) seem to be comparable to the loss reported with the Ahmed valve placed in the sulcus. Hypotony and the presence of peripheral anterior synechiae seem to be risk factors for endothelial loss with this implant.

CONCLUSIONS

Conclusions

8.1 Ultrastructural analysis of the Preserflo Microshunt with FESEM and experimental measurement of flow dynamics

1. The internal and external diameters of the Preserflo implant analyzed with scanning electron microscopy (FESEM) are comparable with the measurements provided by the manufacturer, validating the theoretical calculations of the resistance to flow (R_{THEO}) and pressure drop (Δp_{THEO}), as well as the experimental results of fluid dynamics.
2. The theoretical resistance to flow through Preserflo, calculated using Hagen Poiseuille's Law for an average flow of aqueous humor production of 2 µL/min, is R_{THEO} = 1.3 mmHg/(µL/min), and the pressure drop is Δp_{THEO} = 2.6 mmHg.
3. It is possible to experimentally measure the flow through Preserflo despite its reduced dimensions and hydrophobic material, considering that the flow through the tube in the experiment was laminar and operated at Reynolds numbers below the transition to turbulent flow.
4. The relationship between mass vs. time and flow vs. pressure was linear in the gravity-driven flow experiments through Preserflo. The experimental volumetric flow found was Q=35.22 (µL/min), and the experimental resistance to flow was R_E=1.806 mmHg/(µL/min).
5. The experimental linear relation between pressure and flow found through Preserflo, allows the calculation of flow through this implant for any intraocular pressure, a tool that could be useful for future research about the fluid dynamics of glaucoma implants.
6. The resistance to flow under physiological conditions (taking into account the viscosity of the aqueous humor in patients with glaucoma) calculated from the resistance found experimentally is R_{PHYS}=1.3 mmHg/µL/min and was found to be in excellent agreement with the theoretical resistance to flow R_{THEO}=1.3 mmHg/µL/min, validating the experimental method used in this study and its results.
7. The experimental pressure drop, calculated using Hagen Poiseuille's Law accounting for the resistance to flow under physiological conditions (R_{PHYS}= 1.3 mmHg/µL/min) and the mean flow of aqueous humor in humans (2 µL/min), was Δp_E = 2.6 mmHg, comparable to the theoretical pressure drop (Δp_{THEO}= 2.6 mmHg).
8. The resistance to flow through Preserflo (R_{PHYS}= 1.3 mmHg/µL/min) should not be redundant for the circulation of aqueous humor between the anterior chamber and the bleb once the pressure equilibrium has been reached because the flow is laminar. The resistance to flow through other tubes, as small in diameter as Preserflo, has been shown to offer lower resistance to flow than the trabecular meshwork, supporting the reduction of the tube diameter without undermining the hypotensive efficacy.
9. The theoretical pressure drop (Δp_{THEO}) and the experimental pressure drop in physiological conditions Δp_{PHYS} are comparable (Δp_{THEO}= Δp_{PHYS} = 2.6 mmHg) and lower than the numerical value (5 mmHg) considered numerical hypotony by the World Glaucoma Association.
10. Given that the pressure drop across the Preserflo implant is lower than 5 mmHg, it can be affirmed that this implant was not specifically designed to prevent hypotony due to its physical properties.
11. Despite the theoretical inability of the Preserflo implant to prevent hypotony according to its design, the rate of clinical hypotony is comparable to that of its main competitor XEN45, lower than that of traditional drainage devices (Ahmed valve, Baerveldt implant, and Paul Glaucoma Implant) and far below that of trabeculectomy, in the short and especially in the long term.

8.2 Morphology and geometry of the filtering bleb after surgery with the Preserflo Microshunt in the early postoperative period

1. The IBAGS clinical classification of the Preserflo blebs in the early postoperative period shows that most of them have a low or moderate height, a horizontal extension of approximately two clock hours, medium vascularity and a 0% incidence of avascularity and leaks.
2. The most frequent morphological type of filtering bleb analyzed with AS-OCT in the early postoperative period (24 hours to 3 months) after surgery with the Preserflo implant together with MMC 0.2 mg/ml, applied with sponges for 2 minutes (dose 11.9 to 17 µg), is multiform with a multilayered appearance (multiple parallel layers of tissue separated by hyporeflective zones, sign of the presence of aqueous humor) and episcleral fluid cavities in a high percentage of patients. Subepithelial microcysts are more frequent in the immediate postoperative period and tend to decrease over time. Multilayer morphology and the presence of episcleral fluid cavities are positive predictive factors for the long-term success of the trabeculectomy bleb.
3. The most frequent type of bleb observed in the early postoperative period of the Preserflo implant with the use of MMC 0.02% seems to be comparable to the morphology of the trabeculectomy bleb with MMC 0.04%, as published in the literature by other authors.
4. Compared to its main competitor, XEN45, both implants seem capable of forming multiform blebs with episcleral fluid cavities comparable to trabeculectomy, as long as the conjunctiva-Tenon is dissected to avoid tube obstruction and the dose of MMC applied in the case of XEN45 is higher to compensate for the greater resistance to flow.
5. The results of the studies on the morphology of the filtering bleb and the clinical efficacy of the new microfiltering devices that restrict the flow by a reduction of the inner diameter (Preserflo and XEN45) suggest that the success rate is higher when the conjunctiva is opened to place the end of the tube underneath Tenon´s capsule. A 3.4-fold increase in resistance to flow through XEN45 compared to that through Preserflo appears to increase the risk of fibrosis from 9% to 32% with the open conjunctival technique.
6. The geometrical analysis of the Preserflo bleb in the early postoperative period shows a significant increase in the horizontal and vertical diameters of the intrableb fluid cavity from the first 24 hours to the third month. The mean vertical diameter is lower than the horizontal diameter, explaining the clinical findings observed with the IBAGS classification (diffuse low-elevation blebs).
7. The Preserflo blebs have low vertical diameters and low intrableb pressures, considering the mean intraocular pressure at 3 months (10.9 ± 1.8 mmHg). According to Laplace's Law, the reduction in the vertical diameter together with low intrableb pressures decreases shear stress on the bleb wall, below the closing pressure of the capillaries of the conjunctiva (20 mmHg), reducing hypoxia and excessive extracellular matrix deposition (main protein collagen). Low pressures initiate a fibroproliferative response of the tissues that leads to the formation of porous and permeable capsules. The decrease in shear stress reduces the overexpression of proinflammatory cytokines (TGF-b), which is related to tissue damage during filtering glaucoma surgery. Preserflo´s flow restriction seems to be capable of initiating the process toward the formation of functioning filtering blebs by reducing their vertical diameter and pressure.
8. The position of the tube in the anterior chamber remained stable from the endothelium and iris from 24 hours to 3 months, with the exception of the cases that developed hypotony where the distances were reduced.
9. The reduction in the IOP was significant from baseline to all postoperative visits. The percentage of reported needling was 3.5%, and the need for open surgical revision was 9%. The percentage of hypotony reported during the first month was 11%.

8.3 Effect of the Preserflo Microshunt on the cornea: topography, refraction and biometry

1. The decrease in IOP in the combined and noncombined surgery groups was significant at all postoperative visits up to the third month ($p<0.01$).
2. Best-corrected visual acuity decreased significantly in both groups in the first week after surgery, recovering preoperative values at 3 months.
3. The anterior chamber depth increased significantly in the combined surgery group as a consequence of phacoemulsification and intraocular lens implantation but remained stable in the noncombined surgery group at the third postoperative month. The axial length decreased significantly in both groups.
4. Astigmatism increased in both groups (noncombined surgery and combined surgery) in the first week, showing a progressive reduction until the third month. The increase in anterior surface astigmatism and total astigmatism was greater in the first week in the combined surgery group (1D vs. 0.7D; 1.2D vs. 0.8D). At 3 months, the change in total corneal astigmatism was 0.4D in the noncombined surgery group and 0.2D in the combined surgery group.
5. The elevation of the anterior and posterior surface of the cornea increased in the first week in both groups, recovering preoperative values at 3 months.
6. Preserflo and trabeculectomy increase the vertical curvature of the cornea in the early postoperative period, but in the case of Preserflo, the change in the total astigmatism observed 3 months after surgery seems to be lower than that reported with trabeculectomy.
7. With respect to other filtering glaucoma surgery techniques, according to the results of this study and studies published by other authors, trabeculectomy and Express induce a greater change in astigmatism than deep sclerectomy and Preserflo, which are comparable to each other.
8. There are no published studies on the refractive effect of the XEN45 implant for comparison with Preserflo. The evidence about the refractive effects of GDDs is scarce, offering no comparable results to those reported with Preserflo.
9. The slight and transitory refractive changes observed after glaucoma surgery with the Preserflo implant could be related to the absence of a scleral flap, deep sclerectomy and sutures, as well as the morphology of the filtration bleb, reported to be diffuse, located away from the limbus at a posterior location, and with absence of avascular blebs.
10. As an additional finding, a significant positive correlation was reported between the elevation of the posterior surface of the cornea and the nonmedicated intraocular pressure. This finding was previously reported as a possible new biomarker of the biomechanical modifications of the cornea in glaucoma patients.

8.4 Effect of the Preserflo Microshunt on the posterior surface of the cornea: endothelial cell loss

1. The percentage of endothelial decrease in the total study population (combined surgery and noncombined surgery in pseudophakic patients) one year after surgery was 7.4%, and the mean monthly reduction in endothelial cell density was -14,6 cells/mm^2.
2. The loss of endothelial cells begins in the immediate postoperative period and continues over time in the same way it does after surgery with traditional drainage devices, a sustained loss over time that is greater than the physiological loss associated with aging in the human being. The loss seems to be lower than that reported with GDDs. The ECD loss with Preserflo differs from that with trabeculectomy and phacoemulsification, where the loss is related to surgery and is subsequently stabilized.

3. The endothelial cell loss 3 months after surgery seemed to be higher after trabeculectomy (ECD loss of 13.9%, mean monthly reduction of -265 cells/mm^2) than after Preserflo (ECD loss of 2.3% and MMR of -150 cells/mm^2).
4. Compared to its main competitor, XEN45, 3 months after surgery, the endothelial loss appears to be comparable (2.1%: XEN45, 2.3%: Preserflo) and lower than that with trabeculectomy in both cases (trabeculectomy: 10%). Preserflo and XEN45 appear to reduce ECD to the same extent in the long term (comparable yearly ECD loss).
5. The loss of endothelial cells that occurs after combined surgery is comparable to the reported loss associated with phacoemulsification. One year after surgery, the loss of ECD is higher than the physiological loss of ECD reported in humans with aging in both groups (combined surgery and standalone surgery), suggesting that the presence of the tube in the anterior chamber and the flow of aqueous humor through the tube are the causes for endothelial cell loss in the long term.
6. Compared to traditional drainage devices with the tube in the anterior chamber, the percentage of loss one year after surgery appears to be lower with the Preserflo (Ahmed valve: 10.5%-15.3%, Preserflo: 7.4%).
7. A shorter distance between the distal end of Preserflo and the endothelium accelerates endothelial cell loss, as has been reported for the Ahmed and Baerveldt implants.
8. Endothelial cell loss after Preserflo seems to be comparable to that reported after the Ahmed valve was implanted in the sulcus (Preserflo: 14.6 cells/mm^2, Ahmed: 15.3 cells/mm^2).
9. Mixed model linear regression analysis showed that a reduction in the tube-endothelium distance increases endothelial cell loss. The loss of endothelial cells in the immediate postoperative period is higher with shorter distances. During follow-up, the rate of endothelial loss decreases for all tube positions, but the mean loss reported is higher with the tubes located closest to the endothelium.
10. The analysis of the tube-endothelium distance by group supports the linear regression findings. The endothelial cell loss one year after surgery was higher in tubes located <200 µm (17.9%) than in those located >500 µm (1.1%).
11. A tube-endothelium distance >600 µm appears to be a protective factor for endothelial cell loss 6 months after surgery.
12. A shallower anterior chamber depth and the presence of peripheral anterior synechiae as a consequence of hypotony in the early postoperative period are related to a greater loss of endothelial cells.

Final Conclusions

1. The experimental measurement of the fluid dynamics of the Preserflo Microshunt has demonstrated that this device was not specifically designed to avoid hypotony since the pressure drop across the tube is lower than 5 mmHg.
2. The fluid dynamics of the Preserflo implant favor the formation of multiform and low-vertical-diameter blebs in the early postoperative period, factors that could contribute to the reduction of fibrosis during the filtration bleb maturation process.
3. The morphology and geometry of the filtration bleb derived from the flow restriction associated with Preserflo improves intraocular pressure control with respect to trabeculectomy, the gold standard in glaucoma-filtering surgery.
4. According to the early bleb morphology, the standardization of flow through this implant could increase the rate of functioning filtering blebs in the long term. Along with an easier surgical technique with fewer complications, Preserflo could offer an advantage over trabeculectomy by the standardization of results.
5. Preserflo´s bleb morphology, along with the absence of a scleral flap, sutures and sclerectomy, induces mild and transient refractive changes. These findings could be considered an advantage over trabeculectomy by increasing the accuracy of IOL calculations 3 months after surgery and the possibility of using advanced intraocular lens optic designs.
6. Endothelial cell loss reported after surgery with Preserflo appears to be inferior to that after trabeculectomy and comparable to that after XEN45 and the Ahmed valve when it is placed in the sulcus. A smaller distance from the tube to the endothelium and hypotony are possible risk factors for endothelial loss with this implant.

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Article

Evaluation of the Ultrastructural and In Vitro Flow Properties of the PRESERFLO MicroShunt

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Purpose: To measure the in vitro flow properties of the PRESERFLO implant for comparison with the theoretical resistance to flow.

Methods: The PRESERFLO was designed to control the flow of aqueous humor according to the Hagen-Poiseuille (HP) equation. Scanning electron microscopy (SEM) was performed to analyze the ultrastructure, and flow measurements were carried out using a gravity-flow setup.

Results: SEM images of the PRESERFLO showed luminal diameters of 67.73 × 65.95 µm and 63.66 × 70.54 µm. The total diameter was 337.2 µm, and the wall was 154 µm wide. The theoretical calculation of the resistance to flow (R) for an aqueous humor (AH) viscosity of 0.7185 centipoises (cP) was 1.3 mm Hg/(µL/min). Hence, assuming a constant AH flow of 2 µL/min, the pressure differential across the device (ΔP) was estimated to be 2.6 mm Hg. The gravity-flow experiment allowed us to measure the experimental resistance to flow, which was $R_E = 1.301$ mm Hg/(µL/min), in agreement with the theoretical resistance to flow R given by the HP equation.

Conclusions: The experimental and theoretical flow testing showed that the pressure drop across this device would not be large enough to avoid hypotony unless the resistance to outflow of the sub-Tenon space was sufficient to control the intraocular pressure in the early postoperative period.

Translational Relevance: The fluid properties of glaucoma subconjunctival drainage devices determine their specific bleb-forming capacity and ability to avoid hypotony and therefore their safety and efficacy profile.

Introduction

One of the main challenges of glaucoma drainage device surgery is the control of aqueous humor outflow in the early postoperative phase to prevent hypotony. In the absence of a flow restrictor method, the resistance offered by the tissues that surround the glaucoma tube plate cannot prevent hypotony until at least approximately six weeks after surgery.[1] In this period, there is a risk of severe hypotony, choroidal hemorrhage[2] and anterior chamber flattening, all of which are vision-threatening situations.

In 1969, Anthony Molteno was the first to introduce pioneering concepts regarding the need for a large surface area to disperse the aqueous humor (AH) and the advantage of draining the aqueous away from the limbus.[3] All the long-tube drainage devices currently available are based on the concept of the Molteno implant, which has a tube attached to a large explant placed 9 to 10 mm away from the limbus. The original Molteno implant (Molteno Ophthalmic Limited, Dunedin, New Zealand) has been modified over the years in two ways: the development of valved mechanisms and surgical maneuvers to improve the control of aqueous humor outflow to reduce hypotony, and the enlargement of the plate[4,5] to obtain lower postoperative intraocular pressure (IOP).

The Ahmed glaucoma valve (AGV; New World Medical, Rancho Cucamonga, CA, USA) was the first glaucoma drainage device with a flow resistor made of folded silicone membranes pretensioned by the plate, designed to open and close at a certain pressure level to prevent hypotony.[6] In 2008, Moss and Trope,[7] using a gravity-driven flow test, found that half of the AGV valves tested showed a closing pressure below 6 mm Hg. The Baerveldelt implant (BGI; Abbott Medical Optics, Santa Ana, CA, USA) was introduced in 1990 without a flow restrictor.[5] The placement of temporary sutures around or within the lumen of the tube,[8,9] or a two-stage procedure were proposed to control the outflow. Despite these modifications, the rates of hypotony reported for both implants were similar (12% for the Molteno with the modified technique, 14% for the AGV[10]) and also clinically relevant, regarding the possible devastating consequences to the eye.

The search for adequate control of aqueous humor outflow continues today with the design of new implants based on the Hagen-Poiseuille formula for flow restriction. The resistance to flow (R) and the pressure drop ($\triangle p$) can be modified by means of the length and width of a tube according to the Hagen-Poiseuille equation, which governs the fluid properties of a cylindrical pipe for noncompressible Newtonian fluids.[11] The outflow resistance and therefore the pressure differential increase linearly in relation to the length of the tube and decrease to the fourth power of the lumen radius. Thus two new "miniaturized" tubes have been released, the XEN glaucoma implant (XEN-GGM; Allergan Plc, Parsippany, NJ, USA) and the PRESERFLO MicroShunt (Santen Pharmaceutical Co., Osaka, Japan). They are designed to be implanted either ab interno (XEN) or ab externo (PRESERFLO) to connect the anterior chamber with the subconjunctival space without a valved mechanism or a plate located at the end of the tube. They differ in their length and luminal diameter and therefore in their theoretical resistance to flow and pressure drop, which should be higher for XEN 45 (6 mm long, 45 µm internal diameter) than for the PRESERFLO (8.5 mm long, 70 µm internal diameter). The XEN 45 implant has been reported to be able to maintain backpressure above hypotony (an experimental steady-state pressure of 8.9 mm Hg with a calculated value of 10.98 mm Hg),[12] whereas PRESERFLO has not yet been proven to do so. In a laboratory study performed by the manufacturer,[13] the authors explained that the luminal diameter had to be greater than the diameter of a sloughed endothelial cell (40–50 µm) and that the empirical data generated by rabbit studies from Arrieta et al.[14] helped to "fine-tune" the lumen diameter to 70 µm. The authors hypothesized that the Poiseuille equation "breaks down" at small tube diameters in extremely hydrophobic materials such as poly(styrene-block-isobutylene-block-styrene) (SIBS), which is the material of the PRESERFLO.

To the best of our knowledge, the real flow through this implant, as well as its resistance to flow, and the pressure drop have not been reported. In the current study, we analyze the ultrastructure of PRESERFLO with scanning electron microscopy (SEM) to confirm that the luminal diameter is consistent with the specifications provided by the manufacturer. In addition, we calculate the theoretical resistance to flow and pressure differential for PRESERFLO, XEN 45, and other glaucoma implants using the de Hagen-Poiseuille equation, and finally we perform an experimental fluid-flow test using a gravity-flow setup at different simulated IOPs through the PRESERFLO. Thus the aim of this study is to evaluate the theoretical and experimental flow properties of the PRESERFLO implant.

Methods

The two different samples of the PRESERFLO MicroShunt that were used for each of the analyses (SEM and flow studies) were provided directly by the manufacturer, they were brand new, without any previous surgical or experimental use. The manufacturer-provided dimensions of the device are as follows: total length: 8.5 mm; external diameter: 350 µm; internal diameter: 70 µm; and 1 mm^2 wings located 4.4 mm posterior to the beveled tip of the implant designed to avoid migration from the initial location Figure 1. For the SEM analysis, the PRESERFLO MicroShunt was cut transversally with a 45° cataract blade.

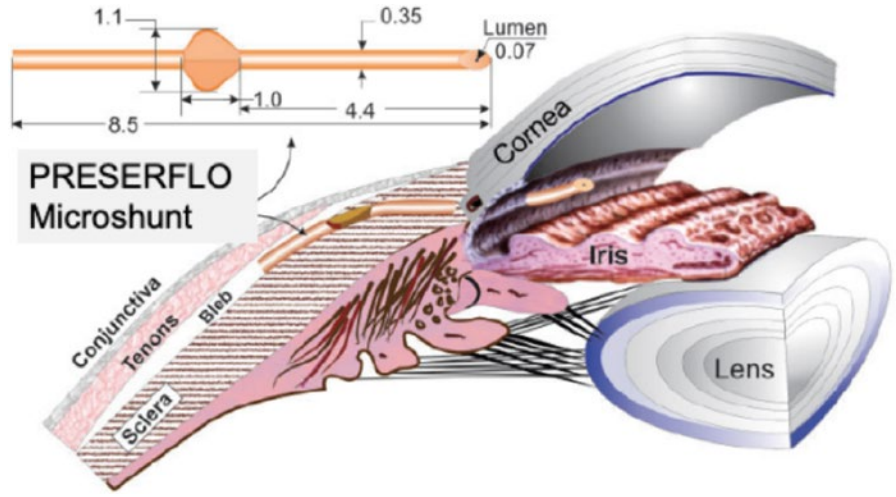

Figure 1. The PRESERFLO MicroShunt dimensions (mm) and location. The distal end is placed in the sub-Tenon space, and the proximal end is placed in the anterior chamber.

Scanning Electron Microscope Analysis

The PRESERFLO luminal diameter was measured with a Quanta FEG 250 scanning electron microscope, which is a field-emission SEM (Fig. 2). Different measures were taken from the lumen, including edge to edge (vertically and horizontally), and edge to shadow. Measures from the lumen, wall, and total diameter are shown in Figure 3.

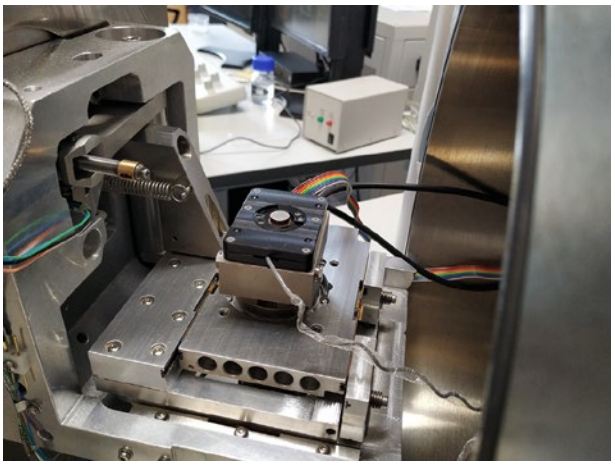

Figure 2. Image of the Quanta FEG 250 scanning electron microscope.

Theoretical Calculation of the Resistance to Flow

The Hagen-Poiseuille equation[11] was used to calculate the theoretical resistance to flow (R, mm Hg/[µL/min]) and the pressure differential or pressure drop ($P = \triangle p = p_1 - p_2$, mm Hg) through PRESERFLO for a known volumetric flow rate or AH production (Q, µL/min) and an AH viscosity of 0.7185 centipoises (cP) at 36°C for primary open-angle glaucoma.[15] In our flow experiments, we used the known dynamic viscosity of water $\mu = 0.9775$ centipoises (cP) at 760 mmHg = 1 atm = 101325 Pa.s (SI) and T = 21°C. Note that the dynamic viscosity of the AH is only 2% higher than the dynamic viscosity of water at the same temperature. We used the version of the Hagen-Poiseuille equation that accounts for the diameter (d^4) and not the radius (r^4). In this case, the proportionality constant number is 128 instead of 8:

$$P = Q \; x \; R$$

$$P = \frac{128 \text{ x } \mu \text{ x L x Q}}{\pi \; x \; d^4}$$

$$R = \frac{128 \text{ x } \mu \text{ x L}}{\pi \; x \; d^4}$$

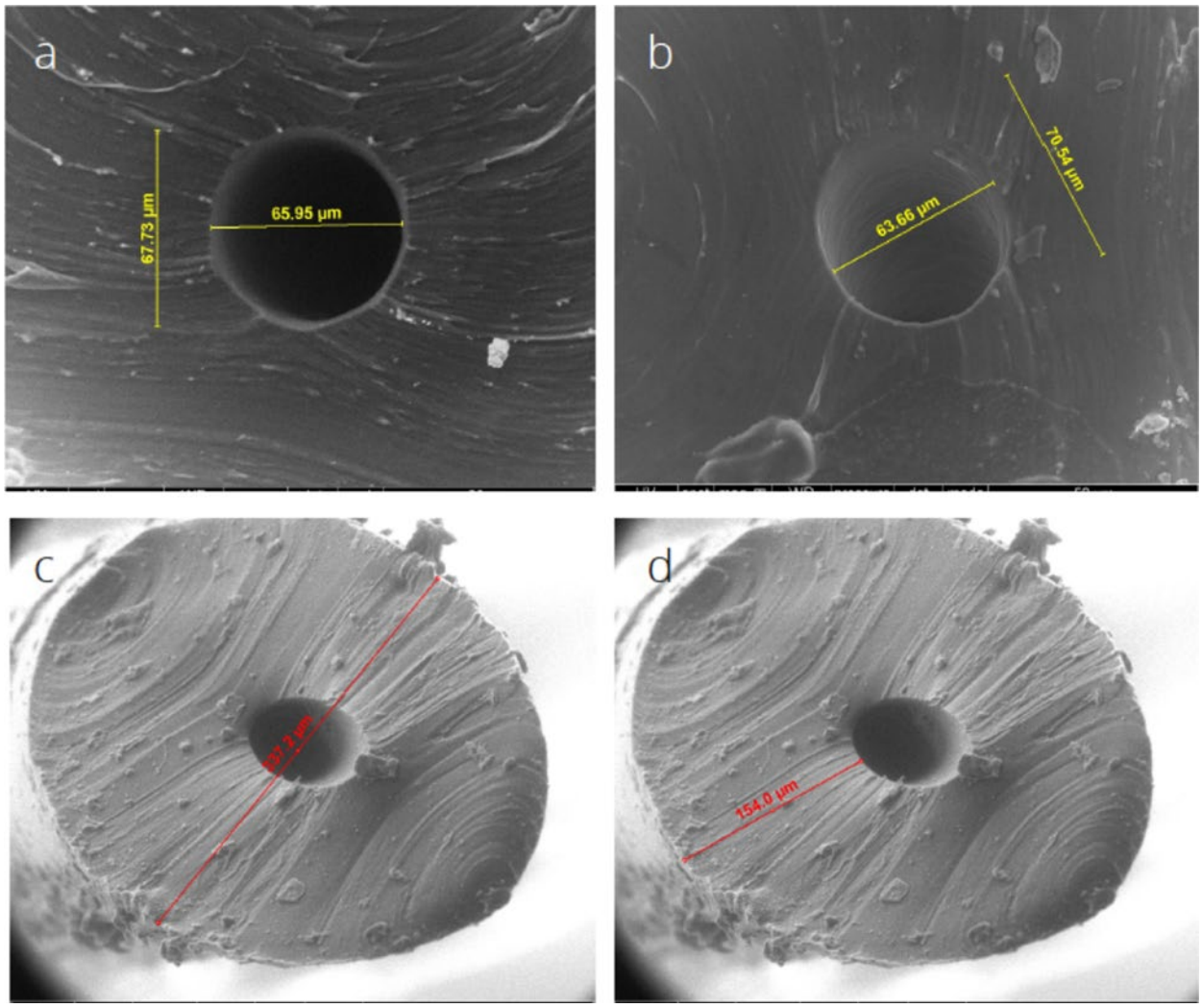

Figure 3. SEM images of the lumen diameter measured with the caliper tool. (a) Luminal diameter 67.73 × 65.95 µm. (b) Luminal diameter 63.66 × 70.54 µm. (c) Total diameter: 337.2 µm. (d) Wall: 154 µm.

$P = \triangle p = p_1 - p_2$ *(pressure drop along the lumen of the tube)*
μ = *dynamic viscosity*
L = *length*
Q = *volumetric flow rate*
R = *resistance to flow*

where R is the resistance to flow of the device, which is expressed in units of mm Hg/(µL/min). From the geometrical characteristics of the device and the dynamic viscosity of the fluid, we can compute the theoretical resistance to flow through the Hagen-Poiseuille flow.

Flow Study

The experimental setup followed the main features of the experiment conducted by Estermann et al.[16] Figure 4 shows the overall experimental setup.

Results

SEM Analysis

The results from the measurements of two SEM images are shown in Figure 3.

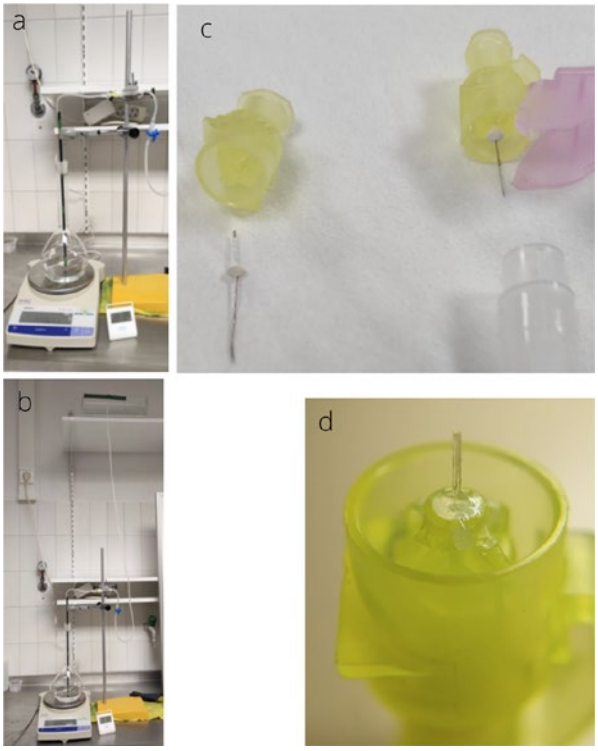

Figure 4. Experimental setup. The PRESERFLO is inserted into a tube with a large diameter ($D_T = 1$ mm), with both ends immersed in a physiological saline solution of sodium chloride used to limit the presence of bubbles in the fluid circuit, connecting two different containers located at different heights. Once the fluid has crossed the implant, it continues through the tube until it reaches the bottom container. The difference in height between the top fluid reservoir and the bottom container induces the hydrostatic pressure P. This hydrostatic pressure is the only motor behind the fluid flow in the circuit according to the Hagen-Poiseuille law. No surface tension mechanisms (such as droplet formation) should affect the dynamics.

Theoretical Calculation of the Resistance to Flow

The results from the theoretical calculation of R and P for each glaucoma implant are shown in Figure 5.

Experimental Flow Study

Figure 6 shows the time evolution of the collected mass as a function of time. The volumetric flow measured was Q = 35.22 (µL/min) for a pressure P of 58.92 mm Hg. From the linear fit ($R^2 = 0.9241$), the estimated resistance to flow was $R_E = 1.806$ mm Hg/(µL/min) with a computed confidence interval of 95% [1.571; 2.041] mm Hg/(µL/min). To compare the results with the parameters present in a physiological environment, we estimated the resistance of the PRESERFLO MicroShunt to be $RE_{PHYS} = R_E \times (\mu_{AQHUM}/\mu_{WATER})$ 1.301 mm Hg/(µL/min), in excellent agreement with the theoretical predicted value $R = 1.3$ mm Hg/µL/min, and the pressure drop was estimated to be 2.6 mm Hg.

With the estimated Reynolds number for the present fluid experiment for a flow of 60 µL/min and an average speed of approximately 0.26 (m/s), our experiment operated for Reynolds numbers in the range between 25 and 60, far below the transition to the turbulent regime. This meant that even for such a high applied pressure, the flow was still laminar, and the Hagen-Poiseuille law was still valid.

Discussion

In recent years, advancements in glaucoma tube development for flow restriction have brought new implants to the glaucoma surgery spectrum that aim not only to prevent hypotony but also to simplify and standardize the surgical technique, with the goal of a lower rate of postoperative complications. The objective is twofold: to reach sufficient flow restriction to decrease the risk of hypotony without undermining the drainage efficacy and to maintain the lowest possible IOP once a filtering bleb has been developed.

Sheybani et al.,[12] in an experimental flow test of the XEN 45, showed that the implant was capable of maintaining the backpressure above numerical hypotony (defined as an IOP ≤ 5 mm Hg, after surgery by the World Glaucoma Association Guidelines[19]). The experimental steady-state pressure reported for XEN 45 by the authors was 8.9 mm Hg, and the resistance to flow was 4.393 mm Hg/(µL/min), 3.4-fold higher than the experimental resistance to flow (R_E = 1.301 mm Hg/]µL/min]) and pressure drop (2.6 mm Hg) measured for the PRESERFLO with our experimental setup, suggesting that PRESERFLO would not be able to avoid hypotony (5 mm Hg) just by means of its flow restriction properties. Given that the clinical rates of hypotony reported for PRESERFLO in the literature are much lower than expected by the results of the laboratory tests, it is reasonable to believe that there must be crucial control of the outflow exerted initially by the Tenon capsule and later by the remodeling of the subconjunctival tissue.

In a study published by Scheres et al.,[20] the incidences of hypotony with the PRESERFLO and the XEN 45 were comparable. In fact, they found that during the first week after surgery, although the rate of hypotony was higher with PRESERFLO (39%) than with XEN 45 (24%), the need for anterior chamber reformation was lower (2% PRESERFLO, 5% XEN

PRESERFLO Microshunt:

$$R = \frac{128 \times 0.7185 \times 1.25 \times 10^{-7} x\ 8.5}{3.1416\ x\ 0.07^4}$$

R= 1.3 mmHg/µL/min

$P = 2\ x\ 1.3$

P= 2.6 mmHg

XEN 45:

$$R = \frac{128 \times 0.7185 \times 1.25 \times 10^{-7} x\ 6}{3.1416\ x\ 0.045^4}$$

R= 5.35 mmHg/µL/min

$P = 2\ x\ 5.35$

P= 10.7 mmHg

Ex-Press P-200:

$$R = \frac{128 \times 0.7185 \times 1.25 \times 10^{-7} x\ 2.56}{3.1416\ x\ 0.377^4}$$

R= 0.0004 mmHg/µL/min

$P = 2\ x\ 0.0004$

P= 0.0008 mmHg

Ahmed AGV, Baeverdelt BGI, Molteno:

$$R = \frac{128 \times 0.7185 \times 1.25 \times 10^{-7} x\ 10}{3.1416\ x\ 0.305^4}$$

R= 0.004 mmHg/µL/min

$P = 2\ x\ 0.004$

P= 0.008 mmHg

Paul Glaucoma Implant:

$$R = \frac{128 \times 0.7185 \times 1.25 \times 10^{-7} x\ 10}{3.1416\ x\ 0.127^4}$$

R= 0.14 mmHg/µL/min

$P = 2\ x\ 0.14$

P= 0.28 mmHg

Figure 5. According to the different internal diameters and lengths of each glaucoma drainage device (PRESERFLO MicroShunt, 70 µm, 8.5 mm; XEN45, 45 µm, 6 mm; Ahmed AGV, Baerveldelt BGI, and Molteno 305 µm, 10 mm; Paul glaucoma implant, 127 µm, 10 mm), we calculated *R* and P for an aqueous humor flow rate of 2 µL/min (the aqueous humor flow rate has been reported to be 2.75 ± 0.63 µL/min [a range of 1.8 to 4.3 µL/min][17]). For the Ex-PRESS implant (Alcon Laboratories, Inc., Fort Worth, TX, USA), the theoretical calculations of flow were performed only for the P200 model (377 µm, 2.56 mm) and not for the P50, which is more commonly used in clinical practice, because the P50 model has a 150 µm diameter bar placed across its lumen,[18] with implications on the fluid properties that prevent the theoretical calculation of the resistance to flow.

45), with the same rate of choroidal detachment for both implants (2%). In addition, after PRESERFLO implantation, Schlenker et al.[21] reported three out of 181 cases of anterior chamber reformation and four out of 181 cases of late choroidal detachment, and Batlle et al.[22] reported 13% shallow anterior chambers and 8.7% choroidal detachments during the first three weeks. In a previous study by our group,[23] we reported an 11% rate of hypotony.

Regarding trabeculectomy, Abbas et al.[24] reported a hypotony rate of 47% at any time during follow-up and 11% of persistent hypotony in two consecutive follow-up visits. In the Tube Versus Trabeculectomy study,[25] the rate of persistent hypotony after trabeculectomy was 23% at three years, increasing to 31% at 5 years.

The Ahmed versus Baerveldt study[10] reported a similar rate of hypotony after one year of follow-up (14%) for both implants. The AGV and the Baerveldt implants share the same resistance to flow ($R = 0.004$ mm Hg/µL/min) but differ in the pressure drop across the tube (0.008 mm Hg for Baerveldt, 13.6 mm Hg, and 6.1 mm Hg opening and closing pressures for AGV[6]) because of the valved mechanism of the AGV. In vitro testing of the AGV model FP7 showed significant variability in the closing pressure, with half closing at IOPs considered potentially problematic in clinical

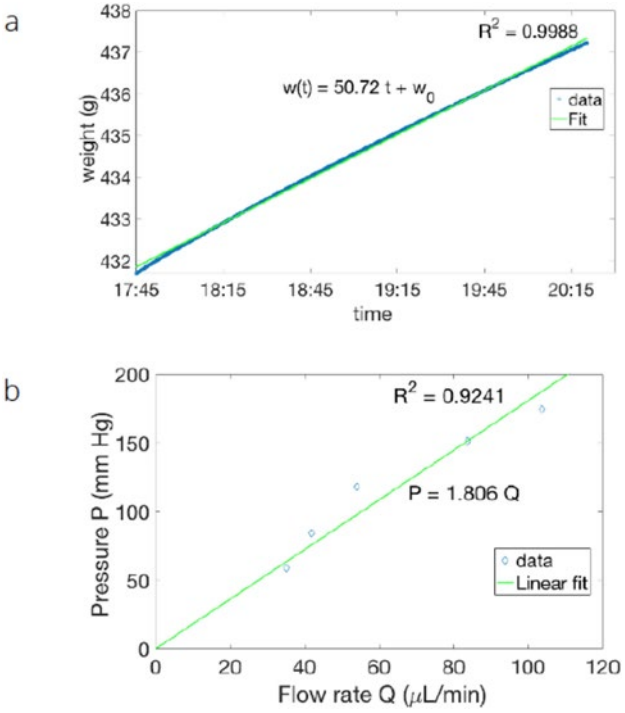

Figure 6. Time evolution of the collected mass as a function of time. (a) Results obtained by moving the upper fluid reservoir and therefore modifying the applied pressure P. According to the Hagen-Poiseuille law, a constant applied pressure difference induces a constant flow, the flow is measured in terms of the collected mass, and the linear growth of the mass as a function of time allows us to determine the flow. The initial difference in height between the top container and the bottom container was 80.1 cm. Assuming that the physiological solution used in our experiment had the same density as water, it was converted into a pressure difference of P = 58.92 mm Hg. The linear fit ($R^2 = 0.9988$) of the collected data was approximated by $w(t) = 50.72\ t + w_0$, where w(t) is the weight in grams and t is the time expressed in a fraction of the 24-hour period. For the fixed pressure, the observed mass flow was given by Q = 50.72 g/(24 h) with a 95% confidence interval of [50.71; 50.74] g/(24 h). The linear fit was excellent; indeed, the confidence interval for the estimated mass flow was very narrow and only affected the fourth significant digit. The volumetric flow was also expressed in more conventional units as Q = 35.22 (μL/min) for a pressure P of 58.92 mm Hg. b) At five different heights, the flow rate (Q) was expressed as a function of the applied pressure (P) through a linear fit ($R^2 = 0.9241$). From the data, the resistance to flow was estimated to be $R_E = 1.806$ mm Hg/(μL/min) with a computed confidence interval at 95% of (1.571; 2.041) mm Hg/(μL/min). Note that error bars are not shown on the data because they are very small (less than the *diamond symbol* size).

situations (1.4, 3.2, 3.5 mm Hg).[7] The FP7 AGV is made of silicone, which is much less stiff than the previous polypropylene, and may probably have a less precise opening pressure.[7]

Compared to the new short tubes (XEN 45, PRESERFLO), only a randomized control trial that included a significant number of patients would show the real clinical differences between them, but the clinical results reported so far suggest that the rates of hypotony expected for them are not higher than those reported for the long-tube drainage devices. The Paul glaucoma implant[26] is an example of the progressive tendency to narrow the luminal diameter of the tube to search for the ideal size to control the outflow in long tubes. This implant does not have a valved mechanism to restrict the flow of aqueous. According to the Hagen-Poiseuille equation, the theoretical pressure drop of this implant would be 0.28 mm Hg, far below 5 mm Hg. Nevertheless the hypotony rates reported are quite similar to those of the Ahmed and Baerveldt drainage devices (14.9% rate of self-limiting shallow anterior chamber and 9.5% hypotony requiring intervention).[27]

The tissue response to the flow rate of aqueous humor and the formation of a characteristic type of filtering bleb could explain the low incidence of hypotony reported for the PRESERFLO implant. In a study published by our group,[23] we analyzed the morphology and geometry of the blebs formed after the implantation of PRESERFLO with anterior segment optical coherence tomography (AS-OCT). From the early postoperative period to the third month, the aqueous humor displaces the tissues to form fluid cavities underneath the Tenon, that showed a measurable horizontal and vertical expansion and a multilayered appearance of the overlying conjunctival stroma in the majority of the cases. The morphology of the blebs in the early postoperative period resembled that of classic trabeculectomy blebs. A longer follow-up of the same patients up to one year showed that the bleb maturation process led to the formation of thick hypo reflective walls, as happened in the maturation process after trabeculectomy (Fig. 7). In contrast, the AS-OCT morphology of the filtering blebs associated with XEN has been described as low-lying, diffuse,[28] or a "filtering conjunctiva,"[27] without a conventional bleb, suggesting that the lower flow through the XEN vs the PRESERFLO probably accelerates the subconjunctival fibrotic response, thus increasing the number of needlings required (43%–71% XEN vs. 8.5% PRESERFLO[21]). The location of the distal end of the implant and the surgical technique ("ab interno" vs. "ab externo") may also determine the ability of a tube to form functioning filtering blebs. Lenzhofer et al.[29] showed with AS-OCT that the XEN gel stents located in deeper locations (the Tenon layer above the outer stent lumen) achieved higher IOP reductions and lower secondary needling rates (68% sub-Tenon, 80% intraconjunctival). The "ab interno" technique used to implant the XEN device has been reported to be less prone to bleb formation because of a higher resistance to flow of the tissues.[30] Most likely,

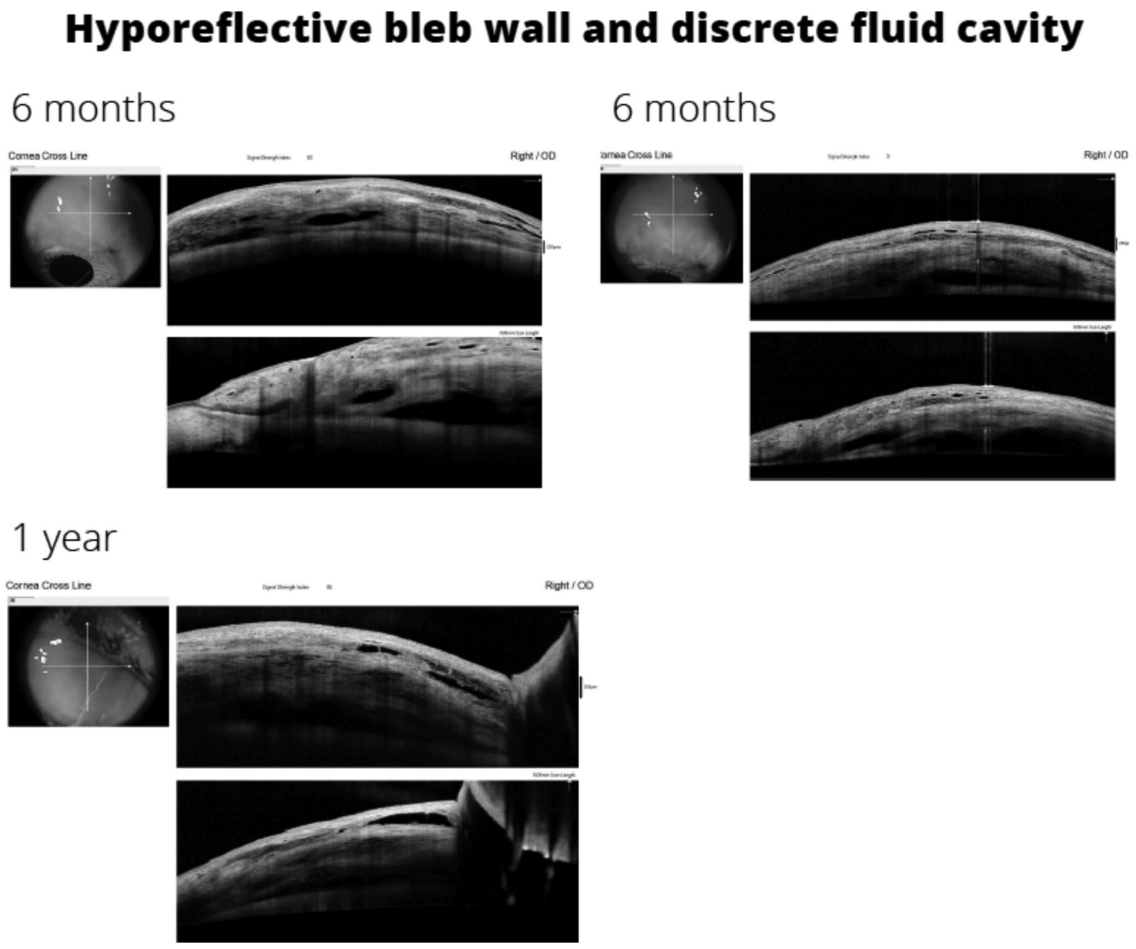

Figure 7. AS-OCT image of the hyporeflective bleb wall and discrete fluid cavity of a PRESERFLO bleb. Evolution from six months to one year.

the "ab interno" approach makes it difficult to identify the layers of the conjunctiva-Tenon complex to place the tip of the implant underneath the Tenon capsule. One of the key aspects of PRESERFLO is the "ab externo" dissection of the virtual space located between the conjunctiva and the Tenon capsule[31] to create a wide pocket where the bleb forming process initiates. Narita et al.[32] suggested the necessity to leave the Tenon capsule as it is during trabeculectomy to facilitate the formation of thick and hypo reflective bleb walls, the same principle followed during the surgical technique used to implant a PRESERFLO. Maintaining the anatomy of the Tenon capsule seems to be an important factor in controlling the outflow in the early postoperative period with this device. The sequence of AH outflow control by the subconjunctival tissue response might well be as follows: the mere presence of the fluid,[33] as well as the presence of inflammatory mediators (cytokines) in the AH of primary open-angle glaucoma patients[34] that initiates the fibrovascular response of the tissues to form the filtering bleb that ultimately modulates AH absorption, takes a few weeks to occur.[1] In the very early postoperative period when the fibrovascular response has not yet been initiated, the resistance to flow offered by the Tenon capsule against the amount of AH delivered by the implant (directly proportional to the preoperative intraocular pressure but restricted by its length and diameter) might be the main factor involved in the prevention of hypotony.

When the AH is released in the sub-Tenon space at a certain flow rate, lower than the capillary pressure of the surrounding tissues, a fibrovascular response is initiated at the capsule toward the formation of a bleb wall,[1] whose porosity and therefore hydraulic conductivity are sufficient to maintain a

constant flow of AH from the anterior chamber, tube, bleb wall and finally the episcleral venous system. According to a study published by Gardiner et al.,[35] the diameter of the tube becomes irrelevant when the pressure between the anterior chamber and the bleb reaches equilibrium. Lim[6] dissents somehow from Gardiner's et al. hypothesis, advocating that neither the XEN 45 nor the PRESERFLO address the problem of "resistor in series," meaning that once bleb resistance has occurred, the flow restrictor within the tube actually becomes redundant and even detrimental to achieving the lowest possible IOP.

Based on the outcomes of the current experiment, which operates at Reynolds numbers in the range of 25 to 60 (far below the transition to the turbulent regime), even for such a high applied pressure, the flow is still laminar, and the Hagen-Poiseuille law is still valid for measuring the resistance to flow through the PRESERFLO MicroShunt.

In conclusion, despite the theoretical and experimental inability of the PRESERFLO MicroShunt to protect against hypotony, the clinical results reported suggest that the tissue response to the particular flow rate of the aqueous humor provided by this implant provides enough resistance to flow to maintain the intraocular pressure above hypotony in most cases.

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Anterior-segment optical coherence tomography of filtering blebs in the early postoperative period of ab externo SIBS microshunt implantation with mitomycin C: Morphological analysis and correlation with intraocular pressure reduction

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ABSTRACT.

Purpose: **To analyse the morphological evolution of filtering blebs with anterior-segment OCT (AS-OCT) and its correlation with intraocular pressure after ab externo SIBS microshunt implantation with mitomycin C (MMC) during a 3-month follow-up period.**

Methods: **Twenty-eight filtering blebs of 28 patients with open-angle glaucoma were measured horizontally and vertically in the sub-Tenon space with AS-OCT after ab externo SIBS microshunt implantation with MMC. The intraocular pressure (IOP) was monitored simultaneously at each visit. Maturation of and morphological changes in the blebs and correlations with the IOP were recorded.**

Results: **The average median preoperative IOP of 20.7 (range, 12–30) mmHg decreased to 8.5 (range, 4–17), 8.9 (range, 5–17), 10.4 (range, 8–16) and 10.9 (range, 9–15) mmHg at 24 hr, 1 week, 1 month and 3 months, respectively ($p < 0.001$). A multiform morphology on AS-OCT prevailed at all time points, with a 3.5% rate of a uniform bleb morphology at the first week. The horizontal and vertical diameters of the blebs increased from baseline to the third month. The horizontal expansion (406 ± 127 µm on day 7, $p = 0.04$, 712 ± 211 µm on day 30, $p = 0.02$ and 952 ± 218 µm on day 90, $p < 0.001$) was greater than the vertical expansion (16 ± 18 µm, $p = 0.3$ on day 1, 63 ± 27 µm, $p = 0.02$ on day 30 and 137 ± 34 µm, $p < 0.001$ on day 90) without correlation with the IOP ($r = -0.3$, $p = 0.2$).**

Conclusion: **Anterior-segment OCT (AS-OCT) of the filtering blebs formed after ab externo SIBS microshunt implantation showed progressive horizontal and vertical expansion of the blebs in the sub-Tenon space, with a significant peak at the first month not significantly correlated with the decrease in the IOP.**

Key words: glaucoma – POAG – PRESERFLO – AS-OCT – MIGS – Bleb – IOP – mitomycin C

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Introduction

In recent years, new surgical approaches for glaucoma surgery that bypass the trabecular meshwork by shunting aqueous humour to the subconjunctival and sub-Tenon space have been developed (Francis et al. 2011). The aim of these new procedures is to improve the safety and predictability compared with those of trabeculectomy (TB), the gold

standard since its introduction in 1968 (Cairns 1968).

Although these new devices (XEN® 45 and XEN®63 Gel Stent, Allergan Inc., Dublin, Ireland) share the same mechanism of action that depends on the formation of a functioning filtration bleb, the PRESERFLO™ MicroShunt (Santen, Osaka, Japan) is composed of a biocompatible biomaterial called poly(styrene-block-isobutylene-block-styrene), or 'SIBS', which has been shown to induce minimal scarring and reduce chronic inflammation (Acosta et al. 2006; Pinchuk et al. 2008; Arrieta et al. 2011). It is designed to maintain a constant aqueous outflow through the lumen of the tube to a posterior location at least 7 mm away from the limbus under Tenon's capsule, with no need for a scleral flap or sutures that determine the amount and direction of the aqueous flow. Poor aqueous humour outflow underneath the scleral flap might increase the risk for scarring and intraocular pressure (IOP) elevation. In fact, the most important risk factor for bleb failure is a fibrotic response at the wound site in the subconjunctival tissue (Hitchings et al. 1983). Although clinical classification systems currently in use (Picht & Grehn 1998]; Indiana bleb Appearance Grading Scale [Cantor et al. 2003], Moorfields Bleb Grading System [Hamanaka et al. 2013]) remain useful tools to determine bleb height, extension and vascularization, anterior-segment optical coherence tomography (AS-OCT) enables assessment of the ultrastructure and differentiation of the type of bleb according to the type of surgery and the presence or absence of fibrosis. There are many published studies that describe fibrosis and scarring as the presence of areas of high reflectivity within the bleb, as shown by AS-OCT images after trabeculectomy (Wells et al. 2004; Ciancanglini et al. 2008; Tominaga et al. 2010; Napoli et al. 2014; Güven et al 2015; Kokuban et al. 2016; Meziani et al. 2016). Other studies have described specific AS-OCT characteristics of the blebs formed after ab interno gel microstent implantation, including a complete absence of significant subepithelial fibrosis, flatter blebs and epithelial thickening (Teus et al. 2019), as well as a uniform bleb morphology rate of 48% (Lenzhofer et al., 2019a,2019b). To our knowledge, the specific ultrastructural characteristics of the blebs obtained after PRESERFLO implantation are currently unknown, as are the possible correlations between the bleb morphology and IOP.

In this study, we used the AS-OCT software of the AngioVue OCT system (Optovue, Inc., Fremont, CA, USA) to compare the blebs described in the literature resulting from trabeculectomy and the implantation of other subconjunctival glaucoma surgery devices (XEN-GGM, Allergan Plc, Parsippany, NJ, USA) with those resulting from PRESERFLO MicroShunt implantation. The sub-Tenon position of the tube was our reference parameter to measure the ultrastructural changes of the bleb from the early preoperative period through the third month and to correlate the changes to changes in the IOP.

Methods

Patients

This was a prospective, interventional, single-centre case series of consecutive patients receiving the ab externo SIBS microshunt with mitomycin C (MMC). All surgeries were performed by the same experienced surgeon (M.I.B). The protocol adhered to the tenets of the Declaration of Helsinki, and approval was obtained for the study protocol from the ethics committee at our institution before enrolment. Informed consent was provided by all participants prior to their inclusion in the study. All patients who underwent surgery were previously diagnosed with primary open-angle glaucoma (POAG) at the Glaucoma Unit of the HLA Hospital Universitario Moncloa, Oftalvist Group, Madrid, Spain. Primary open-angle glaucoma (POAG) was defined as the presence of an open iridocorneal angle, signs of glaucomatous optic nerve damage and visual field (VF) defects. primary open-angle glaucoma (POAG) patients who were on maximum tolerable medical therapy and showed progressive visual loss were included in the study. The inclusion criteria were as follows: best-corrected visual acuity (BCVA) of 20/200 or better; uncontrolled glaucoma under maximum tolerated medication, with an IOP values ranging from 12 to 45 mmHg; phakic or pseudophakic patients treated with intracapsular lens implantation; and individuals who showed rapid and significant loss of visual function [visual function index (VFI), mean deviation (MD) and glaucoma progression analysis (GPA) with the Humphrey Visual Field Analyzer, Carl Zeiss AG, Germany]. Both eyes could be included if necessary, with one month between procedures for uncomplicated cases.

The exclusion criteria were as follows: angle closure, congenital, pseudoexfoliative, pigmentary, uveitic or neovascular glaucoma. Eyes with a history of previous filtering surgery (trabeculectomy) were only included when no sign of filtration was present at the slit-lamp examination (flat, vascularized bleb) and with the AS-OCT (flat bleb, uniform hyperreflective stromal pattern, no microcyst and no bleb drainage pathway). In these cases, the PRESERFLO MicroShunt was implanted in a different quadrant, avoiding the previous filtration site. If the bleb dimensions were as big as to make the OCT measurements impossible, the patient was followed and the OCT examination was repeated in a posterior visit, so only the data that could not be obtained were not analysed. This happened in just one visit of one eye that developed a tenon's cyst. Patients who had neck or back problems were excluded because of their inability to perform AS-OCT. The PRESERFLO MicroShunt was implanted with or without combined cataract surgery. Twenty-eight eyes of 28 patients were enrolled.

Baseline preoperative characteristics were collected from all patients, including demographics (age, sex, date of surgery, laterality and diagnosis), type of surgery (combined or stand-alone) and ocular parameters (BCVA assessed with the Snellen chart, IOP at which the decision for surgery was made measured using a calibrated Goldmann applanation tonometer, central corneal thickness [CCT]) and number of glaucoma medications at the preoperative visit). During follow-up, IOP, BCVA and AS-OCT measures of the tube and the size of the bleb (explained in detail in the AS-OCT section) were analysed (24 hr, 1 week, 1 month and 3 months). The number of patients with hypotony, the number of patients who required glaucoma medication and the number of medications were also collected.

Surgical technique

All surgeries were performed under sub-Tenon's anaesthesia in the inferonasal quadrant. A traction suture on the superior cornea was used to expose the superonasal conjunctiva to perform conjunctival peritomy and careful Tenon dissection over two clock hours, liberating all the attachments between Tenon's capsule and the episclera and creating a posterior pocket between the superior and medial rectus muscles. A diathermy probe was applied to the sclera to control bleeding and to obtain a clear surgical field. Three circular surgical sponges soaked in MMC (0.2 mg/ml) provided by the manufacturer were placed under Tenon's layer away from the limbus for 2 min and then removed, followed by gentle washing with balanced salt solution. A mark with trypan blue was placed with the tip of the calliper 3 mm away from the limbus, and a 1-mm-wide scleral preincision was created with a microknife until the tip was not visible. The scleral tunnel was created parallel to the surface of the sclera with a 25-G needle entering the anterior chamber (AC) at the trabecular meshwork. The PRESERFLO MicroShunt was then introduced in the tunnel until it reached the AC, and its position was visually checked to ensure that it was not too close to the iris or endothelium and was placed with the bevel facing up. If the position of the tube was considered inadequate, the tube was removed and inserted again through a new tunnel placed next to the first tunnel. Located half-way down the tube, there is a planar fixation structure resembling the fins of an arrow that seals the device in the pocket, preventing leakage around the tube and preventing the tube from migrating into the eye. The fins were placed at the end of the scleral tunnel to ensure that it was inside the scleral tunnel. Flow through the implant was confirmed by injecting balanced salt solution (BSS) from the distal side of the tube with a 23-G cannula; usually, a small air bubble could be seen advancing to the anterior chamber, and drop-by-drop flow was confirmed from the end of the tube with a surgical sponge. None of the shunts had to be replaced intraoperatively due to an obstruction of the lumen. Tenon's layer was advanced prior to the conjunctiva to ensure that the implant was not caught in it, and then the conjunctiva was sutured watertight over Tenon's layer with 10-0 nylon. A side-port incision was created at the end of the surgery to inject 0.1 ml of cefuroxime (1 mg/0.1 ml) into the AC. For combined surgery, the surgical technique did not change and was performed at the end of the phacoemulsification and intraocular lens (IOL) implantation procedure.

Postoperative management

In both groups of patients, that is those treated with the combined and standalone procedure, postoperative care included prophylactic antibiotics in the first week after surgery and decreasing doses of topical corticosteroids over 8 weeks. Patients were seen at day 1, 1 week, 1 month and 3 months postoperatively. All visits included slit-lamp examination, with a Seidel test and fundus examination to evaluate choroidal detachment in the case of clinical signs of hypotony.

AS-OCT

Anterior-segment OCT (AS-OCT) was performed in all patients with the AS-OCT software of the Avanti Widefield OCT with AngioVue Angiography system (Optovue, Inc., Fremont, CA, USA), which is commonly used for the assessment of perfusion in various retinal layers. The optical source of the device was centred on 840 nm with a bandwidth of 50 nm, a scan speed (A-scan) of 70 000, an axial and transverse resolution of 5 and 15, an imaging depth of 2.0–3.0 mm, and a theoretical acquisition time of 2.7 s × 2, 4.6 s × 2 (HD mode). The AS-OCT software allows imaging and the recording of short videos of the anterior chamber with the anterior chamber adapter provided by the manufacturer. The patient was instructed to look down while the upper eyelid was pulled up to expose the filtering bleb. All images were captured by the same examiner without exerting any additional pressure on the eye. We used two different modes, '3D cornea' and 'Cornea Crossline', of the anterior-segment display of the OCT system software. The '3D Cornea' mode works with a 4.5 × 4.5-mm square that scans the X- and Y-axes that can be rotated and moved to be centred over the implant in the orientation desired until the image of the tube under Tenon's capsule can be seen. Several scans were obtained, and the best of them was selected for the study. From each scan, it was possible to obtain a short video, and serial images could be extracted to use those with the best quality, in which the bleb appeared to be larger, and in which the tube could be easily identified under Tenon's capsule. Usually, that spot was quite posterior, next to the distal end of the tube. The 'Cornea Crossline' mode offers higher resolution images but does not record video. We used this option to centre the raster over the tube in the anterior chamber to measure the distance of the tube from the iris and endothelium and to obtain high-quality images of the blebs to identify the different layers (conjunctival and Tenon layers) and the bleb structure. In both cases, the calliper tool of the OCT system was used to measure the tube-iris/tube-endothelium distance and the horizontal and vertical diameters of the bleb. The tube was considered to be significantly displaced when a difference ≥500 μm was found as a result of subtracting the lowest from the highest total distance (tube-endothelium + tube-iris) at every time point. For bleb measurements, in those cases where the bleb width exceeded the AS-OCT display, the horizontal measure was performed from one side to the other of the image even though the borders could not be identified (Fig. 1). The presence of a multiform versus a uniform morphology and the number of blebs with defined vs undefined borders were analysed at 24 hr, 1 week, 1 month and 3 months postoperatively. Multiform blebs were defined by the presence of multilayered stroma above the aqueous humour (AH) cavity, conjunctival microcysts and AH bleb formation around the tube (Fig. 2). Uniform blebs were defined as those in which the stroma around the tube was hyperreflective and smooth without a bleb drainage pathway (Fig. 3A). Multilayered (ML) and microcystic (MC) blebs were classified into three categories (low, moderate and high), and blebs formed around the tube (DP) were categorized as small, medium or diffuse.

Clinical classification of the filtering blebs

Additionally, the bleb morphology was analysed by slit-lamp examination by the same examiner who performed the

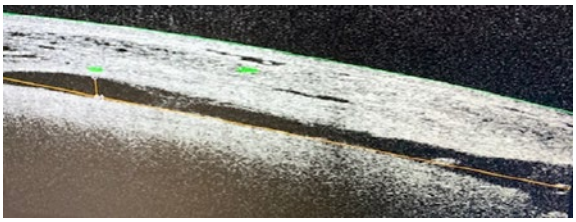


Fig. 1. AS-OCT image of a multilayered bleb where the drainage pathway exceeds the borders of the display. The tube can be identified on the right side of the image. Horizontal and vertical diameters were measured with the calliper tool.

OCT examination. Photographs were obtained at each visit to classify the blebs according to the Indiana Bleb Appearance Scale (IBAGS) (Cantor et al. 2003).

Statistical analysis

The statistical analysis was performed using Stata® 15.1 (Statistics Data Analysis, StataCorp, Texas, USA). The Shapiro–Wilk test was used to verify the normality of the data distribution. Mixed models were selected for the analysis of repeated measures using the Akaike and Bayesian information criteria to test the quality of the statistical model. The Satterthwaite equation was employed to calculate the degrees of freedom, the Scheffe test was used for multiple comparisons, Fisher's exact test was used for the analysis of binary variables, and the Spearman correlation coefficient and linear regression were used to analyse the relations between variables.

Results

Study population and baseline characteristics

A total of 28 eyes of 28 patients were included. The mean age of the study population at the time of the operation was 77.7 ± 8 years; laterality (RE/LE), 13/15; sex (M/F), 12/16; median preoperative IOP treated with antiglaucoma medications, 20.7 (range, 12-30 mmHg); mean BCVA, 0.65 ± 0.3; and mean number of eye drops pre-surgery, 2.8 ± 0.7. Combined surgery was performed in 4 patients (14%), 23 patients were pseudophakic (82%) and one was phakic (4%). The mean CCT was 533 ± 59 µm. Two patients required surgical revision due to a high IOP (at 24 hr and 1 month). Filtering blebs were successfully formed after revision. In one of the cases, a very

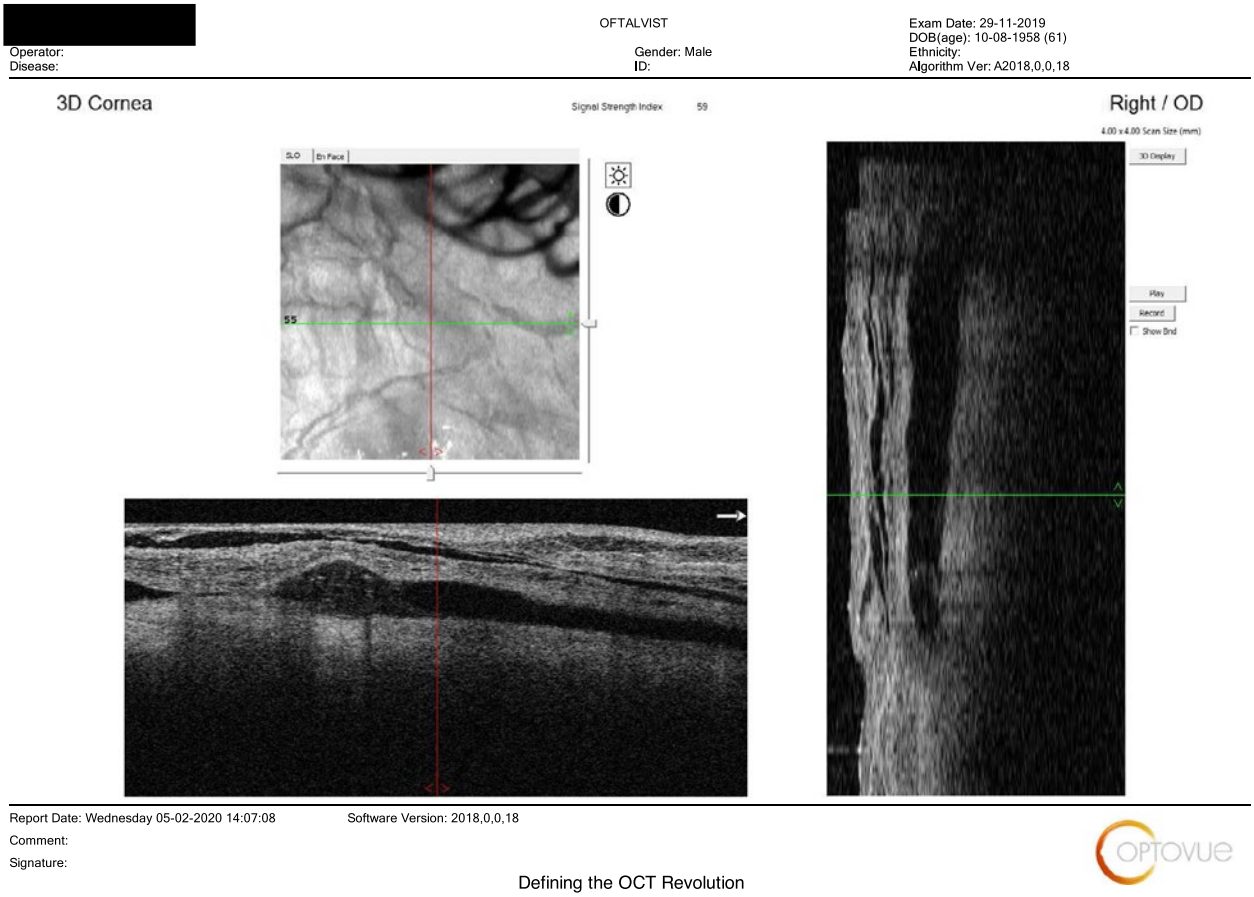
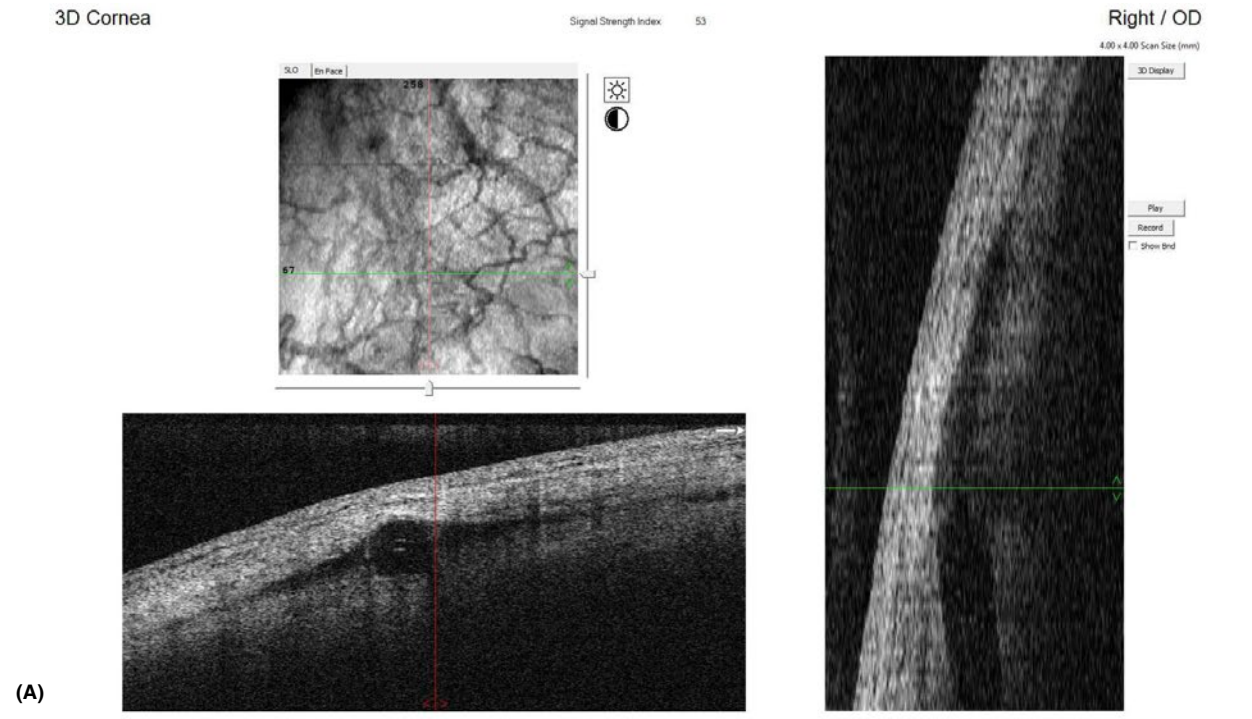
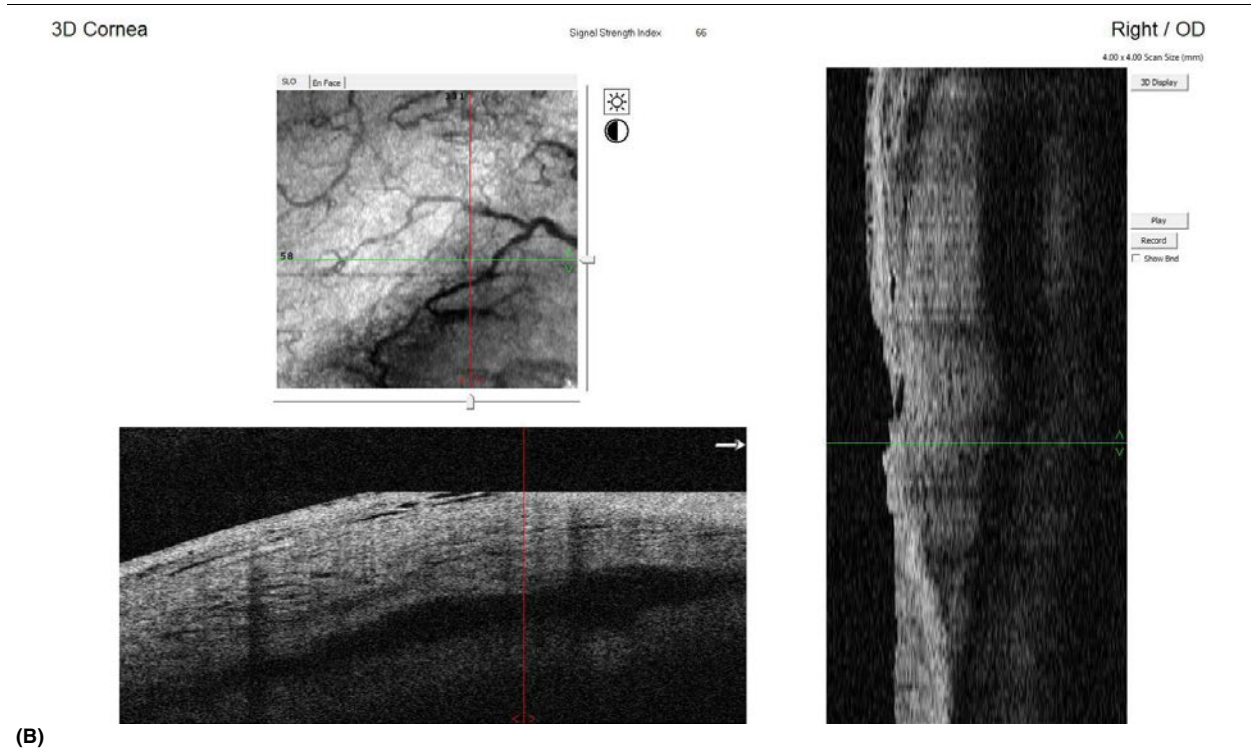


Fig. 2. AS-OCT image of a multiform PRESERFLO bleb, including multilayered stroma above the aqueous humour cavity and the bleb drainage pathway around the tube.



(A)



(B)

Fig. 3. A) AS-OCT image of a uniform bleb. The stroma above the tube is hyperreflective and smooth, and there is little AH around the tube. B) Image from the same patient after open surgical revision, including multilayered stroma and aqueous bleb around the tube.

elevated Tenon's cyst was observed in the first month visit (Fig. 4), and the OCT data from that visit first could not be obtained. Needling at the slit-lamp examination was not performed in any of the cases, and open surgical revision was preferred to sort out bleb failure.

Effect of the PRESERFLO MicroShunt on IOP

PRESERFLO MicroShunt implantation induced a decrease in the IOP from a median baseline (treated) of 20.7 (range, 12–30) mmHg to 8.5 (range, 4–17) mmHg at 24 hr, 8.9 (range, 5–17) mmHg at 1 week, 10.4 (range, 8–16) mmHg at 1 month and 10.9 (range, 9–15) mmHg at 3 months. The variation from baseline was significant at all time points (-12.2 ± 0.7 mmHg at 24 hr, -11.8 ± 0.7 mmHg at 1 week, -10.3 ± 0.7 mmHg at 1 month and -9.8 ± 0.7 mmHg at 3 months, $p < 0.001$). Pairwise comparisons of the IOP by age (<75 versus ≥75 years) or sex did not show significant differences ($p = 0.4$; $p = 0.7$). The Shapiro–Wilk test showed that the IOP did not follow a normal distribution. The IOP measurements are shown in Fig. 5. The rate of hypotony in this series was 11% (3 cases). One patient developed hypotony in the first week with athalamia and choroidal detachment. The evolution was positive with conservative treatment (occlusion and atropine eye drops 1%), with resolution in the first month. Another two patients showed moderate hypotony in the first week with a narrow AC but without choroidal detachment or hypotony maculopathy.

One patient required topical medication before the third month. In this case, even though the IOP was 16 mmHg without medication in the first month, we decided to introduce a prostaglandin analog to reach values in the low teens instead of performing surgical revision. The IOP prior to surgery was 26 mmHg with topical medication (latanoprost, timolol 0.5% and dorzolamide). Visual function damage was not severe (MD, −8.2 dB), and the microshunt apparently maintained flow through the tube. A filtering bleb was clearly observed on slit-lamp examination, and a small drainage pathway around the tube with a superior cap and lateral margins could be identified by AS-OCT (Fig. 6).

Clinical classification

Clinical classification of the blebs of the patients in the study was obtained following the Indiana Bleb Appearance Grading Scale (IBAGS) (Cantor et el. 2003) at all visits (Table 1), and slit-lamp images were captured to document the description of each bleb.

Position of the tube in the anterior chamber

The distance from the tube to the endothelium remained stable from the first visit to the third month (24 hr,

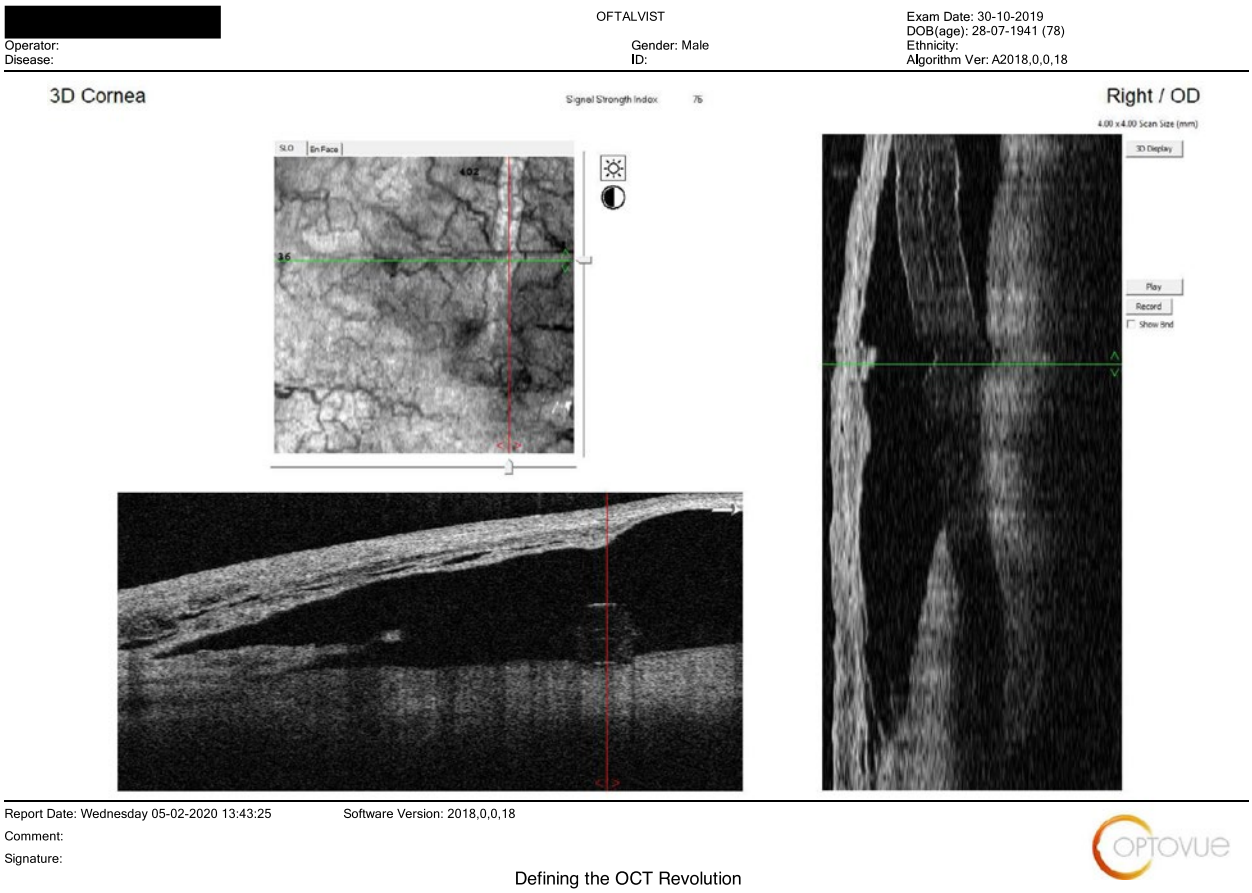


Fig. 4. AS-OCT image of an incipient Tenon cyst around the tube.

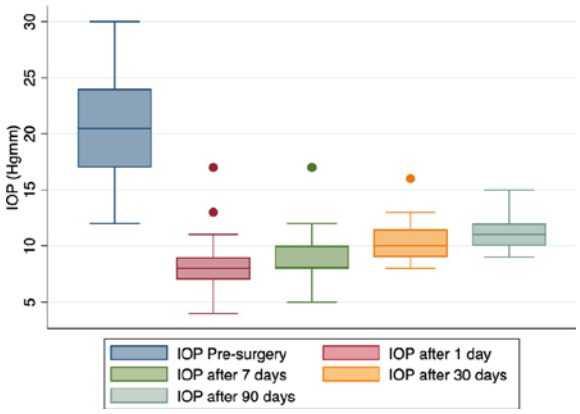


Fig. 5. Box plot of the evolution of the IOP after PRESERFLO implantation: preoperatively (treated, blue box) and 1 day (red box), 7 days (green box), 30 days (orange box) and 90 (light blue box) days postoperatively. The IOP decreased significantly on day 1, with a gradual increase from day 1 to day 90.

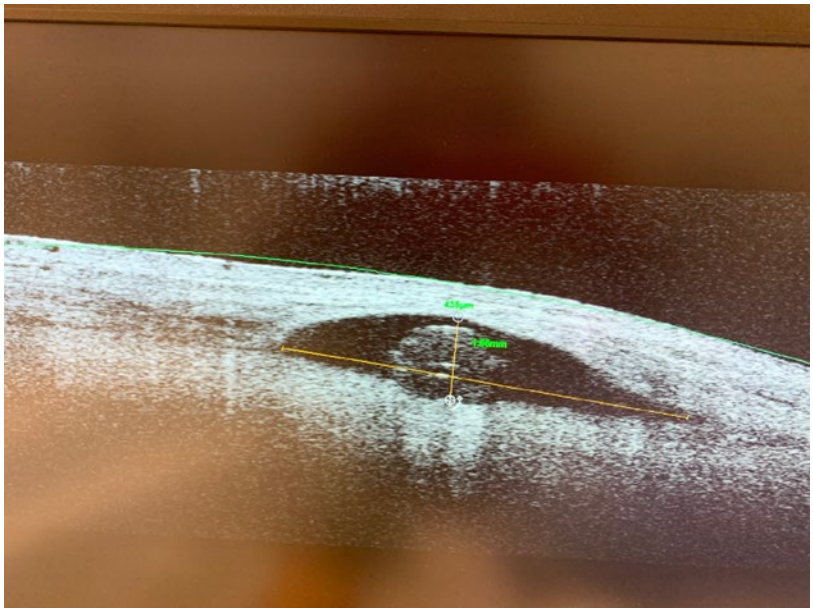


Fig. 6. AS-OCT image of a small bleb around the PRESERFLO MicroShunt in a patient who required topical medication before the third month. The IOP was 16 mmHg without treatment.

531 ± 341 μm; one week, 491.5 ± 339 μm; one month, 514 ± 290 μm; three months, 514 ± 306 μm). The tube-iris distance also remained stable, with a slight decrease in the first week due to the three cases of hypotony, one with athalamia and the other two with a moderately flat AC (24 hr, 760 ± 331 μm; 1 week, 700 ± 398 μm; 1 month, 821 ± 340 μm; 3 months, 812 ± 352 μm). Tube displacement (≥500 μm) was found in 3 cases (11%), the three cases of hypotony encountered in our series (a cross-sectional image of a tube too close to the iris and its measures are shown in Fig. 7). Fisher's exact test did not show significant differences in the position of the tube in the anterior chamber in relation to sex, laterality or age.

AS-OCT classification, bleb measurements and correlations with IOP

The morphological AS-OCT classifications are shown in Table 2. A multiform morphology prevailed at all time points, with a very low proportion of uniform blebs (0% at day 1, 3.5% at 1 week, and 0% at 1 and 3 months). The degree of a multilayered stroma decreased from 24 hr to 3 months (35.7% moderate MLs at day 1 versus 10.7% at month 3; 39.2% high MLs at day 1 versus 0% at 3 months), while the percentage of microcysts increased (moderate MCs 10.7% at 24 hr versus 64.2% at 3 months).

Regarding the horizontal and vertical diameters of the bleb, the Shapiro–Wilk test showed that the data followed a normal distribution. Mixed models with the Satterthwaite method were used to analyse the results. The mean horizontal bleb diameter varied from 1964 ± 929 μm at 24 hr to 2370 ± 852 μm, 2676 ± 932 μm, and 2916 ± 940 μm at 1 week, 1 month and 3 months, respectively (Fig. 8). The increase from baseline (day 1) was significant for all postoperative visit values (406 ± 127 μm at day 7, $p = 0.04$, 712 ± 211 μm at day 30, $p = 0.02$ and 952 ± 218 μm at day 90, $p < 0.001$). Furthermore, using the Scheffe test for multiple comparisons, a significant difference between the horizontal diameter at 1 week and 3 months (545 ± 180 μm) was found. There were no significant differences in the horizontal bleb diameter by sex or age ($p = 0.6$). The vertical bleb dimensions showed less variability, with a gradual increase from baseline (day 1, 294 ± 111 μm) to the first week (311 ± 121 μm), first month (349 ± 150 μm) and third month (427 ± 154 μm) (Fig. 9). The increase in the vertical diameter from baseline was not significant at the first week (16 ± 18 μm, $p = 0.3$), but it was statistically significant at days 30 and 90 (63 ± 27 μm, $p = 0.02$ and 137 ± 34 μm, $p < 0.001$). The Scheffe test for multiple comparisons showed a significant increase in the vertical diameter from day 7 to day 90 (120 ± 34 μm, $p = 0.01$) and from day 30 to day 90 (74 ± 23 μm, $p = 0.03$). There were no statistically significant differences in the vertical bleb diameter by age ($p = 0.9$) or sex ($p = 0.1$).

The Spearman rank correlation coefficient and linear regression analysis were used to study the possible relation between the size of the bleb formed after PRESERFLO implantation and the IOP. With the given sample size (28 eyes), a negative but nonsignificant correlation was found between the horizontal bleb diameter and the IOP at days 1, 30 and 90 ($r = -0.3$, $p = 0.2$ at the 3 time points) and between the vertical diameter and

Table 1. Indiana Bleb Appearance Grading Scale (IBAGS) classification of the PRESERFLO MicroShunt blebs from 24 hr to the third month.

Preserflo Bleb	24 hr	1 week	1 month	3 months
Bleb height				
H0: flat bleb	2 (7%)	2 (7%)	1 (3%)	0 (0%)
H1: low bleb	14 (50%)	13 (46%)	13 (46%)	14 (50%)
H2: medium bleb	10 (36%)	12 (43%)	14 (50%)	13 (46%)
H3: high bleb	2 (7%)	1 (3%)	0 (0%)	0 (0%)
Horizontal extent				
E0: 0 < 1 clock hours	2 (7%)	1 (3%)	2 (7%)	0 (0%)
E1: 1–2 clock hours	24 (86%)	24 (86%)	25 (89%)	27 (96%)
E2: >2 <4 clock hours	2 (7%)	2 (7%)	1 (3%)	1 (3%)
E3:4 or >clock hours	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Bleb vascularity				
V0: avascular white	0 (0%)	0 (0%)	0 (0%)	0 (0%)
V1: avascular cystic	0 (0%)	0 (0%)	0 (0%)	0 (0%)
V2: mild vascularity	1 (3.5%)	14 (50%)	22 (78%)	24 (86%)
V3: moderate vascularity	26 (93%)	13 (46%)	6 (21%)	4 (14%)
V4: extensive vascularity	1 (3%)	1 (3%)	0 (0%)	0 (0%)
Seidel test				
S0: no leak	28 (100%)	28 (100%)	28 (100%)	28 (100%)
S1: multiple pinpoint leaks	0 (0%)	0 (0%)	0 (0%)	0 (0%)
S2: streaming leak (within 5 s)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

the IOP at day 7 ($r = -0.07$) and day 30 ($r = -0.2$). Linear regression analysis between the bleb size and the IOP showed that only 10% of the IOP encountered on day 1 was explained by the size of the bleb ($R^2 = 0.10$).

Discussion

In this prospective study, we analysed the specific characteristics of the filtering blebs produced by an ab interno SIBS microshunt (PRESERFLO) implanted to treat POAG. The most frequent type of bleb found in these patients was localized under Tenon's capsule posterior to the limbus, with an AH drainage pathway around the tube expanding beyond the measurable limits of the AS-OCT system in 28% of the cases at 24 hr and 68% at 3 months. The connective tissue above the AH cavity showed a multilayered, loosened subconjunctival structure with fluid spaces and subepithelial microcysts in the majority of cases. A uniform bleb morphology (no fluid cavities under the conjunctiva and a hyperreflective stroma) was very infrequent in our series (3.5% at the first week), in contrast to the observed rate of 20.8% reported by Nakano (Nakano et al. 2010) and 48% by Lenzhofer et al., (2019a, 2019b) 2 weeks after TB and XEN implantation, respectively. In the first week, only one of our patients showed the absence of AH around the tube (AS-OCT, Fig. 3A) and a flat bleb on slit-lamp examination. After surgical revision, an AH drainage pathway was identifiable under Tenon's capsule (Fig. 3B).

The rate of AH flow under Tenon's capsule in a posterior location provided by the PRESERFLO may explain the low percentage of uniformity compared to that of TB and XEN implantation. A multiform bleb morphology was the most frequent AS-OCT pattern encountered at all time points (100% at 24 hr, one month and 3 months and 96.4% at 1 week), with the presence of multilayered, loosened stroma (Fig. 2) being more frequent in the very early postoperative period than later on during follow-up, probably due to the fibrotic response induced by surgical trauma. Microcysts showed a tendency to increase over time, following what may be termed the 'bleb maturation' process.

The horizontal bleb diameter observed after PRESERFLO implantation increased significantly from the very early postoperative period until the third month (28.5% undefined borders at 24 hr versus 67.8% at 3 months). The horizontal and vertical diameters of the bleb measured on AS-OCT did not show a significant correlation with the decrease in the IOP, and only 10% of the postoperative IOP at 24 hr could be explained by the bleb size. Most likely, not only is the size of the bleb around the tube responsible for the significant decrease in the IOP, but the bleb morphology (most of the blebs showed a multiform pattern on AS-OCT) may also play a role in the IOP decrease.

Smaller blebs have been related to higher IOP levels after TB using computational modelling of fluid flow, as reported by Gardiner (Gardiner et al 2010). In smaller TB blebs, the fluid must travel further from the bleb before it can be completely absorbed, but the fluid movement is limited by a lower hydraulic conductivity that induces an increase in the IOP. In the same model, larger blebs were predicted to induce lower IOP levels but with a significant risk of hypotony in TB blebs with a lack of definable cut-off values. In our study, 64% of the blebs formed around the tube had undefined horizontal borders at the 1-month examination, and there were only 3 cases of hypotony (11%). The expected rate of hypotony after PRESERFLO implantation, according to the bleb morphology that we encountered in the majority of cases, should be higher according to Gardiner's model designed for TB. The explanation for the low rate of hypotony despite the high frequency of large horizontal blebs could be the constant flow rate provided by the PRESERFLO, the resistance to flow through the lumen of the implant, and the surgical technique, different from TB and other devices, such as the XEN 45.

According to the Hagen–Poiseuille equation (McEwen 1958), the flow rate depends mainly on the diameter of the lumen and the length of the tube. For an AH flow rate (Q) of 2 µl/min, [2.75 ± 0.63 µl/min (range, 1.8–4.3 µl/min)] (Brubacker 1991), and a viscosity of human aqueous humour in POAG of 0.7185 (Vass et al. 2004), the resistance to flow through the PRESERFLO (lumen diameter, 70 µm; length, 8.5 mm) is predicted to be 2 mmHg/µl/min, whereas for the XEN 45 (lumen diameter, 45 µm; length, 6 mm), it is 8.5 mmHg/µl/min, which is fourfold higher than that for the PRESERFLO. These two devices differ not only in their resistance to flow but also in the surgical approach. The ab interno technique used with the XEN implant has been demonstrated to result in significantly ($p = 0.004$) increased

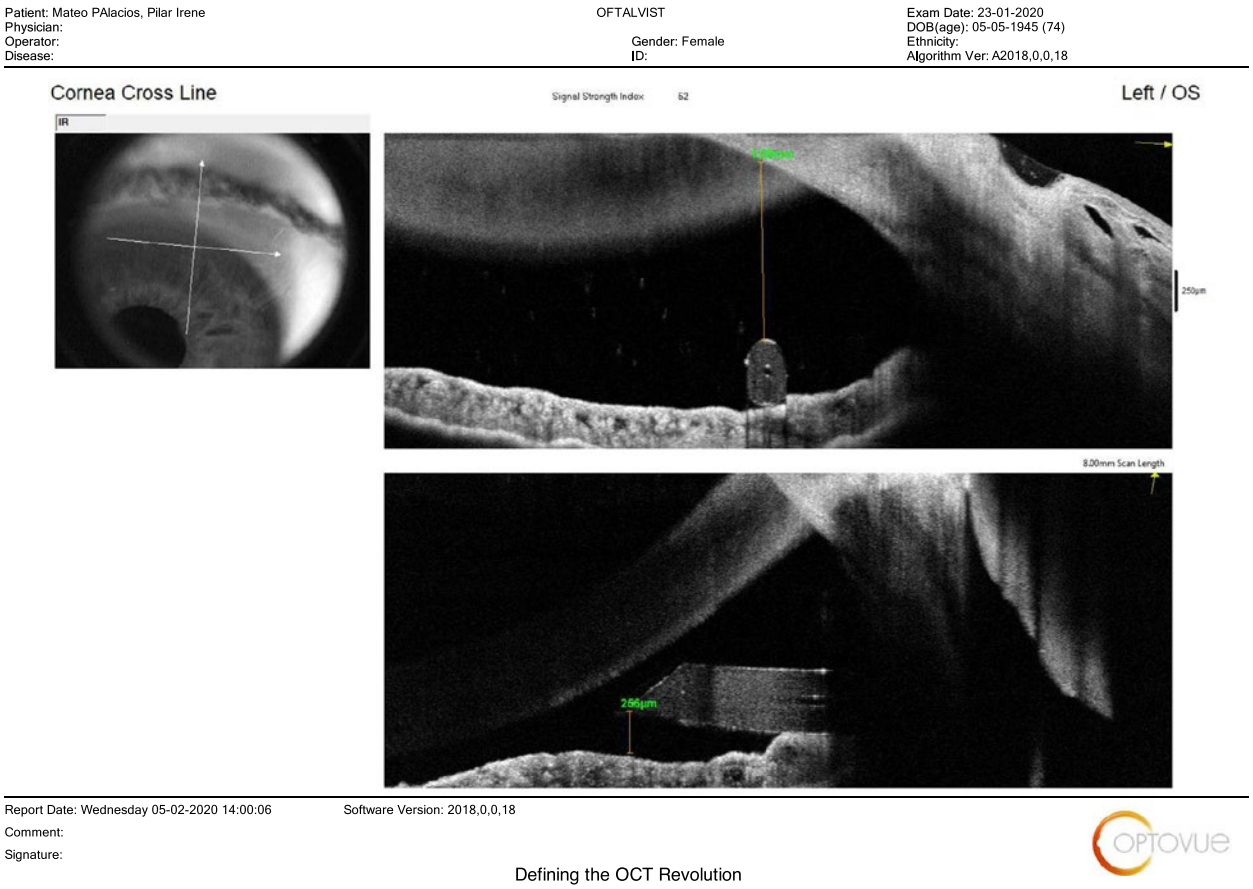


Fig. 7. Cross-sectional AS-OCT image of a PRESERFLO implant that was placed too low in the AC and was in contact with the iris.

Table 2. Morphological AS-OCT classification of PRESERFLO filtration blebs at 24 hr, 1 week, 1 month and 3 months.

	Uniform	Multiform	ML none	ML low	ML moderate	ML high	MC none	MC low	MC moderate	MC high	DP none	DP small	DP medium	DP diffuse
Day 1	0%	100%	0%	25%	36%	39%	25%	64%	11%	0%	0%	43%	28%	28%
Week 1	3%	96%	11%	25%	61%	3%	11%	71%	18%	0%	3%	7%	53%	36%
Month 1	0%	100%	7%	57%	28%	7%	7%	46%	46%	0%	0%	3%	32%	64%
Month 3	0%	100%	14%	75%	11%	0%	7%	28%	64%	0%	0%	7%	25%	68%

DP = drainage pathway, MC = microcysts, ML = multilayer.

outflow resistance (0.8 ± 0.11 mmHg/µl/min) compared with the ab externo implantation of the same device (0.42 ± 0.05 mmHg/µl/min), as reported by Lee et al. (2019). In addition, the resistance to flow and therefore the efficacy rate of the ab interno technique for the XEN implant has also been reported to be higher with deeper placement (intra–sub-Tenon) than with more superficial placement (intraconjunctival) (Lenzhofer et al., 2019a,2019b). The higher outflow resistance associated with the ab externo technique used for the XEN implant, especially when it ends up being implanted intraconjunctivally, along with the higher resistance to flow through the tube, may explain the uniform bleb rate of 48% reported by Lenzhofer et al., (2019a, 2019b), as well as the results reported by Teus et al. (2019), including significantly flatter XEN blebs (417 ± 183 µm) compared to those resulting from TB (618 ± 256 µm), a 50% rate of 'cystic area' formation versus 80% after TB, and the development of a 'filtering conjunctiva' after XEN implantation instead of a conventional, localized bleb. Nevertheless, it should be noted that in the study conducted by Teus, both the XEN implantation and TB surgeries had been performed one year before AS-OCT analysis, preventing a direct comparison of the results with those of the current study. Our findings suggest that the blebs that developed after PRESERFLO implantation resemble those resulting from TB more than those resulting from XEN implantation, even though the XEN and PRESERFLO implants are tubes with a constant fluid flow. Most likely, the lower resistance to flow through the

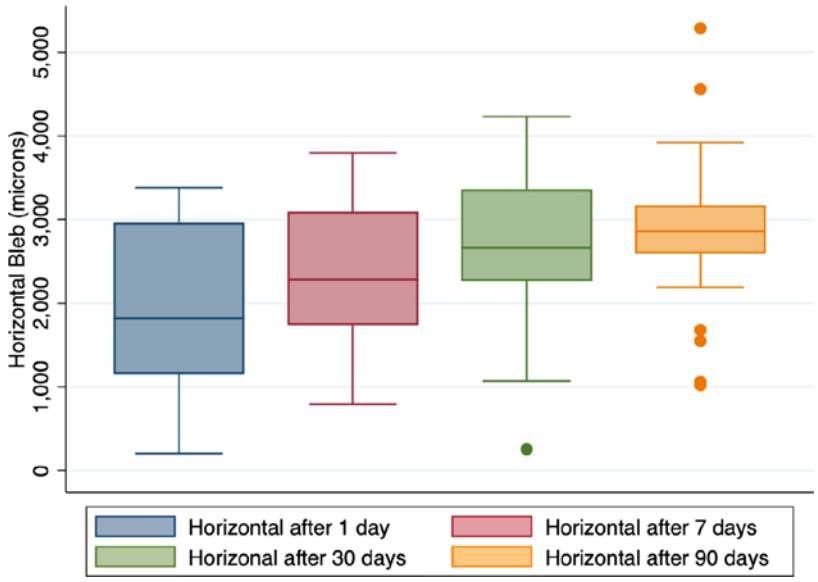


Fig. 8. Box plot of the evolution of the horizontal bleb diameter from day 1 to day 90. Blue box: Horizontal diameter at day 1; Red box: Horizontal diameter at 7 days; Green box: Horizontal diameter at 30 days; Orange box: horizontal diameter at 90 days.

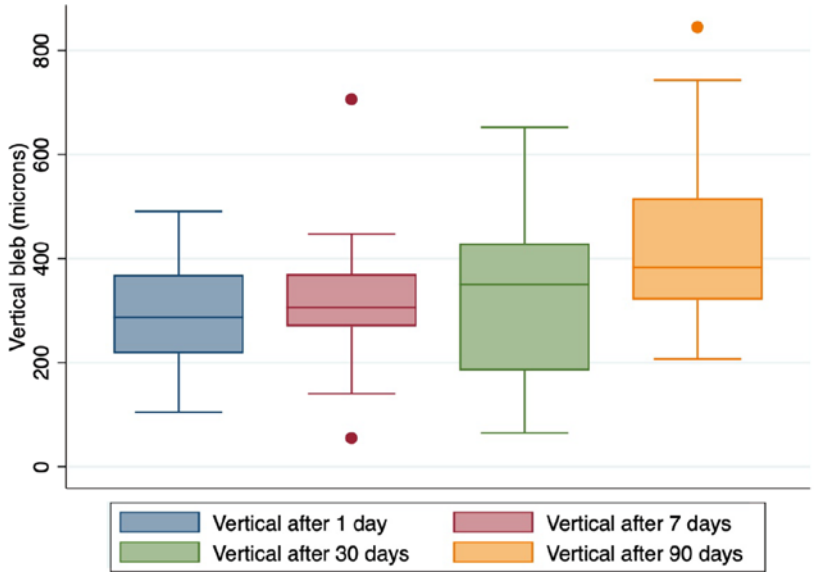


Fig. 9. Box plot of the evolution of the vertical bleb diameter from day 1 to day 90. Blue box: Vertical diameter at day 1; Red box: Vertical diameter at 7 days; Green box: Vertical diameter at 30 days; Orange box: Vertical diameter at 90 days.

PRESERFLO than the XEN and the surgical technique (ab externo), which induces a decrease in the outflow resistance and allows greater control by the surgeon over positioning the tube underneath Tenon's capsule at a posterior location (approximately 7 mm from limbus), produce intermediate aqueous humour drainage at a low pressure (8.5 ± 3 mmHg at 24 hr) throughout the third month (10.8 ± 2 mmHg), thereby allowing the formation of wide and diffuse drainage pathways, as shown by AS-OCT analysis. When the pressure inside the bleb is lower than the capillary pressure of Tenon's capsule, it is insufficient to initiate the avascular fibrodegenerative response that induces the formation of thick, impermeable capsules (Molteno et al. 2003). Scar formation at low pressures is initiated above the bleb, where the pressure is higher, as reported by Gardiner (Gardiner et al. 2010), but not on the horizontal borders of the bleb, where the pressure is lower. The bleb can therefore expand its horizontal limits slowly and progressively up to at least the third month if the pressure remains low and stable. The horizontal expansion seems to be higher than the vertical expansion after PRESERFLO implantation and has been shown to be related to the low levels of controlled IOP in these patients. The scar

formation above the bleb at low pressures would limit the tissue's exposure to hypoxia (induced at higher fluid pressures), delaying the adverse fibrodegenerative response that leads to the formation of thick, vascularized walls that have been reported to be associated with uncontrolled IOP (Azuara-Blanco et al. 1998). In light of these findings, it is reasonable to hypothesize that the standardized ab externo technique used for PRESERFLO implantation, in addition to the deep and posterior localization of the distal end of the tube and its lower resistance to flow compared to that of the XEN 45, may be related to the higher number of multiform blebs and lower rate of surgical revision in our series.

For TB, it is not possible to calculate resistance to flow with the Hagen–Poiseuille law, which only gives the pressure drop for laminar flow through a cylindrical pipe of a constant cross-section. During trabeculectomy, the tension of the scleral sutures is crucial to control the outflow (Samsudin et al. 2016), and it depends on the surgical technique and skills of the surgeon. If the sutures are loose, the AH will displace the normal interstitial tissue fluid at a pressure that exceeds the intracapillary pressure in the deeper layers of Tenon's capsule, which stimulates the fibrodegenerative process in response to hypoxia. In contrast, if the sutures are too tight, insufficient aqueous humour flow results in the formation of a uniform layer of avascular and modestly cellular connective tissue 20–60 μm thick that reaches its final thickness by 4 weeks after the operation and remains unchanged thereafter (Molteno et al. 2003). Most likely, the intermediate and constant flow rate provided by the PRESERFLO (higher than that of the XEN 45 and lower than that of TB with loose sutures) induces the formation of filtering blebs that resemble those described for functioning TB blebs but with a repeatable morphology in the majority of patients (multiform, wide horizontal extension with a thin cap). Other authors did not find a significant correlation between the height or extent of the filtering bleb cavity and the IOP after TB. Pfenninger (Pfenninger et al. 2011) reported a significant direct correlation between the reflectivity of the fluid-filled cavity and the IOP, while Tominaga et al. (2010) found a significant inverse correlation between the thickness of the bleb wall and the IOP.

On the other hand, inflammation is an important contributing factor for glaucoma surgery failure. Investigators have reported the presence of inflammatory mediators in glaucomatous AH (Chang et al. 2000), with increased rates in uveitic and neovascular glaucoma. A decrease in the inflammatory response due to different factors, such as an advanced age, the use of steroids and the use of MMC, may be involved in the higher rate of success after the formation of filtering blebs. In the case of the PRESERFLO, the material of which it is composed is another factor that appears to decrease the inflammatory response. The polymer SIBS, initially used for coating cardiac stents (TAXUS® stent), induces less scarring and chronic inflammation, with no biodegradation and minimal tissue reaction described in studies of this implant in cardiology (Silber et al. 2009). Most likely, the presence of multiform, wide blebs with low IOPs in the early postoperative period, in combination with a polymer that induces minimal inflammation and MMC to control the fibrotic response, could be a predictive factor of success in the long term. However, further investigation is required.

The position and length of the tube in the anterior chamber were analysed in our study to determine its anatomical relation to the iris and endothelium and provide surgeons a tool to prevent future damage to ocular structures (endothelial cell loss and pigment dispersion). The correlation between the position of the tube in the anterior chamber and the IOP has already been reported to be nonsignificant. A study of three different MIGS implants (XEN 45, XEN 63 and PRESERFLO) showed a minimal (nonsignificant) effect of the position of the tube on the IOP (<0.1 mmHg) (Bachar et al. 2020). The independence of these two variables can be explained by the fact that the fluid pressure inside the bleb is effectively the same as the IOP, to within 1 mmHg, according to Gardiner´s computational modelling of fluid flow and IOP for glaucoma drainage devices (GDDs) (Gardiner et al. 2010). Therefore, the position of the tube in the anterior chamber does not affect the IOP but could have a negative effect on the endothelial count when positioned too close to the cornea or induce pigment dispersion when located too close to the iris. It is possible to measure the real position of the tube in the AC on AS-OCT, thereby helping the surgeon decide whether revision is necessary to change its position. Intraoperative OCT could also be a helpful tool for the implantation of this kind of device.

To our knowledge, this is the first study to describe the ultrastructural AS-OCT changes in the subconjunctival and sub-Tenon space after PRESERFLO MicroShunt implantation for glaucoma. We demonstrated that the blebs formed with this implant are diffuse, posterior and low and that they increase in size from the first visit through the third month after implantation of the PRESERFLO MicroShunt. One of the limitations of the study is that the AS-OCT examinations, bleb and tube measurements and clinical evaluations were performed by only one observer. However, the observer followed the same protocol in all cases, centred the images using the same reference points, discarded low-quality scans and repeated the examinations as many times as necessary to obtain high-quality OCT images. Another limitation of this study is the sample size. Our preliminary results need to be validated by the collection of a higher number of subjects and observation over a longer follow-up period to strengthen the statistical power of the results and extend the analysis of the bleb maturation process.

The results of this study suggest that PRESERFLO implantation for glaucoma induces the formation of deep and posterior blebs that are localized under Tenon's capsule and show a multiform morphology on AS-OCT in the majority of cases. The horizontal expansion of these filtering blebs occurs from the early postoperative period until the third month. Long-term follow-up is needed to evaluate the bleb maturation process and its influence on IOP control.

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CHANGES TO CORNEAL TOPOGRAPHY AND BIOMETRICS AFTER PRESERFLO MICROSHUNT SURGERY FOR GLAUCOMA

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Precise: Glaucoma surgery with the Preserflo Microshunt shows mild and transient changes in the corneal astigmatism, the corneal elevation and biometrics in the early postoperative period. The posterior corneal elevation tends to be higher in POAG.

Abstract

Purpose: To determine the changes in the corneal keratometry, astigmatism and elevation, refraction, axial length and anterior chamber depth and volume after the implantation of the Preserflo Microshunt in patients with primary open angle glaucoma (POAG) in the early postoperative period.

Methods: Patients diagnosed with primary open angle glaucoma (POAG) who underwent an ab-externo SIBS (poly(styrene-block-isobutylene-block-styrene) microshunt implantation were recruited. The central corneal thickness (CCT), the intraocular pressure (IOP), best corrected visual acuity (BCVA), refraction, biometrics and corneal topography with a Scheimpflug topographer were analyzed preoperatively and 24 hours, 1 week, 1 month and 3 months after surgery.

Results: A total of 30 eyes of 29 patients were included. In 24 eyes the device was implanted as a standalone procedure and in 6 eyes it was combined with cataract surgery. The results were analyzed separately. The IOP decreased from 21.8±5.2 and 16.5±1.5 mmHg at baseline to 10.9±1.8 and 10.1±1.1mmHg at 3 months in the non-combined and combined groups ($p<0.01$). The anterior, posterior and total corneal astigmatism (ASA, PSA, TCA) increased in each group 0.4±0.3/ 0.2±1.0 D, 0.08±0.1/ 0.03±0.1 D and 0.4±0.3/ 0.2±0.9 D respectively at 3 months. The anterior and posterior corneal elevation (ACE max, ACE min, PCE max) increased on the first week ($p=0.01$) with no significant changes at 3 months in the non-combined group. The changes observed in the combined group were not significant. The axial length (AL) decreased 0.13±0.23 and 0.2±0.07 mm in each group ($p=0.01$). There was a significant correlation

between the IOP and the maximum elevation of the posterior surface of the cornea at the preoperative examination (r=0.93, p= 0.02).

Conclusions: The Preserflo Microshunt implant for glaucoma surgery induces mild and transient changes in corneal astigmatism, axial length and anterior chamber depth in the early post-operative period.

Keywords: Glaucoma, Preserflo Microshunt, MIGS, astigmatism, Scheimpflug.

Introduction

Minimally invasive glaucoma surgery (MIGS) is a term that includes a group of relatively new surgical interventions to lower the intraocular pressure (IOP), aiming for a faster recovery, a lower risk of complications and fewer refractive and visual changes than classic trabeculectomy[1]. There are three possible categories of MIGS depending on the targeted anatomical structure of the outflow pathway: the Schlemm´s canal, the suprachoroidal space and the subconjunctival-sub-Tenon space. Recently, the European Glaucoma Society has excluded the implantation of subconjunctival devices from the definition of MIGS, which refers to only ab interno bleb-less implants[2].

Subconjunctival implants for the surgical treatment of glaucoma are basically shunts that allow the aqueous humor to drain from the anterior chamber into an area underneath the conjunctiva, creating a filtering bleb in the same manner as trabeculectomy since it was described by Cairns 53 years ago[3]. In an effort to reduce hypotony, new ab interno and ab externo, cylindrical implants have been designed according to the Hagen-Poiseuille equation[4], to limit the aqueous humor (AH) flow into the subconjunctival space. The different flow rates through those tubes may lead to specific morphological characteristics of the resultant blebs[5], as compared with those described for trabeculectomy.

Ocular biometric changes[6], and more specifically corneal keratometric and topographical changes induced by trabeculectomy and other bleb-forming procedures such as the Ex-Press® glaucoma implant (Alcon Laboratories, Inc, Fort Worth, TX, USA), and nonpenetrating deep sclerectomy (NPDS), have been widely reported in the literature[7-13]. Changes to the corneal curvature may have an impact on refraction, visual acuity and thus the IOL power calculation. The cause of these changes may be related to the removal of tissue under the scleral flap in trabeculectomy[8], tight sutures[11], large drainage or avascular blebs and ptosis[14].

The Preserflo Microshunt (Santen Pharmaceutical Co., Osaka, Japan) consists of an 8.5 mm long SIBS (poly(styrene-block-isobutylene-block-styrene) tube with an outer diameter of 350 µm and a lumen diameter of 70 µm that was designed for a simple bleb-based procedure that does not require a scleral flap15. It is placed ab-externo through a 3 mm-long scleral tract made using a 25-G needle to shunt aqueous humor from the anterior chamber to the sub-Tenon space approximately 6.5 mm away from the limbus. Hypothetically, a sutureless and nonscleral flap-dependent technique, as well as deeper and more posterior filtering blebs5, could be advantages over trabeculectomy, NPDS or Ex-Press® by reducing the amount of induced corneal and refractive changes. The lack of data about the ocular changes induced by this implant prompted us to evaluate the ocular changes, as evaluated using the Pentacam and the IOLMaster 700, after glaucoma filtering surgery performed using the Preserflo Microshunt.

Materials And Methods

Patients:

This was a prospective, interventional, single-center case series of 30 eyes of 29 patients. Patients were consecutively recruited from among patients diagnosed with POAG who were attended at the Glaucoma Unit of the HLA Hospital Universitario Moncloa (Oftalvist Group, Madrid, Spain) and underwent glaucoma surgery with the Preserflo Microshunt.

The protocol adhered to the tenets of the Declaration of Helsinki and it was approved by the Institutional Review Board of the HLA Moncloa Hospital (Madrid, Spain). Informed consent was provided by all participants prior to their inclusion in the study.

The inclusion criteria were as follows: diagnosis of POAG and poor glaucoma control with an IOP higher than 21 mmHg or less than 21 mmHg and progression of glaucoma damage observed in complementary examinations. Patients with cataracts and visual acuity of 0.5 were included and combined surgery was performed (cataract extraction and implantation of Preserflo Microshunt). In pseudophakic patients or those without cataracts, only Preserflo Microshunt implantation was performed. Pseudophakic patients in which cataract removal had been performed less than 3 months before the glaucoma procedure, were excluded.

The exclusion criteria were as follows: angle closure and congenital, pseudoexfoliative, pigmentary, uveitic or neovascular glaucoma. Eyes with a previous history of filtering surgery were only included when total bleb failure was previously demonstrated clinically and by AS-OCT (flat bleb, uniform hyperreflective stromal pattern, no microcyst and no bleb drainage pathway). In these cases, the Preserflo Microshunt was implanted in a different quadrant, avoiding the previous filtration site.

Baseline preoperative characteristics were collected from all patients, including demographics (age, sex, date of surgery, laterality), type of surgery (combined or stand-alone) and ocular parameters (best-corrected visual acuity [BCVA] assessed with the Snellen chart, intraocular pressure [IOP] at which the decision for surgery was made measured using a calibrated Goldmann applanation tonometer, central corneal thickness [CCT] and number of glaucoma medications at the preoperative visit). The following indices were collected, pre and postoperatively (1 day, 1 week, 1 month and 3 months): IOP, axial length (AL) using a noncontact method (IOLMaster 700, swept-source biometry, Carl Zeiss Meditec AG), spherical equivalent (SE), Pentacam HR (Oculus, Inc.) and measurements of the anterior and posterior corneal curvatures in the central 8-mm-diameter area, including anterior surface astigmatism (ASA), ASA axis (flat), posterior surface astigmatism (PSA), PSA axis (flat), total corneal astigmatism (TCA, 4 mm), anterior corneal radius flat, steep, and mean [Rf (A), Rs (A), Rm (A)], posterior corneal radius flat, steep, and mean [Rf (B), Rs (B), Rm (B)], anterior corneal elevation (ACE max, ACE min, ACE central), posterior corneal elevation (PCE max, PCE min, PCE central) and anterior chamber depth (ACD).

Surgical technique:

All surgeries were performed by the same surgeon (MIB), under sub-Tenon anesthesia in the inferior nasal quadrant. A traction suture on the superior cornea was used to expose the upper nasal conjunctiva in order to perform conjunctival peritomy and careful Tenon dissection over two clock hours, liberating all the attachments between the Tenon capsule and episclera and creating a posterior pocket between the superior and medial rectus muscles. A diathermy probe was applied to the sclera to control bleeding and to ob-

tain a clear surgical field. Mitomycin C (MMC) 0,2 mg/ml was used in all cases via introducing three soaked surgical sponges provided by the manufacturer under Tenon´s layer for 2 minutes, avoiding limbus, and then gently washing with balanced salt solution. A mark with trypan blue was placed with the tip of the caliper 3 mm away from the limbus and a 1-mm-wide scleral preincision was created with a microknife until the tip was not visible. The scleral tunnel was created parallel to the surface of the sclera with a 25-gauge needle entering the anterior chamber (AC) at the trabecular meshwork. The Preserflo Microshunt was then introduced in the tunnel until it reached the AC, and its position was visually checked, ensuring that it was not too close to the iris or endothelium and was placed with the bevel facing up. Located half-way down the tube, there is a planar fixation structure resembling the fins of an arrow that seals the device in the pocket, preventing leakage around the tube and preventing the tube from migrating into the eye1. The fins were placed at the end of the scleral tunnel to ensure that it was inside the scleral tunnel. Flow through the implant was confirmed by injecting BSS from the distal side of the tube with a 23-G cannula, usually a small air bubble was observed advancing to the anterior chamber and drop-by-drop flow was confirmed from the end of the tube with a surgical sponge. Tenon´s layer was advanced prior to the conjunctiva to ensure that the implant was not caught in it, and then the conjunctiva was sutured watertight over Tenon´s layer with 10-0 nylon. (Figure 1). A side-port incision was created at the end of the surgery to inject 0.1 ml of cefuroxime (1 mg/0.1ml) into the AC. For combined surgery, the surgical technique did not change and was performed at the end of the phacoemulsification and IOL implantation procedure.

Postoperative management:

In both groups of patients, i.e., those treated with the combined and stand-alone procedure, postoperative care included prophylactic antibiotic (moxifloxacin) 4 times a day in the first week after surgery and decreasing doses of topical corticosteroids over 10 weeks (dexamethasone 4 times a day the first 15 days, 3 times a day for one month, 2 times a day, 15 days and 1 time a day for 15 days). Patients were seen at 24 hours, one week, one month and three months. All visits included slit lamp examination with a Seidel test, Goldmann applanation tonometry to measure IOP and fundus examination to evaluate for choroidal detachment in the case of clinical signs of hypotony.

Pentacam examination:

The Pentacam HR (Oculus, Inc.) is an eye tomography system that uses a 360-degree rotating Scheimpflug camera to obtain cross-sectional images of the anterior segment and is illuminated with a monochromatic slit-light source (blue-light-emitting diode [LED]; wavelength, 475 nm). The device obtains up to 50 images in 2 seconds with 138.000 evaluated measuring points. In this study, 25 images were captured, and the examination was repeated until an acceptable scan could be obtained according to the quality scan index. Elevation maps were viewed by comparing the data to a standard reference surface (best-fit-sphere) in the 8-mm zone that appeared best for clinical interpretation13.

Statistical analysis:

The Stata 17® (StataCorp LLC, Texas, USA) was used for statistical analysis. The Shapiro-wilk Test was used to test for normality of each variable of the study. Paired Student´s t-test was used for comparison of the normally distributed variables and the

Wilcoxon signed-rank test was used for data that did not follow a normal distribution. To analyze relationships between variables, the Pearson correlation coefficient was used to analyze the normally distributed variables and the Spearman correlation coefficient for data that did not show a normal distribution. Prob-

abilities were considered significant at < 0.05. The statistical power was calculated for each of the tests used for comparison of the variables.

Results

Study population and baseline characteristics:

A total of 30 eyes of 29 patients were included. Combined surgery was performed in 6 eyes (20%). The results from this subgroup of patients were analyzed separately (group 1: non-combined; subgroup 2: combined). The mean age of the study population at the operation was 77.7 ± 8 (non-combined) and 69.8±4 years (combined); laterality (RE/LE), 14/15; sex (M/F), 12/17, mean preoperative IOP treated with antiglaucoma medications was 21.8±5.2 mmHg (group 1) and 16.5±1.5 mmHg (subgroup 2); mean BCVA 0.6±0.3 (group 1) and 0.8±0.1 (subgroup 2); number of eye-drops pre-surgery were 2.8 (1-4), group 1, and 2.3 (1-4), subgroup 2. Postoperatively at each follow-up visit, only one patient from group 1 required medication (3.6%). This patient received monotherapy with a prostaglandin analog 60 days after surgery. At the last follow-up visit, 96.4% of patients were free of medication. In the non-combined group, 23 patients were pseudophakic (82%), and one was phakic (4%). The mean CCT was 530±34µm and 525±11 (group 1 and subgroup 2). Three out of 24 patients in the non-combined surgery group required surgical revision (11%). Two of them due to the increase in IOP at 24 hours and 1 month respectively and one case associated with the development of a Tenon´s cyst on the first month. Needling at the slit lamp examination was not performed in any of the cases, and open surgical revision was preferred to sort out bleb failure. There were 3 patients with a history of previous glaucoma surgery (Ex-Press®, two cases, XEN®, one case) and one patient that referred a selective laser trabeculoplasty (SLT) performed 5 years before the preoperative visit. Baseline demographics are summarized in Table 1.

Effect of the Preserflo Microshunt on IOP:

The reduction of the IOP from baseline at every postoperative visit and the statistical significance is shown in Table 2 (a) non-combined group and (b) combined subgroup and in Figure 2. The variation from baseline was significant at all time-points ($p<0.01$) for both groups. There was a 60% reduction of the IOP in group 1 at 24 hours and 50% at 3 months. In subgroup 2, the reduction was 44% and 39% respectively.

Hypotony was defined according to the numeric definition of an IOP ≤ 5 mmHg after surgery suggested by the World Glaucoma Association Guidelines16. The rate of hypotony for this series was 10% (3 cases), 2 of them were from group 1. One of the patients developed hypotony in the first week with a flat anterior chamber and choroidal detachment. The evolution was positive with conservative treatment (occlusion and atropine eye drops 1%) with resolution in the first month. The other two patients showed moderate hypotony in the first week with a narrow AC but without choroidal detachment or hypotony maculopathy.

One patient required topical medication before the third month. In this case, even though the IOP was 16 mmHg without medication in the first month, a prostaglandin analog was prescribed to reach a value in the low teens instead of performing surgical revision. The IOP prior to surgery was 26 mmHg with topical medication (latanoprost, timolol 0.5% and dorzolamide). Visual function damage was not severe (MD, -8.2 dB), and the microshunt apparently maintained flow through the tube. A filtering bleb was clearly seen on the slit lamp examination, and a small drainage pathway around the tube with a superior cap and lateral margins could be identified by AS-OCT.

Comparison of preoperative and postoperative visual acuity, refraction and biometrics:

The changes observed in the best-corrected visual acuity (BCVA), spherical equivalent (SE), refractive sphere and cylinder, anterior chamber depth (ACD and axial length (AL) are shown in Table 2 (a,b) for group 1 and subgroup 2. BCVA decreased significantly from baseline to the first week in both groups, showing no differences at 3 months. The change in SE was not significant in group 1 (0.2±0.8), but it was significant (-2.79±1, $p<0.01$) in the combined surgery subgroup. The refractive cylinder showed no significant changes from baseline to the third month in both groups. The ACD remained unchanged in the non-combined group but increased significantly in the combined surgery subgroup (1.7±0.2 mm, $p<0.01$). The axial length showed a significant decrease in both groups (0.1±0.2, $p=0.01$ group 1; 0.2±0.07, $p<0.01$ subgroup 2).

Corneal changes analyzed with the Pentacam® Sheimpflug Camera:

The results from the change in the anterior and posterior surface astigmatism as well as the total corneal astigmatism are shown in Table 3 (a) Non-combined group, (b) combined subgroup and in Figure 3. On the first week, there was an increase in the anterior, posterior and total corneal astigmatism in group 1, significant for ASA and TCA ($p=0.01$). In subgroup 2 (combined surgery), the increase was significant only for TCA ($p=0.02$). ASA and TCA remained elevated with respect to baseline at one month in both groups (not significant in the non-combined group, significant for TCA in the combined group) and decreased on the third month. The total increase in ASA and TCA from pre-op to three months was 0.4±0.3 ($p=0.03$ and $p=0.05$ respectively) in group 1 and 0.2±1.0 ($p=0.2$) and 0.2±0.9 ($p=0.4$) respectively in subgroup 2. The increase in the PSA on the first week in both groups did not reach statistical significance and no differences were found at three months with respect to baseline. There was a non-significant with-the rule (WTR) shift from baseline to the third month in the axis of ASA and PSA. In Figure 3, the boxplot diagram of the results from ASA and PSA shows the increase of astigmatism on the first week and decrease towards the third month in both groups.

The changes in the flat, steep and mean radius (Rf, Rs, Rm) of the anterior and posterior cornea are shown in Table 4 (a) group 1 and (b) subgroup 2. On the first week, there was a significant increase in the Rf of the anterior surface and a significant decrease in the Rs and Rm of the posterior surface in the non-combined group. In the combined subgroup there were no significant changes except for the decrease of Rs on the first week. At three months, there were no significant changes in the corneal radius (anterior and posterior) in group 1, and a statistically significant but not clinically relevant (0.02±0.06, 0.04±0.03) decrease in Rs and Rm of the anterior surface in subgroup 2.

The results from the analysis of the anterior and posterior corneal elevation are shown in Table 5 (a) Non-combined group, (b) combined subgroup and in Figure 4. The anterior and posterior corneal elevation (max, min, central) increased on the first week in both groups except for the PCE central in subgroup 2 that decreased not significantly. The increase was significant for ACE max and min, and PCE max in group 1. At three months, the differences from baseline were not significant for any of the variables studied. In figure 4, the boxplot diagrams show the same tendency for the changes in the corneal elevation in both groups.

The correlation analysis between the IOP and the different variables of the study (astigmatism, corneal elevation, AL and ACD) showed no statistically significant correlation. Even though it was not a primary objective of this study, a significant correlation between the baseline nonmedicated IOP and the preoperative corneal posterior maximum elevation ($r=0.93$, $p=0.02$) was found.

Discussion

In this study, the effect of the Preserflo Microshunt implantation on the anterior and posterior corneal surfaces (astigmatism and elevation) and other biometric parameters (AL and ACD) was analyzed from the early postoperative period to the third month. In this sense, an increase of 0.7 D in the anterior corneal astigmatism and 0.8 D in the total corneal astigmatism at one week postoperatively in the group of non-combined surgery was observed. In the subgroup combined-phaco-Preserflo Microshunt, slightly higher changes were noticed (1 D of increase in the anterior surface astigmatism and 1.2 D in the total corneal astigmatism). In both groups, the increase observed on the first week decreased towards the third month showing a residual difference from baseline of 0.4 D of ASA and TCA in the non-combined group and 0.2 D in the combined subgroup.The changes observed in the posterior surface astigmatism were similar for the combined and non-combined patients on the first week, without significant differences from preoperative values at 3 months. The anterior and posterior corneal elevation showed a tendency to increase on the first week and decrease throughout the third month in both groups, as it is shown in the boxplot diagram for ACE and PCE (Figure 4). Most likely, the sudden drop in the IOP in the very early postoperative period induces an increase in the corneal curvature and a consecutive increase in astigmatism and corneal elevation.

Several studies have examined the effect of glaucoma surgery on astigmatism. The type and amount of astigmatism induced by trabeculectomy was first reported by Hugkulstone[7] using keratometry in 10 patients after a 28-day follow-up period. He reported a statistically significant reduction in the vertical corneal radius from a mean of 7.71 mm preoperatively to 7.36 mm at day 1 postoperatively and at all time points up to the last follow-up visit. This steepening of the vertical meridian or induction of WTR astigmatism was confirmed by Cunliffe et al[8], who reported a reduction in the vertical corneal radius from 7.69 mm to 7.56 mm at week one, persisting at weeks 3 and 8 but returning to preoperative values at 10 months. The findings of the current study concerning the corneal radii showed similar changes in comparison to those reported for trabeculectomy, with a decrease in the steep radius (7.6 to 7.5 mm) on the first week and a change of the anterior flat axis of the astigmatism from 105° to 135° (steep axis from 15° to 45°), which suggest that trabeculectomy and Preserflo Microshunt implantation seem to induce the same kind of vertical corneal steepening in the early postoperative period. Still, the methodology used to measure the changes on the corneal radii and astigmatism have evolved from the studies published with keratometry to the actual topographers.

Claridge et al[9], using computer-assisted corneal topography in 1995, described complex regional changes in the corneal curvature that had not been detected by alterations in refractive or keratometric parameters and reported that the topographic changes after trabeculectomy lasted for at least 12 months after surgery. Rosen et al.[10] reported that the amount of induced steepening was underestimated by keratometry compared to topography, finding a WTR shift in 8 eyes of 1.5 to 2.5 D up to 12 weeks postoperatively. Dietze et al[11], using computerized video keratography, reported that the mean central corneal astigmatism increased by 1.4 D along the surgical meridian at 1 week postoperatively and was within ± 1 D by 12 weeks. In our study, we used the Pentacam system to measure the corneal astigmatism, finding a change of 0.8 D in the total corneal astigmatism in the very early postoperative period after Preserflo Microshunt implantation that decreased up to 3 months postoperatively, with a residual difference of 0.4 D from baseline which seems to be lower than previous reports and clinically not significant. An underestimation of the corneal astigmatic change with Scheimpflug tomography is very unlikely, given that the Pentacam has demonstrated its superiority to Placido topography because not only the anterior but also the posterior corneal surfaces are measured with high precision[17]. Even when compared with the first commercially

available tomography technology (scanning slit; Orbscan, Bausch & Lomb), Scheimpflug tomography gives more precise anterior and posterior corneal measurements[18]. Therefore, we believe that our results observed after the Preserflo Microshunt implantation are reliable.

Regarding other glaucoma surgery techniques beyond trabeculectomy and their effect on the cornea, Hammel et al.[12] examined 19 eyes undergoing EX-PRESS implantation with the Pentacam system, finding a transient increase in both the anterior and posterior corneal astigmatism (2.6 to 4.7 D anterior; 0.4 to 0.9 D posterior), higher than the increase found in our series with Preserflo Microshunt. The authors reported that IOP fluctuations were correlated with corneal changes, a point that was not confirmed in our study. Egrilmez and colleagues13 found less WTR astigmatism induction after deep sclerectomy with a nonabsorbable implant (T-Flux) vs trabeculectomy (0.62 D vs .1.06 D) in the early postoperative period and less of an ATR shift thereafter (0.62 D vs. 1.24 D at 6 months). Similar results were reported by El-Saied19 (0.67 D at 6 months) in a group of eyes treated with deep sclerectomy and MMC, and although slightly lower in magnitude, also by Corcostegui et al.[20] after combined surgery with deep sclerectomy plus placement of an absorbable implant (SKGel) (less than 0.5 D and not significant). In summary, published studies suggest that trabeculectomy and Ex-Press implantation seem to induce greater corneal astigmatism than deep sclerectomy and Preserflo Microshunt. Concerning the changes observed on the axial length after glaucoma surgery, this implant seems to induce a comparable reduction to trabeculectomy with MMC. Using a noncontact biometry device to obtain the measurements on the third month the results seem to be comparable (-0.14 ± 0.13 mm for trabeculectomy[6], 0.13±0.23 mm for non-combined and 0.2±0.07 mm for combined surgery).

There are relatively few publications on other glaucoma surgical devices such as MIGS and glaucoma drainage devices (GDD). Ioannidis et al.[21] analyzed the refraction, visual acuity and manifest astigmatism, represented by the astigmatism component of a power vector in patients undergoing combined femtosecond laser-assisted cataract surgery and the insertion of two iStent inject (Glaukos, San Clemente, CA, USA) trabecular micro-bypass. Mean cylinder changed from 0.91 pre-op to 0.41 at 4 weeks with a significant reduction in J45 and B values in the vector-based values. To our knowledge, there are presently no studies examining refractive outcomes following GDD implantation. Tzu et al.[22] examined the refractive outcomes after cataract-only and combined cataract- trabeculectomy with 0.4 mg/ml MMC, or either Ahmed (New World Medical Inc., CA) or Baerveldt (Abbott Laboratories Inc., IL) implants. They reported 1.31 ± 0.86 (+ 0.26 to + 3.76) change in cylinder in the combined group vs 0.99 ± 0.72 (0 to + 2.75) in the cataract-only group (p=0.1) but this did not differentiate between the combined trabeculectomy and GDD populations. To the best of our knowledge, there are no studies published that report the corneal and refractive changes after the implantation of the XEN gel stent. A summary of the studies published in the literature is shown in Table 6.

Different mechanisms have been proposed to explain the change in the corneal astigmatism with different glaucoma surgeries. Egrilmez[13] explained that the thin layer of tissue left intact in deep sclerectomy may help reduce the amount of induced corneal steepening. Cunliffe et al.[8] postulated that the sclerostomy performed in trabeculectomy allows the edge of the cornea to retract, reducing the vertical corneal radius. Rosen et al.[10] proposed cauterization at the time of surgery as one of the main factors for corneal curvature changes. Dietze[11] suggested that overtight scleral flap sutures at 12 o'clock could cause local tissue compression, steepening the vertical meridian. Large drainage blebs, ptosis and hypotony have also been reported as possible mechanisms of astigmatism induction[14] as well as the use of mitomycin C, which appears to be associated with longer lasting astigmatic changes. In a study published by Hong et al.[23], there was less WTR astigmatism induction in the early postoperative period and longer lasting ATR astigmatism

up to 12 months in the trabeculectomy-MMC group (0.2mg/ml). Kook et al.24 found a WTR change of 1.23D at 3 months, persistent at 6-12 months (0.94 and 0.65D) after trabeculectomy with MMC 0.4 mg/ml. In contrast, Delbeke et al[25] reported a lower WTR change with 0.2 mg/ml MMC (0.35 D at 1 month, which had almost disappeared at 3 months, 0.18 D). In our series, with a 0.2 mg/ml concentration of MMC, the changes observed in the corneal astigmatism seem to be comparable to those reported for trabeculectomy in the early postoperative period for the same concentration. A longer follow-up of our cases will show if there are longer lasting corneal effects associated with this implant and MMC concentration, even though our results suggest a tendency to regain preoperative values close to the third month.

The Preserflo Microshunt implant avoids the need for scleral flap creation and sutures (both risk factors for astigmatism induction). Furthermore, the most frequent type of filtering bleb (analyzed clinically and by AS-OCT) has been reported to be multilayered, low and posterior in relation to the limbus in the majority of the cases[5] with no reported cases to date of large or avascular blebs that have been related to changes in the corneal curvature[14]. Even though it is a penetrating procedure, the lack of a sclerectomy and the narrow external diameter of the tube (350 µm) placed across the angle probably induce very small changes in the angular morphology. These findings may be involved in the transient and mild astigmatic change observed after surgery with this implant.

A potential bias to the study is the presence of three cases of refractory glaucoma. In these cases, the Preserflo Microshunt was implanted as the second glaucoma surgery procedure due to the fibrosis of the filtering bleb, confirmed clinically and by AS-OCT. The surgery was performed in another quadrant (nasal-superior was preferred to leave the temporal-superior quadrant for a future GDD if required) to avoid the scar tissue, aiming to decrease the inflammation and fibrosis. Still, an unexpected fibrotic response could alter the maturation process of the bleb with a subsequent influence on the corneal curvature.

Even though it was not a initially the main purpose of the study, we found a significant correlation between the nonmedicated mean IOP and the posterior corneal elevation at the preoperative baseline visit, suggesting that mechanical compression of the stroma occurs in POAG eyes with a subsequent increase in the posterior corneal elevation. This finding, which could be a new marker for the biomechanical corneal modifications that occur in glaucoma patients, has been previously described by Arranz et al[26]. Most likely, corneal hysteresis (CH) and the way it responds to changes in the IOP are involved in the changes encountered in the posterior corneal elevation. Corneal hysteresis remains stable with diurnal variations in the IOP[27] or transient corneal swelling induced by contact lenses[28], thus maintaining biomechanical integrity under these conditions[29]. Nevertheless, changes in the CH do occur in association with structural changes in the cornea, such as in Fuchs dystrophy, after LASIK, in keratoconus[30] and in glaucoma patients (POAG or normal tension glaucoma [NTG])[31]. Under these conditions, the CH is lower than normal. Furthermore, in glaucomatous eyes, a lower CH has been identified as a risk factor for progressive worsening of the visual field[32]. In addition, the CH seems to recover to baseline values after the IOP is decreased using antiglaucoma medication[29]. Thus, a lower CH and smaller central corneal thickness (CCT) have been described as independent risk factors for glaucoma progression[32]. This hypothesis suggests that a more deformable cornea may be linked to more deformable posterior ocular structures (sclera, lamina cribrosa) and thus might be associated with a higher risk for glaucoma development and progression. It would be interesting to investigate whether the higher posterior corneal elevation shown in glaucoma patients is also correlated with a worsening VF and therefore glaucoma progression.

Based on our results, the Preserflo Microshunt technique seems to improve the refractive changes caused by classic filtering surgery. Compared to trabeculectomy, which has been reported to induce visually significant changes in the AL, ACD and keratometric parameters, in some cases, lasting more than a year, the

effect of Preserflo Microshunt implantation on ocular biometrics appears to be clinically not significant, inducing low and transient corneal and biometric changes only in the very early postoperative period. The mild refractive changes induced by Preserflo Microshunt may benefit IOL calculations for combined surgery or even for the implantation of toric and extended depth-of-focus IOLs in specific cases. Although the current study did not initially aim to find new glaucoma biomarkers in the cornea, we confirmed that POAG eyes seem to have a higher posterior corneal elevation since a positive correlation was demonstrated between the baseline, nontreated IOP and posterior corneal elevation. Further investigation on the posterior corneal elevation in POAG and VF worsening is clearly needed, as well as a longer follow-up period to elucidate the long-term effects of the Preserflo Microshunt implantation on the cornea and refraction.

Disclosure

The authors declare no conflict of interest.

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Table 1. Baseline characteristics of the study population in the non-combined group and combined subgroup. Best corrected visual acuity (BCVA); selective laser trabeculoplasty (SLT).

Baseline demographics	Non-combined (24 eyes)	Combined (6 eyes)
Age	77.7±8	69.8±4
Central corneal thickness	530±34µm	525±11µm
BCVA (non-combined)	0.6±0.3	0.8±0.1
N° of meds pre-surgery	2.8 (1-4)	2.3 (1-4)
N° of meds post-surgery	0.2	0
% of patients with post-op meds	3.6%	0
Mean time (days) to post-op meds	60	0
Pseudophakic	23 (82%)	0
Phakic	1(4%)	0
Needling	0	0
Mean time to needling	0	0
N° of patients with surgical revision	3 (11%)	0
Time to surgical revision	22.3 (7-35)	0
Previous glaucoma surgery	3	0
Previous SLT	1	0

Table 2. Comparison of preoperative and postoperative intraocular pressure, visual acuity, refraction and biometrics. Anterior chamber depth (ACD); axial length (AL); best-corrected visual acuity (BCVA); intraocular pressure (IOP); spherical equivalent (SE). (a) Non-combined group (b) Combined subgroup.

Parameters	Preoperative	One week	*p/z* value	One month	*p/z* value	Three months	*p/z* value	Difference pre-3M	*p/z* value
IOP	21.8±5.2	8.7 (7-17) (60% reduction)	<0.01*	10.4 (8-16) (52% reduction)	<0.01*	10.9±1.8 (50% reduction)	<0.01*	10.8±5.13	<0.01*
BCVA, Snellen	0.6±0.3	0.4±0.3	0.01*	0.5±0.3	0.1	0.6±0.2	0.8	0.009±0.2	0.8
SE	0.08±1	-	-	-	-	0.3±1	0.1	0.2±0.8	0.1
Refractive Sphere	-0.5±1	-	-	-	-	-0.2±1	0.05*	0.3±0.7	0.05*
Refractive Cylinder	1.2±0.8	-	-	-	-	1.1±0.7	0.8	0.01	0.8
ACD	3.9±0.9	-	-	-	-	3.9±0.7	0.9	0.00006±0.7	0.9
AL	24.5±2.7	-	-	-	-	24.3±2.7		0.13±0.23	0.01*

Table 2 (a) Non-combined group.

Parameters	Preoperative	One week	*p/z* value	One month	*p/z* value	Three months	*p/z* value	Difference pre-3M	*p/z* value
IOP	16.5±1.5	9.3±2.5 (44% reduction)	<0.01*	10.1±1.3 (29% reduction)	<0.01*	10.1±1.1 (39% reduction)	<0.01*	6.3±0.8	<0.01*
BCVA, Snellen	0.8±0.1	0.6 (0.1-0.9)	0.04*	0.78±0.1	0.05*	0.8 (0.4-1)	0.7	+0.025±0.1	0.7
SE	-1.8±1.4	-	-	-	-	0.9±0.6	<0.01*	-2.79±1	<0.01*
Refractive Sphere	-2.4±1.2	-	-	-	-	0.25±0.5	<0.01*	-2.6±1	<0.01*
Refractive Cylinder	1.2±0.9	-	-	-	-	1.4±0.8	0.4	-0.25±0.7	0.4
ACD	2.4±0.3	-	-	-	-	4.2±0.3	-	1.7±0.2	<0.01*
AL	24.1±0.6	-	-	-	-	23.8±0.2	-	0.2±0.07	<0.01*

Table 2 (b) Combined subgroup.

Table 3. Comparison of preoperative and postoperative astigmatism. Anterior surface astigmatism (ASA); anterior surface astigmatism axis (ASA axis, flat); posterior surface astigmatism (PSA); posterior surface astigmatism axis (PSA axis, flat); total corneal astigmatism (TCA, 4 mm); statistical power of the test (pwr). (a) Non-combined group (b) Combined subgroup.

Parameters	Preoperative	One week	*p/z* value	One month	*p/z* value	Three months	Difference pre-3M	*p/z* value
ASA	1.4±0.9	2.1 (1.5-3.4)	0.01*	2.1±0.8	0.2	1.8±1	0.4±0.3	0.03*
ASA axis (flat)	104.7±72	118.2 (6.3-171.5)	0.3	127 (14.6-166)	0.4	135.2 (60-174)	30.5±64	0.2
PSA	0.3±0.1	0.4 (0.3-0.6)	0.06	0.3±0.09	0.1	0.4±0.1	0.08±0.1	0.2
PSA axis (flat)	87.2 (1.4-176)	162.5 (141.8-169.6)	0.3	172±7	0.6	170.7±4.7	83.5±91	0.07
TCA, 4 mm	1.5±1.1	2.3 (1.8-3.8)	0.01*	2.5±1	0.2	1.9±1.1	0.4±0.3	0.05*

Table 3 (a) Non-combined group.

Parameters	Preoperative	One week	*p/z* value	One month	*p/z* value	Three months	Difference pre-3M	*p/z* value
ASA	1.1 ± 1	2.2 ± 1.7	0.07	1.7 ± 1	0.1	1.4 ± 0.8	0.2±1.0	0.2
ASA axis (flat)	101.5±67	126.8±53	0.2	114.5±66	0.3	117.8±56	7.3±76.2	0.6
PSA	0.3±0.1	0.2±0.4	0.09	0.4±0.3	1	0.3±0.2	0.03±0.1	0.3
PSA axis (flat)	66.1±71	102.4±77	0.1	106.1±79	0.3	105.3±78	16±89	0.4
TCA, 4 mm	1.1±1	2.3±1.5	0.02*	1.7±0.8	0.03*	1.5±0.7	0.2±0.9	0.4

Table 3 (b) Combined subgroup.

Table 4. Comparison of preoperative and postoperative corneal radii for the non-combines group (a) and combined subgroup (b).; Radius flat (Rf); Radius steep (Rs); Radius mean (Rm); anterior surface (A); posterior surface (B); * statistically significant; statistical power of the test (pwr).

Parameters	Preoperative	One week	*p* value	One month	*p* value	Three months	Difference pre-3M	*p* value
Rf (A)	7.8±0.4	7.9±0.4	0.03*	7.8±0.1	0.2	7.8±0.4	0.008±0.1	0.7
Rs (A)	7.6±0.4	7.5±0.4	0.1	7.5±0.3	0.2	7.5±0.3	0.04±0.1	0.2
Rm (A)	7.7±0.4	7.75±0.3	0.9	7.71±0.3	0.6	7.6±0.4	0.01±0.09	0.4
Rf (B)	6.55±0.2	6.57±0.3	0.7	6.6±0.2	0.2	6.5±0.2	0.0004±0.1	0.9
Rs (B)	6.2±0.2	6±0.3	<0.01*	6.1±0.2	0.2	6±0.2	0.01±0.1	0.5
Rm (B)	6.39±0.2	6.32±0.2	0.03	6.38±0.2	0.3	6.2±0.2	0.02±0.08	0.2

Table 4(a) Non-combined group (Group 1)

Parameters	Preoperative	One week	*p* value	One month	*p* value	Three months	Difference pre-3M	*p* value
Rf (A)	7.6±0.2	7.6±0.1	1	7.6±0.4	0.8	7.6±0.2	0.01±0.01	0.1
Rs (A)	7.39±0.3	7.25±0.4	0.01	7.2±0.5	0.9	7.31±0.36	0.02±0.06	0.02*
Rm (A)	7.5±0.2	7.4±0.4	0.2	7.4±0.4	0.6	7.45±0.3	0.04±0.03	0.01*
Rf (B)	6.3±0.2	6.3±0.2	0.5	6.4±0.3	0.4	6.4±0.2	0.05±0.1	0.3
Rs (B)	6.08±0.1	5.9±0.3	0.1	6.04±0.3	0.02*	6.07±0.03	0.01±0.03	0.5
Rm (B)	6.23±0.2	6.1±0.2	0.5	6.2±0.2	0.1	6.25±0.2	0.02±0.04	0.3

Table 4(b) Combined subgroup (Subgroup 2)

Table 5. Comparison of preoperative and postoperative corneal elevation. Anterior corneal elevation (ACE, max, min, central); posterior corneal elevation (PCE, max, min, central); *statistically significant. (a) Non-combined group. (b) Combined subgroup.

Parameters	Preoperative (µm)	One week (µm)	*p/z* value	One month (µm)	*p/z* value	Three months (µm)	*p/z* value	Difference pre-3M (µm)	*p/z* value
ACE max	26 (20-31)	40 (29-51)	0.01*	32 (22-43)	0.01*	29 (23-34)	0.1	3.2	0.1
ACE min	-19 (-23/-14)	-27 (-37/-17)	0.01*	-21 (13-29)	0.03	-21 (-27/-13)	0.8	1.3	0.5
ACE central	0.6 (-0.9-2)	0.9 (-2/4)	0.7	1 (-0.6-3)	0.7	0.7 (-0.5-2)	0.2	0.04	0.7
PCE max	53.5 (40-67)	67 (46-88)	<0.01*	56 (40-73)	<0.01	59.8 (41-78)	0.3	6.3	0.7
PCE min	-34 (-45/-23)	-44 (-61/-27)	0.5	-38 (-57/-19)	0.2	-31(-39/-24)	0.9	-3	0.8
PCE central	4.6±4.8	5.2±4.3	0.3	3.7±5	0.2	3.4±4.7	0.8	0.6±0.5	0.2

Table 5 (a) Non-combined group

Parameters	Preoperative (µm)	One week (µm)	*p/z* value	One month (µm)	*p/z* value	Three months (µm)	*p/z* value	Difference pre-3M (µm)	*p/z* value
ACE max	22±15.6	40.5±9.7	0.09	36.2±13.9	0.8	27.3±11.2	0.3	5.3±9	0.2
ACE min	-14.3±7.5	-19±4.5	0.2	-25±8.2	0.2	-18.5±9	0.01*	4.1±8.1	0.2
ACE central	1.6±0.8	2.5±2.3	0.5	2.5±1.9	0.4	1.5±1	0.2	0.1±0.3	0.6
PCE max	47.1±24.4	75±22.9	0.1	54.2±20.6	0.4	46.3±18.4	0.4	0.8±11.5	0.8
PCE min	-25.6±17	-60.7±60.5	0.4	-33.2±7.4	0.8	-29.1±4.7	0.1	3.5±19	0.6
PCE central	2.9±3.4	1.2±3.9	0.5	2.7±2.5	0.4	1.8±2	0.3	1.1±3.4	0.4

Table 5(b) Combined subgroup

Table 6. Summary of the studies published in the literature for the changes in refraction and astigmatism after different glaucoma surgery techniques. Mitomycin C (MMC); against-the-rule (ATR); with-the-rule (WTR).

	Surgical technique	Intraoperative antimetabolite	Follow-up	Findings	Astigmatism induction
Hugkulstone[7]	Trabeculectomy	None	7 months	Vertical corneal radius reduction in the first postoperative day (from 7.71 to 7.36) with a gradual increase until the 7th week	WTR at all time points up to 28 days
Cunliffe et al[8]	Trabeculectomy	None	10 months	WTR astigmatism shift (reduction in vertical radius from 7.69 to 7.56 at week one) that returned to the preoperative values within 10 months.	
Claridge et al[9]	Trabeculectomy	None	3 months	Two types of topographic variations: superior corneal steepening and flattening, with WTR astigmatism in both cases	WTR induction
Rosen et al.[10]	Trabeculectomy	None	3 months	Mean WTR astigmatism of 1.24 D at 3 months and WTR shift in 8 eyes of 1.5 to 2.5 D up to 12 weeks postoperatively	+1.5-2.5 D
Dietze et al[11]	Trabeculectomy	None	3 months	WTR astigmatism at 1st week (1.4D) which returned to within 1 D of preoperative values on 12th week	+0.4 ± 0.3
Hong et al.[23]	Trabeculectomy	With or without MMC 0.2	12 months	Less WTR astigmatism induction in the early postoperative period and longer lasting ATR astigmatism up to 12 months in the trabeculectomy-MMC group.	0.71 ± 0.54 induced WTR astigmatism
Kook et al[24]	Trabeculectomy	MMC 0.4	12 months	WTR change of 1.23D at 3 months, persistent at 6-12 months (0.94 and 0.65D)	1.23 at 3 months
Delbeke et al[25]	Trabeculectomy	MMC 0.2	3 months	WTR change of 1.23D at 3 months, persistent at 6-12 months (0.94 and 0.65D)	0.35 D at 1 month, 0.18 at 3 months
Hammel et al[12]	Ex-Press	None	3 months	Transient increase in both anterior and posterior corneal astigmatism (from 2.6 to 4.7 D anterior; 0.4 to 0.9 D posterior	ACA change from 2.6 to 2.2 D
Egrilmez et al[13]	Trabeculectomy and deep sclerectomy	None	6 months	Less WTR astigmatism induction after deep sclerectomy with a nonabsorbable implant (T-Flux) vs trabeculectomy (0.62 D vs .1.06 D) in the early postoperative period and less of an ATR shift thereafter (0.62 D vs. 1.24 D at 6 months)	0.62 D (DS) 1.06 D (TB)
El-Saied[19]	Deep sclerectomy vs trabeculectomy	MMC	6 months	0.67 D at 6 months	0.67 D (DS) 0.82 D (TB)
Corcostegui et al[20]	Combined cataract+ deep sclerectomy	Reticulated hyaluronic acid implant	3 months	The refractive change after phacoemulsification combined with deep sclerectomy was mild and not clinically significant.	WTR change < 0.5 D range (0.03-1.67 D)
Ioannidis et al[21]	iStent + catarac surgery			Mean absolute difference from SE target refraction was 0.36 0.25D with 73.9% of eyes within 0.5D of the refractive target with 98.9 eyes within 1.00D of the predicted refractive target.	Keratometric astigmatism not analyzed
Tzu et al[22]	Cataract vs combined (trabeculectomy or Ahmed/Baerveldt)	MMC 0.4 mg/ml		1.31 0.86 (+ 0.26 to + 3.76) change in cylinder in the combined group vs 0.99 0.72 (0 to + 2.75) in the cataract-only group (p=0.1)	1.31 ± 0.86 (+ 0.26 to + 3.76)

Figure 1. Image of the Preserflo Microshunt implanted on the superior nasal quadrant 1 week after surgery. Fornix-based conjunctival dissection. The conjunctiva is sutured watertight with two 10/0 nylon stitches close to the limbus.

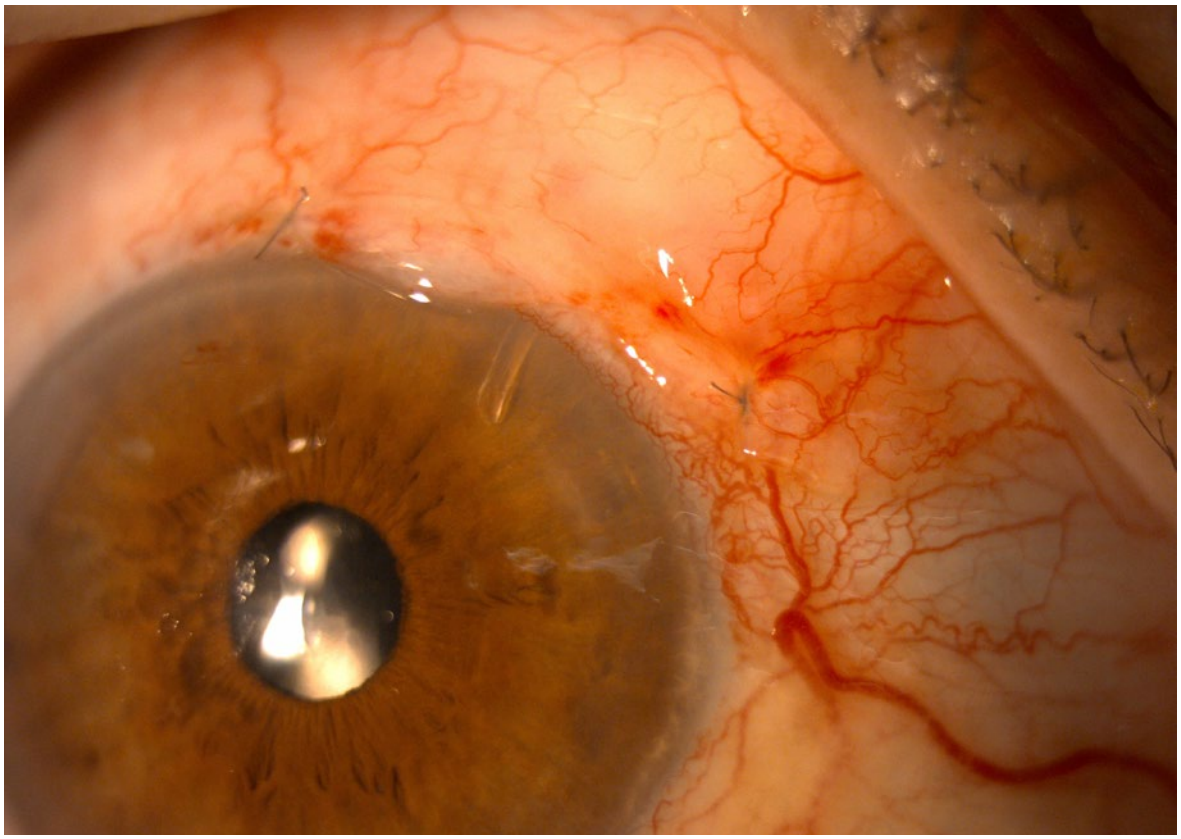


Figure 2. Box plot diagram from the evolution of the IOP after the Preserflo Microshunt implantation in the non-combined group and combined subgroup. Intraocular pressure (IOP); interquartilic range (IQR). IOP preoperative: 21.8±5.2, 16.5±1.5 mmHg (group 1, subgroup 2); one week: 8.7 (7-17), 9.3±2.5 mmHg (group 1, subgroup 2); one month: 10.4 (8-16) ,10.1±1.3 mmHg (group 1, subgroup 2); 3 months 10.9±1.8, 10.1±1.1 mmHg (group 1, subgroup 2).

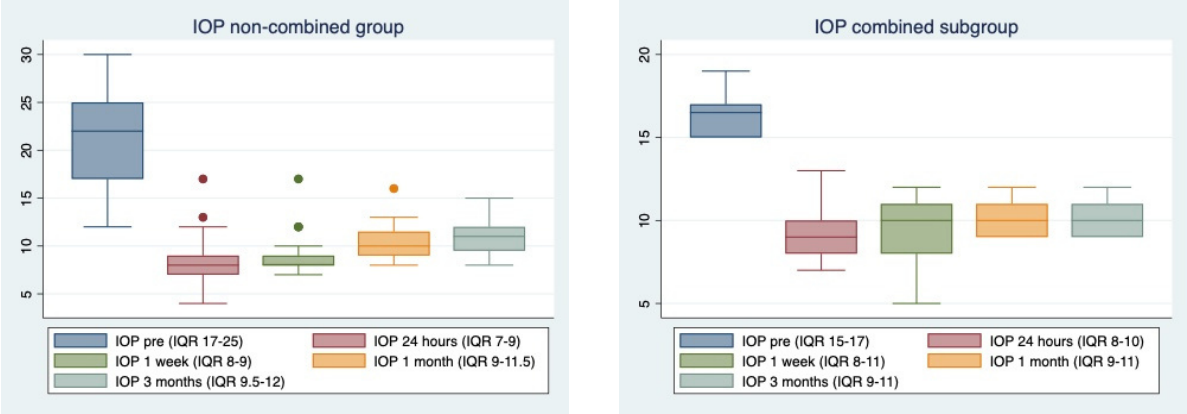
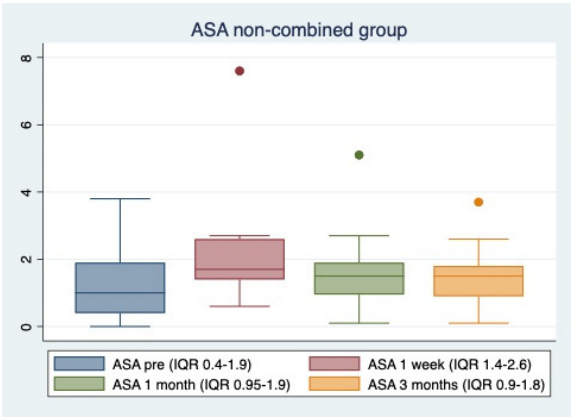
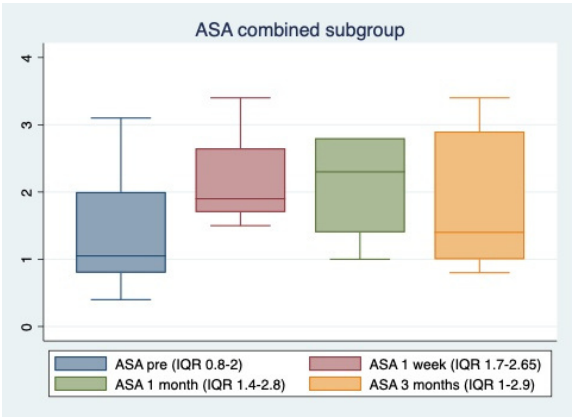
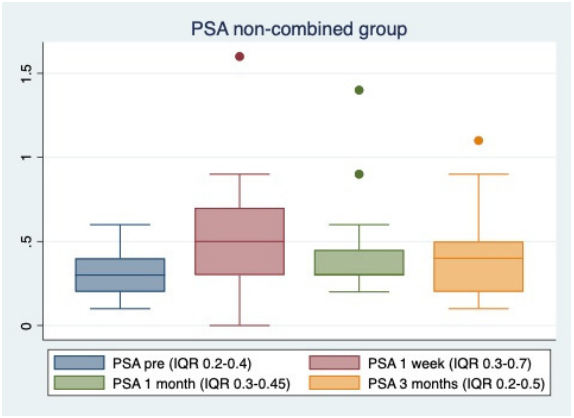
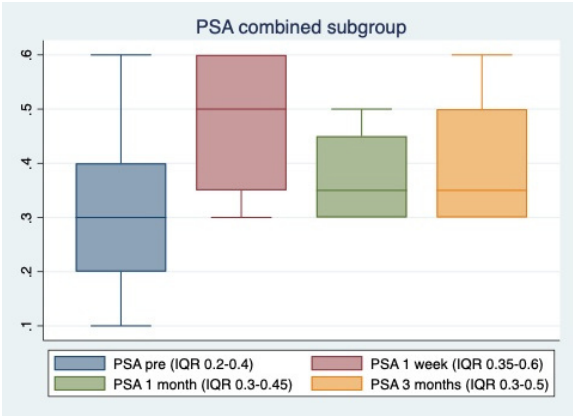


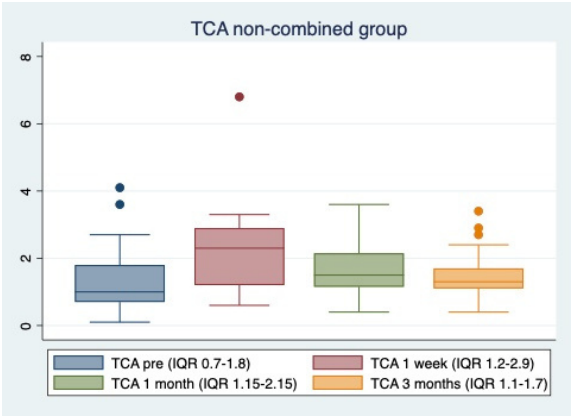
Figure 3. Box plot diagrams from the changes observed in the anterior and posterior corneal astigmatism for the combined and non-combined groups. Anterior surface astigmatism (ASA); posterior surface astigmatism (PSA); total corneal astigmatism (TCA); interquartilic range (IQR). The results from ASA, PSA and TCA are also summarized in Table 3 (a) non-combined and (b) combined.











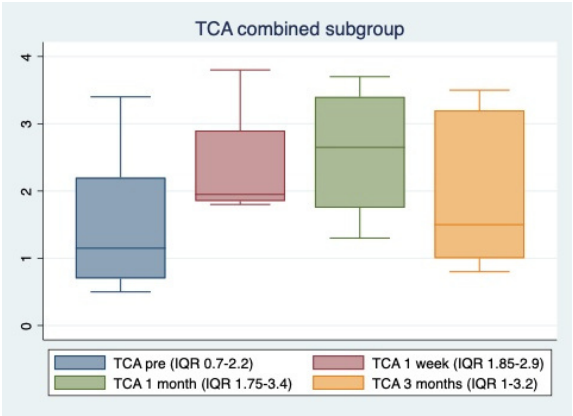
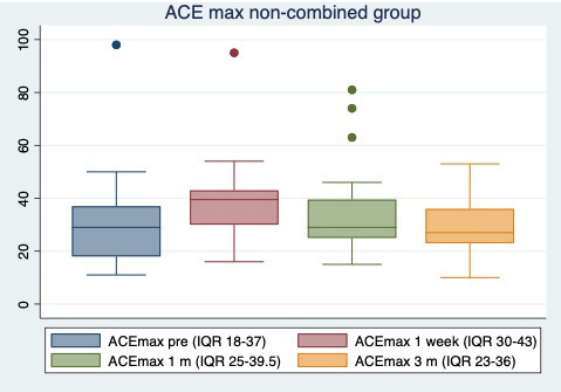
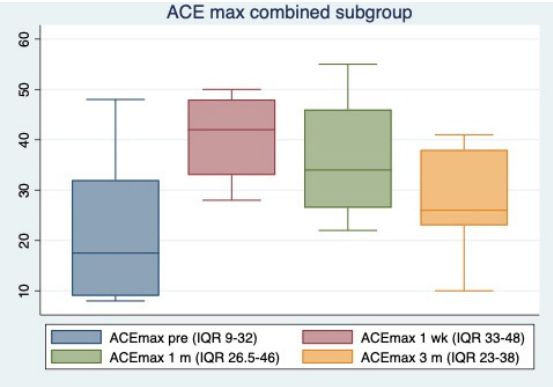
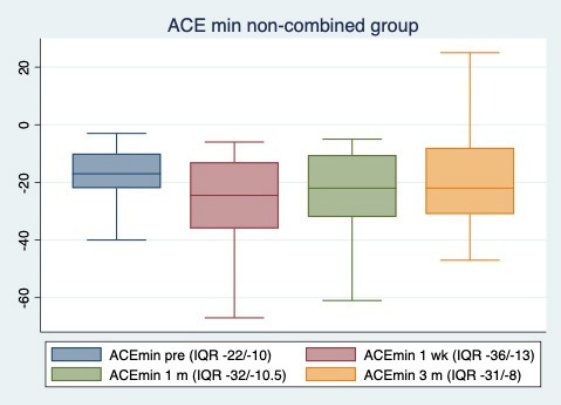
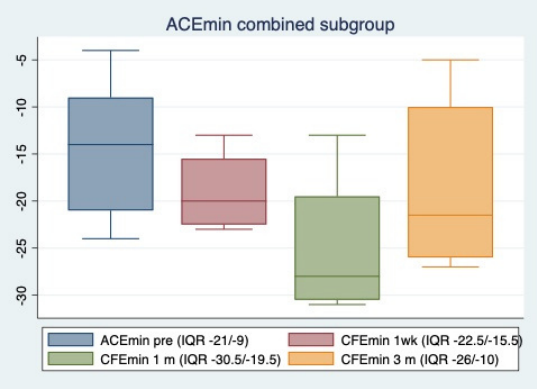


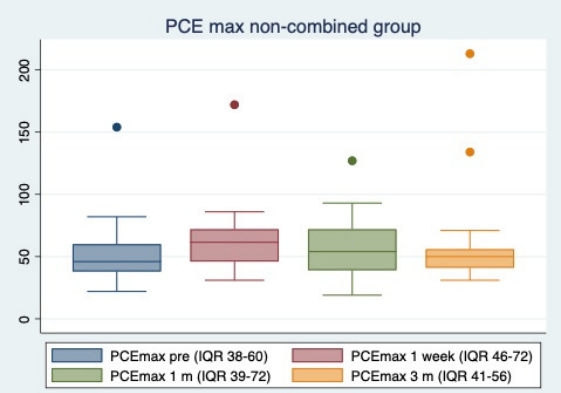
Figure 4. Box plot diagrams from the changes observed in the anterior and posterior corneal elevation for the combined and non-combined groups. Anterior corneal elevation max (ACE max); anterior corneal elevation min (ACE min); posterior corneal elevation max (PCE max); posterior corneal elevation min (PCE min); interquartilic range (IQR). The results from ACE and PCE are also summarized in Table 4 (a) non-combined and (b) combined.

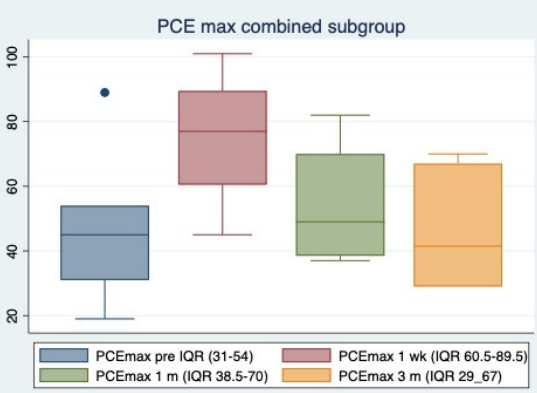


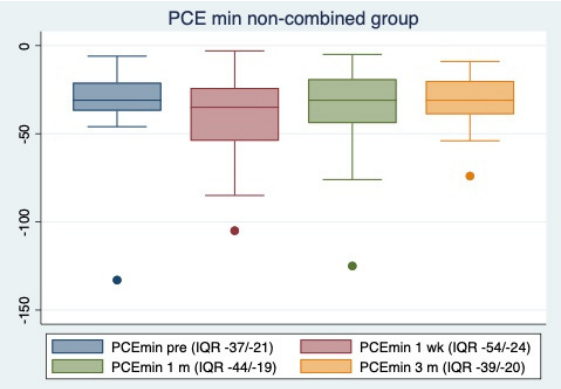


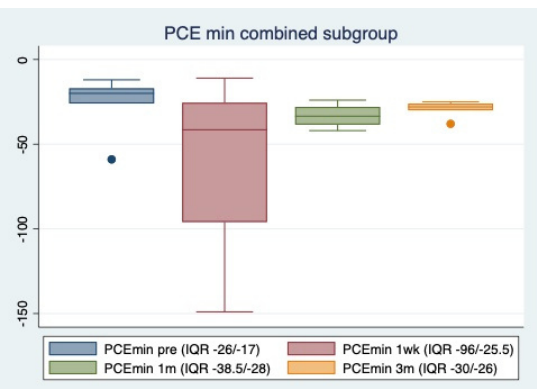












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ORIGINAL RESEARCH

Corneal Endothelial Cell Loss After PRESERFLO™ MicroShunt Implantation in the Anterior Chamber: Anterior Segment OCT Tube Location as a Risk Factor

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ABSTRACT

Introduction: To analyze the effects of PRESERFLO on corneal endothelial cell density (ECD).

Methods: Forty-six eyes that underwent PRESERFLO implantation were followed up for 12 months. Specular microscopy was performed preoperatively and at 1, 3, 6, and 12 months postoperatively to measure central ECD and mean monthly reduction (MMR). Anterior segment optical coherence tomography (AS-OCT) was applied to measure the tube–endothelium (TE < 200 μm, 201–500 μm, > 500 μm) distance. The relationship between TE distance and ECD was analyzed with a linear mixed-effects model.

Results: Central ECD decreased significantly at 1 year (7.4%, $p = 0.04$), with an MMR of -15 ± 25 cells/mm^2. Regarding TE distance groups, there was an 18% ECD reduction in the < 200 μm group vs. 1% in the > 500 μm group ($p = 0.08$). Endothelial cell loss was related to TE distance (mean 482.9 ± 238 μm), with a higher rate at 1 month in comparison to 12 months for the same tube position in the anterior chamber (-174.8 ± 65.2 cells/mm^2 at 1 month vs. 30.2 ± 11.3 cells/mm^2 at 12 months, $p < 0.01$). From month 6, tubes located > 600 μm from the endothelium showed EC loss close to zero.

Conclusions: The PRESERFLO implant is associated with a loss of EC from the immediate postoperative period that continues over time at lower rates. A shorter TE distance appears to cause more severe ECD loss.

Keywords: AS-OCT; Glaucoma; Endothelial cell loss; Filtering glaucoma surgery; Glaucoma; Glaucoma drainage device; MIGS; PRESERFLO

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Key Summary Points

Why carry out this study?

There is growing concern about the endothelial safety of the new glaucoma implants since the recent market withdrawal of a suprachoroidal device. The rate of endothelial cell loss and the risk factors associated with the PRESERFLO MicroShunt are still not known.

What was the hypothesis of the study?

The main hypothesis of this study was that a short distance of the tip of the PRESERFLO from the endothelium might be associated with greater corneal endothelial cell loss, as has been shown to happen with long-tube glaucoma drainage devices.

What was learned from the study?

What were the study outcomes? A short distance from the tip of the tube from the endothelium appears to cause more severe endothelial cell density (ECD) loss. The tubes located at a distance greater than 600 μm show ECD loss close to zero.

What has been learned from the study? The pattern of ECD loss associated with PRESERFLO resembles the ECD loss associated with long-tube glaucoma drainage devices, an ongoing loss of endothelial cells that occurs over time, but at a slower rate with PRESERFLO. At one year, the mean percentage of ECD loss (7.4%) and the mean monthly reduction (-14.6 cells/mm^2) are comparable to the ECD loss reported for the Ahmed valve located in the ciliary sulcus. A distance from the tip of the endothelium greater than 600 μm appears to protect from endothelial cell loss. Hypotony and peripheral anterior synechiae are risk factors for greater endothelial cell loss in the immediate postoperative period.

INTRODUCTION

Endothelial cell loss (ECL) leading to corneal decompensation is one of the major concerns regarding glaucoma surgical procedures. Many studies have reported the effects of glaucoma surgery on corneal endothelial cells (CECs). In recent years, attention has been focused on the effects that the relatively new techniques, microincisional glaucoma surgery (MIGS) and subconjunctival "miniaturized" tube shunts (XEN 45, Allergan Inc. Dublin, Ireland; PRESERFLO MicroShunt, Santen Pharmaceutical Co., Osaka, Japan), might have on the endothelium. However, the withdrawal of the CyPass supraciliary micro-shunt (Alcon Laboratories, Fort Worth, TX, USA) from the global marketplace as a direct result of adverse effects on endothelial cell density (ECD), with a cumulative 5-year incidence of ECL > 30% [1], has increased concerns about the monitoring and control of the impact of glaucoma surgery on CEC health.

Although the pathophysiology of endothelial cell loss is not well understood, it has been proposed to involve at least three mechanisms: first, mechanical damage derived from the proximity of the implant to the endothelium; second, the high fluid flow of aqueous humor through the tube, inducing ECL proximal to the tube entry site; and third, postoperative inflammation [2, 3]. In the CyPass study, the position of the tube in the anterior chamber (AC) was associated with ECL, with greater ECL in eyes where the implant protruded further and was closer to the endothelium [1]. Based on the Ahmed glaucoma valve (AGV; New World Medical, Rancho Cucamonga, CA, USA), the mean monthly rate of central endothelial cell loss was recently reported to be significantly higher when the tube was located in the AC compared to the ciliary sulcus [4]. Similarly, in a 3-year follow-up study, the position of the tube of the Baerveldt (BV) glaucoma drainage device (GDD) (Abbott Medical Optics, Chicago, Illinois, USA) was found to accelerate EC loss when placed closer to the endothelium, i.e., the shorter the distance, the greater the loss [5].

The PRESERFLO implant is an 8.5-mm-long tube designed to shunt the aqueous humor from the AC to the subconjunctival space in a posterior location, away from the limbus, and underneath Tenon's capsule. For the same tube length, the volume occupied by this implant in the AC is almost half that of a traditional GDD (due to their different external diameters, 350 vs. 630 μm, respectively). In addition, resistance to flow, as calculated with the Hagen–Poiseuille equation [6], is significantly higher through PRESERFLO, increasing inversely to the fourth power of the lumen diameter (70 vs. 305 μm, 1.3 mmHg/μL/min vs. 0.004 mmHg/μL/min). Thus, the fluid flow through PRESERFLO is significantly lower than that through a traditional GDD. Both parameters, namely, high fluid flow through the tube and turbulence present at the tip of the implant producing damage to the endothelium near tube entry, have been proposed as possible mechanisms involved in ECL after GDD implantation [2, 7]. Theoretically, the lower rate of aqueous flow through this new implant might reduce the rate of ECL after surgery, though the effects of the location of PRESERFLO in the AC (distance from the endothelium and the iris and the total length of the tube) on ECD have not yet been analyzed. We hypothesized that a short distance of the tip of the PRESERFLO from the endothelium might be associated with higher rates of CEC loss, as has been shown for the BV glaucoma implant, but probably at a slower rate due to its smaller volume into the AC and its lower flow rate of aqueous humor in comparison with traditional GDDs. The aim of this study was to evaluate the effect of tube location on corneal cell density after implantation of the PRESERFLO MicroShunt.

METHODS

This is an observational, prospective study. It was performed in accordance with the tenets of the Helsinki Declaration of 1964. All the patients gave their informed consent for data collection and further publication of the study outcomes prior to surgery. Approval from the ethics committee was not required, given both the observational nature of the study and that the usual clinical practice was followed.

Study Population

The study included consecutive patients who underwent PRESERFLO implantation in the upper-temporal or upper-nasal quadrant in the AC in the Glaucoma Department of the Oftalvist Clinic-Moncloa HLA Hospital (Madrid, Spain). Both "standalone" and combined cataract phacoemulsification and PRESERFLO procedures were included, but only pseudophakic eyes were considered for "standalone" PRESERFLO implantation. The exclusion criteria were as follows: previous glaucoma surgery with a tube shunt, previous corneal disease, previous corneal transplant, neovascular and uveitic glaucoma, and inability of the patient to cooperate with the tests required for this study.

Surgical Technique

The surgical technique has been previously reported by our group [8]: https://journals.lww.com/glaucomajournal/Fulltext/2021/10000/Changes_to_Corneal_Topography_and_Biometrics_After.8.aspx.

In brief: "All operations were performed by the same surgeon (M.I.B.), with sub-Tenon anesthesia in the inferior nasal quadrant. A traction suture on the superior cornea was used to expose the upper nasal conjunctiva to perform conjunctival peritomy and careful Tenon dissection over two clock hours, liberating all the attachments between the Tenon capsule and episclera and creating a posterior pocket between the superior and medial rectus muscles. A diathermy probe was applied to the sclera to control bleeding and to obtain a clear surgical field. Mitomycin C (MMC) 0.2 mg/ml was used in all cases by introducing three soaked surgical sponges provided by the manufacturer under Tenon's layer for 2 minutes, avoiding the limbus, and then gently washing with balanced salt solution. A mark with trypan blue was placed with the tip of the caliper 3 mm away from the limbus, and a 1-mm-wide scleral pre-incision was created with a microknife until

the tip was not visible. The scleral tunnel was created parallel to the surface of the sclera with a 25-gauge needle entering the AC at the trabecular meshwork. The PRESERFLO MicroShunt was then introduced into the tunnel until it reached the AC; its position was visually checked, ensuring that it was not too close to the iris or endothelium and was placed with the bevel facing up. A planar fixation structure resembling the fins of an arrow that seals the device in the pocket is located half-way down the tube, preventing leakage around the tube and the tube from migrating into the eye. The fins were placed at the end of the scleral tunnel to ensure that it was inside. Flow through the implant was confirmed by injecting BSS [balanced salt solution] from the distal side of the tube with a 23-G cannula; a small air bubble advancing to the AC is usually observed, and drop-by-drop flow was confirmed from the end of the tube with a surgical sponge. Tenon's layer was advanced prior to the conjunctiva to ensure that the implant was not caught in it, and then the conjunctiva was sutured watertight over Tenon's layer with 10-0 nylon. A side-port incision was created at the end of the surgery to inject 0.1 ml of cefuroxime (1 mg/0.1 ml) into the AC. For combined surgery, the surgical technique was the same and performed at the end of the phacoemulsification and IOL [intraocular lens] implantation procedure" [8].

Evaluation of the Anterior Segment

All patients underwent preoperative evaluation of the central corneal thickness (CCT) and noncontact specular microscopy (Topcon SP-1P specular microscope, Topcon Corporation, Tokyo, Japan) for corneal endothelial evaluation prior to PRESERFLO implantation. The photography magnification of this model is $\times 254$, range 0.25×0.55 mm. The automatic segmentation on a data set of in vivo specular microscopy images obtained with this device showed 95.8% correctly merged cells and 2% undersegmented cells [9]. The images were collected by the fully automated capture procedure. To obtain the images, the mode "center" was used.

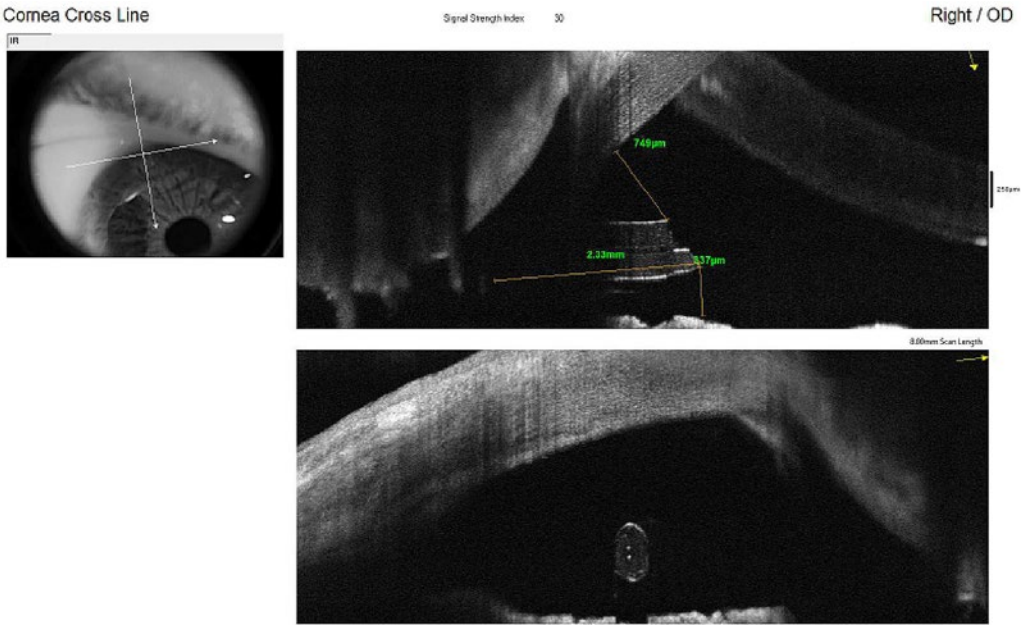

Fig. 1 AS-OCT image of the tube of the PRESERFLO in the AC. The caliper tool of the "Crossline" software of the Optovue Avanti Widefield was used to measure the distance from the tube to the endothelium and the iris and its total length

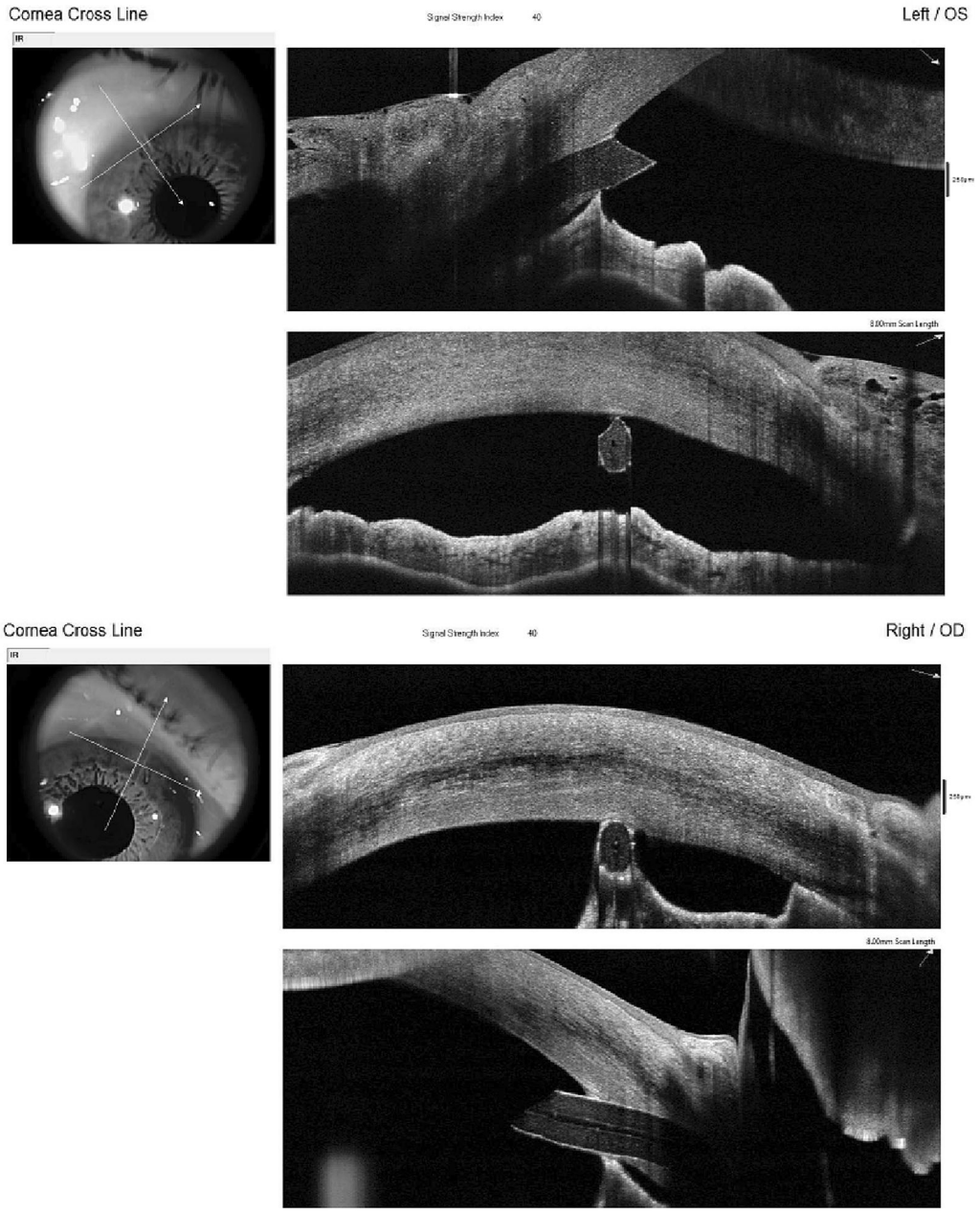

Fig. 2 AS-OCT image of two cases of peripheral anterior synechiae (PAS)

During postoperative visits, these measurements were repeated while accounting for the central ECD. During follow-up, anterior segment optical coherence tomography (AS-OCT) with the Avanti Widefield (Optovue, Inc., Fremont, CA, USA) was used to evaluate the distance of the tube from the endothelium and iris and its length in the AC using the caliper tool of the "Crossline" option. Measurements from the distal superior end of the beveled tip of the tube were performed perpendicular to the internal surface of the cornea (tube–endothelium [TE] distance) [5], and from the distal inferior end of the tube to the iris plane (tube–iris [TI] distance)

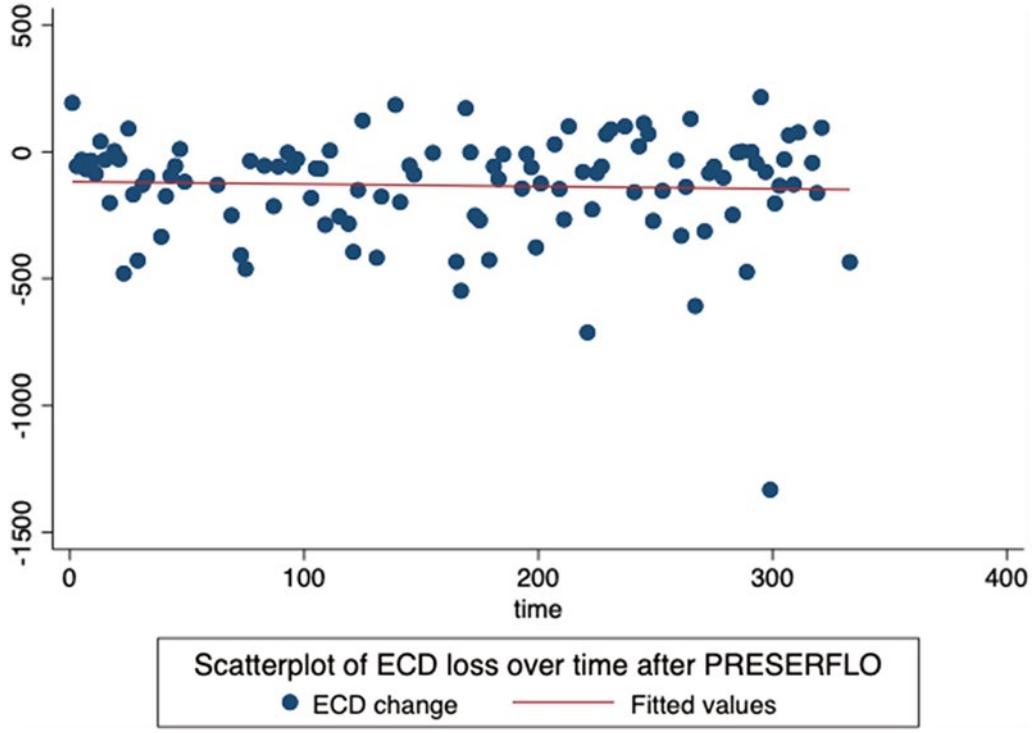

Fig. 3 Scatterplot of endothelial cell density (ECD) loss over time after PRESERFLO implantation into the AC. The horizontal axis shows days after surgery, and the vertical axis shows ECD calculated by subtracting ECD postoperatively from ECD preoperatively (cells/mm^2)

(Fig. 1). These measurements were repeated at 1 week and at 1, 3, 6, and 12 months after surgery.

The length of the tube in the AC was measured from the beveled tip to the angle at 3 months (Fig. 1). Peripheral anterior synechiae (PAS) were evaluated by AS-OCT (Fig. 2).

Statistical Analysis

Graphic analysis of the data distribution was analyzed with scatter plots, box plots, and bar graphs of the total ECD, ECD change (ECD postoperative − ECD preoperative) versus time from surgery (number of days since surgery) (Figs. 3, 4), and the mean monthly reduction (MMR) in ECD of the total population of the study (Fig. 5). The MMR was calculated by dividing the ECD change (preoperative − postoperative) by the number of months since surgery.

The percentage of central ECD loss (mean ± SD) was obtained by dividing the monthly ECD change by the preoperative ECD for the total population of the study at the different follow-up visits. A linear mixed model was employed to analyze the influence of the TE and TI distances on the ECD central change, and the Pearson correlation index was calculated to analyze the correlation between ECD and the TE and TI distances.

Stata® 17 (StataCorp LLC, College Station, TX, USA) was used for statistical analysis.

The sample was divided into different groups for analysis:

1. Two groups divided by type of surgery: Combined phaco-PRESERFLO surgery ("combined" group) vs. pseudophakic eyes that received the implant as a "solo" procedure ("standalone pseudophakic, SPF").

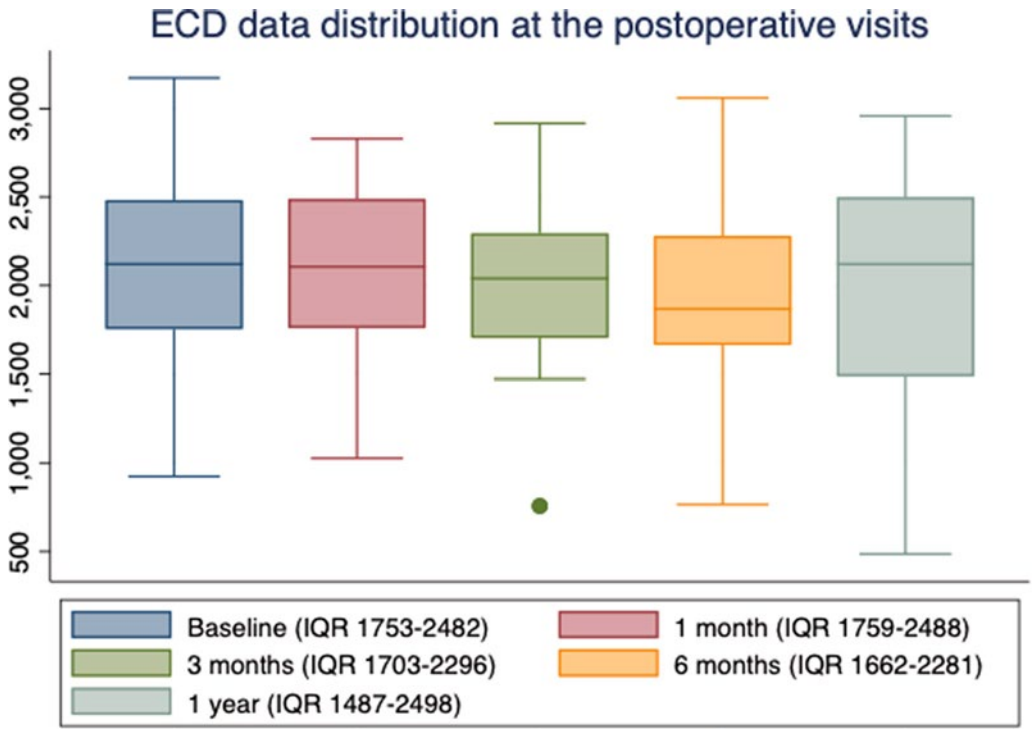

Fig. 4 Endothelial cell density (ECD) distribution at postoperative visits. *IQR* interquartile range

2. Three groups divided by the TE distance: (a) TE distance < 200 μm, (b) TE distance 201–500 μm, (c) TE distance > 500 μm.

No sample size was specifically calculated for the current study, because the published literature about the PRESERFLO implant is so scarce that this study should be regarded as a pilot study.

RESULTS

Baseline Data

Forty-six eyes of 40 patients were included (mean age 74 ± 9 years, 37% female, 52% right eye). Thirty-two eyes were pseudophakic, and 14 underwent combined surgery. Two eyes had pseudoexfoliative glaucoma, 43 had primary open-angle glaucoma, one eye had primary angle-closure glaucoma, and three had undergone a previous glaucoma surgery (trabeculectomy). All patients provided informed consent before surgery.

Mean Central Endothelial Cell Density, Mean Monthly Reduction, and Percentage of Central ECD Loss in the Total Population of the Study Over Time

Preoperatively, the mean central ECD was 2088 ± 527 cells/mm^2. At 12 months, the percentage of total central ECD loss was 7.4%. The results for mean central ECD and mean pachymetry are shown in Table 1.

The MMR in central ECD (calculated as the difference between preoperative and postoperative endothelial measurements divided by the number of months since surgery, mean ± SD cells/mm^2) at the different time points is shown in Table 2 and Fig. 5. MMR was −92.8 ± 165.6 cells/mm^2 in the first month, decreasing progressively in the first year to −14.7 ± 28.4 cells/mm^2.

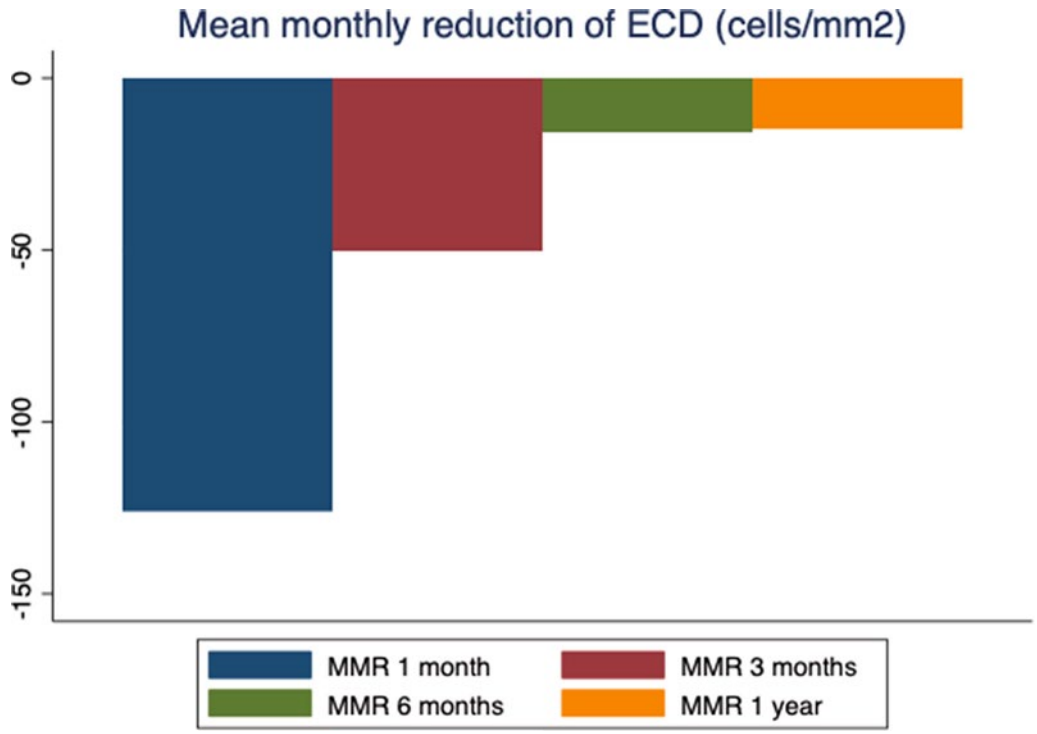

Fig. 5 Bar graph of the mean monthly reduction (MMR) in endothelial cell density (ECD, cells/mm^2) after implantation of the PRESERFLO MicroShunt, as calculated by dividing ECD change (preoperative − postoperative) by the number of months since surgery

Table 1 Central ECD, percentage of total mean decrease of ECD, and mean pachymetry at different time points

	Central ECD (cells/mm^2)	Mean decrease (cells/mm^2)	% decrease ECD (%)	Mean pachymetry (microns)
Baseline	2088 ± 527			515 ± 27
1 month	2087 ± 431	−126 ± 160	0.04	511 ± 31
3 months	2040 ± 484	−151 ± 189	2.3	504 ± 27
6 months	1980 ± 541	−94 ± 180	5.1	495 ± 93
12 months	1933 ± 653	−162 ± 301	7.4	509 ± 35

SD standard deviation

The percentage of central ECD loss (mean ± SD) obtained by monthly ECD change divided by preoperative ECD for the total population of the study at the different follow-up visits is shown in Table 3.

Endothelial Cell Changes Analyzed by Groups

Combined Versus Standalone

There was no statistically significant difference in baseline ECD between the groups (Student's *t* test $p = 0.1$). The mean central ECD (cells/mm^2) per group at consecutive visits and the

Table 2 Mean monthly reduction in the endothelial cell density (total endothelial cell decrease/no. of months since surgery) in the total population of the study

Mean monthly reduction (cells/mm^2)	Mean	SD
1 month	−125.8	160
3 months	−50.2	63
6 months	−15.6	30
12 months	−14.6	25

SD standard deviation

total percentage of loss at 1 year are given in Table 4.

The MMR in ECD per group showed greater endothelial loss in the combined group in the first month (−140 ± 110 vs. −118 ± 181 cells/mm^2) and in the standalone group at 1 year (−16 ± 28 vs. −11 ± 13 cells/mm^2), even though the differences were not significant ($p = 0.6$, $p = 0.3$, respectively; Table 5). Figure 6 shows the progressive MMR in ECD (cells/mm^2) from 1 month to 1 year.

Table 3 Percentage of central ECD loss (mean ± SD) obtained by monthly ECD change divided by preoperative ECD for the total population of the study at the different follow-up visits

% central decrease (MMR/baseline ECD)	Mean	SD (%)
1 month	−0.075	0.07
3 months	−0.02%	0.03
6 months	−0.008%	0.01
12 months	−0.007%	0.01

Comparison of TE Distance Between Groups

The mean distance from the tube to the endothelium was 482.9 ± 238 μm; from the iris, it was 778.5 ± 348 μm. The mean length of the tube in the AC was 2.76 ± 0.4 mm.

The mean central ECD in the groups with TE < 200 μm (Group a), 201–500 μm (Group b), and > 501 μm (Group c) from baseline to 1 year is shown in Table 6. No statistically significant differences were found between the groups at baseline or at different time points (one-way ANOVA, Bonferroni correction, Prob > F 0.4), but ECL over time was greater when the TE distance was less than 500 μm (−134 ± 203 and −209 ± 349 cells/mm^2 in groups < 200 μm and 201–500 μm TE, vs. −36 ± 163 in group > 500 μm TE). According to the percentage of total loss at 12 months between the groups, the shorter the TE distance, the greater the loss (< 200 μm: 18%, 200–500 μm: 11%, > 500 μm: 1%) .

The MMR in ECD based on TE distance groups is shown in Table 7 and Fig. 7. Although the differences were not significant (one-way ANOVA, Bonferroni correction, Prob > F 0.5), MMR in the first month was higher when the TE distance was less than 200 μm (−149 ± 76). At 12 months, the group with TE distance greater than 500 μm had less MMR in ECD (−3 ± 14 cells/mm^2) than the < 200 μm (−11 ± 9 cells/mm^2) and 201–500 μm (−20 ± 29 cells/mm^2) groups.

Table 4 Mean central ECD (cells/mm^2) at baseline, 1, 3, 6, and 12 months by groups; combined and standalone pseudophakic (SPF) and mean difference from baseline

Central ECD	Combined	% decrease (%)	SPF	% decrease (%)
Baseline	2239 ± 428		2022 ± 558	
1 month	2065 ± 365	7.7	2097 ± 467	0
3 months	2096 ± 407	6.3	2018 ± 519	0.2
6 months	2110 ± 529	5.7	1899 ± 550	6
12 months	2114 ± 428	5.5	1869 ± 713	7.5

Table 5 Mean monthly reduction (MMR) in ECD (cells/mm^2) per group; combined and standalone pseudophakic (SPF) at 1, 3, 6, and 12 months post-op

MMR in central ECD (cells/mm^2)	Combined	SPF
1 month	−140 ± 110	−118 ± 181
3 months	−72 ± 41	−41 ± 69
6 months	−19 ± 25	−13 ± 33
12 months	−11 ± 13	−16 ± 28

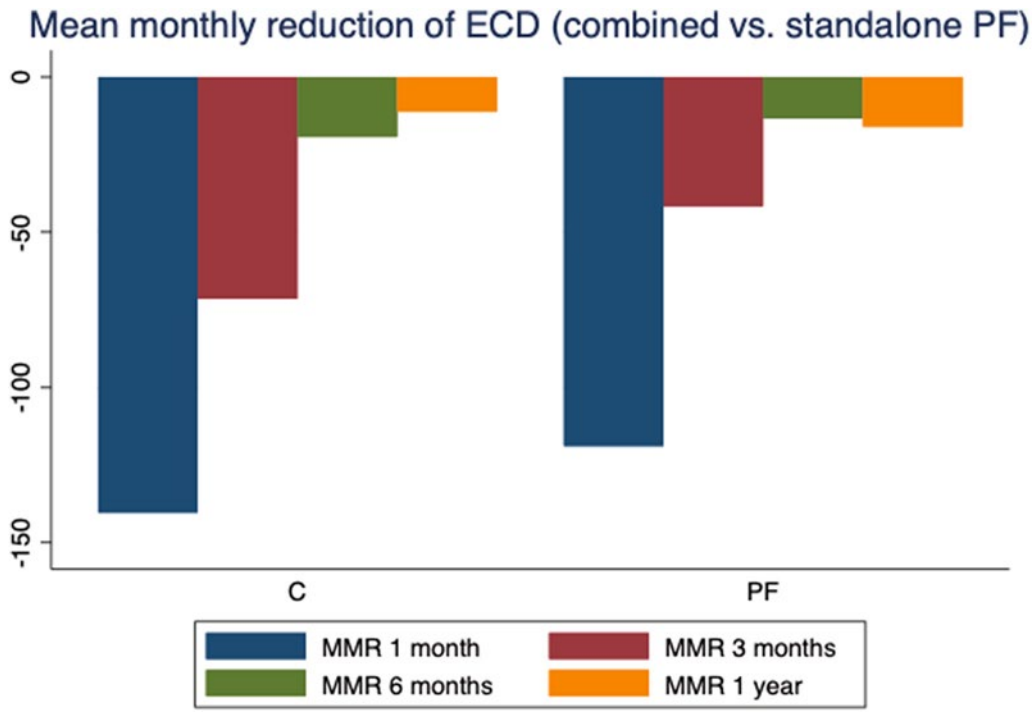

Fig. 6 Mean monthly reduction (MMR) in ECD (cells/mm^2) per group (combined vs. standalone pseudophakic PF)

Mixed-Effects Linear Regression Model Analysis Between the TE Distance and Total Length of the Tube in the AC with Endothelial Cell Loss

A mixed-effects linear regression model ruled by the equation MMR = mean TE coefficient × mean TE constant coefficient showed an inverse linear relationship between the MMR in ECD at 1, 3, 6, and 12 months and the TE distance (Fig. 8). According to this model, all tube locations were associated with endothelial cell loss at 1 and 3 months: the shorter the TE distance, the greater the loss. At 1 month, loss began at −174.8 ± 65.2 cells/mm^2 for a TE distance = 0 µm ($p < 0.01$), decreasing to −62.2 ± 27.8 cells/mm^2 at 6 months ($p = 0.02$). At 6 and 12 months, the linear fit showed a lower rate of loss, which was initiated at −33.9 ± 12.8 cells/mm^2 ($p < 0.01$) and −30.2 ± 11.3 cells/mm^2 ($p < 0.01$) at 6 and 12 months, respectively. From 6 months and thereafter, tubes located further than 600 µm from the endothelium were associated with a

Table 6 Central endothelial cell density (ECD) per group of tube–endothelium (TE) distance at different time points (Group a: 3 eyes; Group b: 21 eyes; Group c: 22 eyes)

Central ECD	< 200 µm TE (Group a)	201–500 µm TE (Group b)	> 501 µm TE (Group c)
Baseline	2073 ± 356	2023 ± 588	2239 ± 478
1 month	2010 ± 383	2029 ± 460	2248 ± 420
3 months	2048 ± 207	2123 ± 490	2143 ± 427
6 months	1928 ± 251	1810 ± 525	2157 ± 622
12 months	1700 ± 349	1810 ± 697	2214 ± 574
Mean diff baseline − 12 months (Prob *p*) and total % loss	−134 ± 203 (17.9%)	−209 ± 349 (10.5%)	−36 ± 163 (1.1%)

Table 7 Mean monthly reduction in the endothelial cell density (total endothelial cell decrease/no. of months since surgery) per group of tube–endothelium (TE) distance

Mean monthly reduction	< 200 µm TE	201–500 µm TE	> 501 µm TE
1 month	−149 ± 76	−129 ± 195	−71 ± 79
3 months	−48 ± 67	−50 ± 69	−37 ± 59
6 months	−38 ± 34	−23 ± 33	−3 ± 21
12 months	−11 ± 9	−20 ± 29	−3 ± 14

very low rate of endothelial cell loss, close to zero (Fig. 8b, c). Using this model, an inverse linear relationship with a significant goodness of fit ($p < 0.01$) was found between the mean TE distance and ECD at 1 year, with a shorter distance from the tube to the iris having a higher ECD (Fig. 9). However, linear regression mixed-model analysis did not find an association between the total length of the tube into the AC and the MMR in ECD at any of the time points.

Pearson's Correlation Coefficient to Measure the Statistical Relationship Between TE Distance, TI Distance, and Tube Length in the AC with Endothelial Cell Density

A positive and significant correlation ($r = 0.38$, $p = 0.05$) between TE distance and ECD and a negative and significant correlation ($r = -0.5$, $p < 0.01$) between mean TE distance and ECD were found 1 year after surgery. Therefore, higher ECD at 1 year was associated with greater distance of the tube from the endothelium and smaller distance of the tube from the iris. The total length of the tube in the AC did not exhibit a correlation with MMR or ECD at any of the time points of the study.

Analysis of Other Risk Factors for Endothelial Cell Loss with the PRESERFLO Implant

Age*:* An inverse and significant correlation was found between age and ECD at 6 months ($r = -0.3$, $p = 0.05$, younger age, higher ECD). The correlation was negative but not significant at the remaining postoperative visits. Linear mixed-effects models showed an inverse linear relationship ($r = -0.15$, $p = 0.03$) between age and MMR at 12 months.

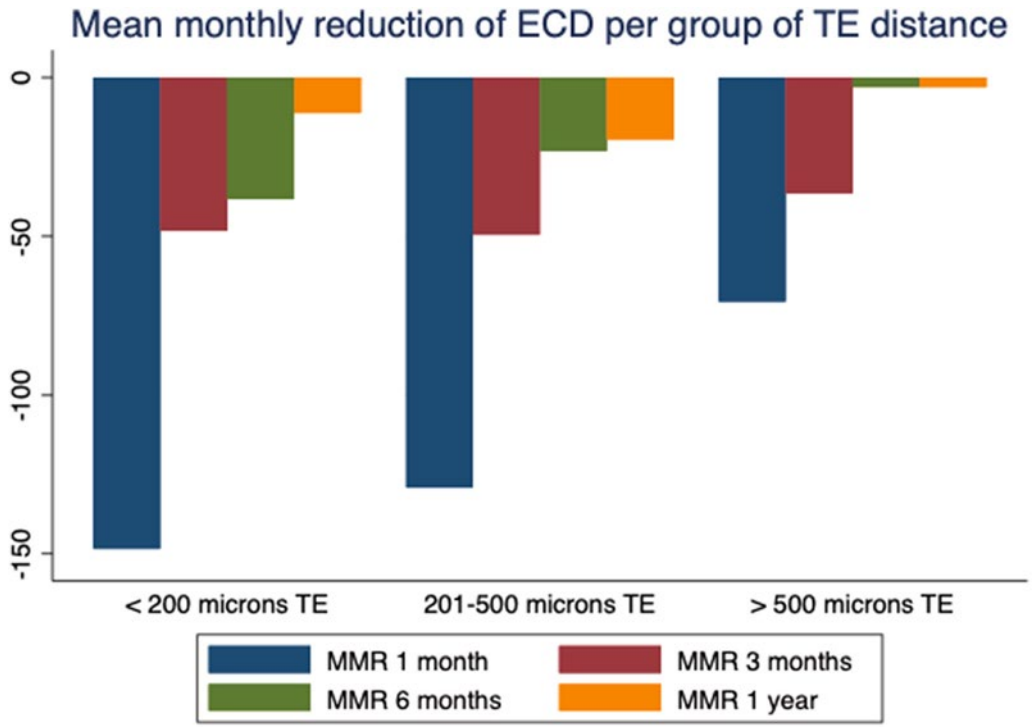

Fig. 7 Mean monthly reduction (MMR) in ECD (cells/mm^2) based on tube–endothelium distance (TE) groups. < 200 µm; 201–500 µm; > 500 µm

AC depth: A positive and significant correlation was found between ACD and ECD at 3 months ($r = 0.3$, $p = 0.05$), but an inverse and significant correlation ($r = -0.4$, $p = 0.01$) was found between ACD and MMR in ECD at 3 months: he higher the ACD at 3 months, the higher the ECD; conversely, the lower the ACD, the higher the MMR in ECD.

PAS: Student's *t* test analysis showed that the MMR in ECD at 1 month was greater (-341 ± 139 vs. -111 ± 28 cells/mm^2) in patients with PAS ($p = 0.02$).

Significant Endothelial Cell Loss Was Observed in One Case of Extremely Short TE Distance and Anterior Corneal Position of the Tube

In the total study population, there was one case of a 78-year-old man who underwent surgery for tube repositioning. AS-OCT images revealed that the tube had been introduced into the AC through the corneal stroma very close to the endothelium (176 µm), with subsequent endothelial cell changes observed from baseline to 1 year. The endothelial cell count decreased from 2173 cells/mm^2 preoperatively to 1755 cells/mm^2 at 3 months, 1459 cells/mm^2 at 6 months, and 783 cells/mm^2 at 1 year. Figure 10 displays the endothelial changes observed from baseline and the very anterior position of the tube into the AC. Mean TE and TI distances in this case were 176.3 ± 81.9 µm and 1210 ± 73.6 µm, respectively, with a total tube length of 2.85 mm in the AC. The tube was repositioned 1 year after surgery when endothelial loss was evidenced.

PAS and MMR of Endothelial Cells

Five eyes were found to have PAS related to transient hypotony in the early postoperative period. MMR in the first month was significantly greater in these eyes (mean -341 ± 139

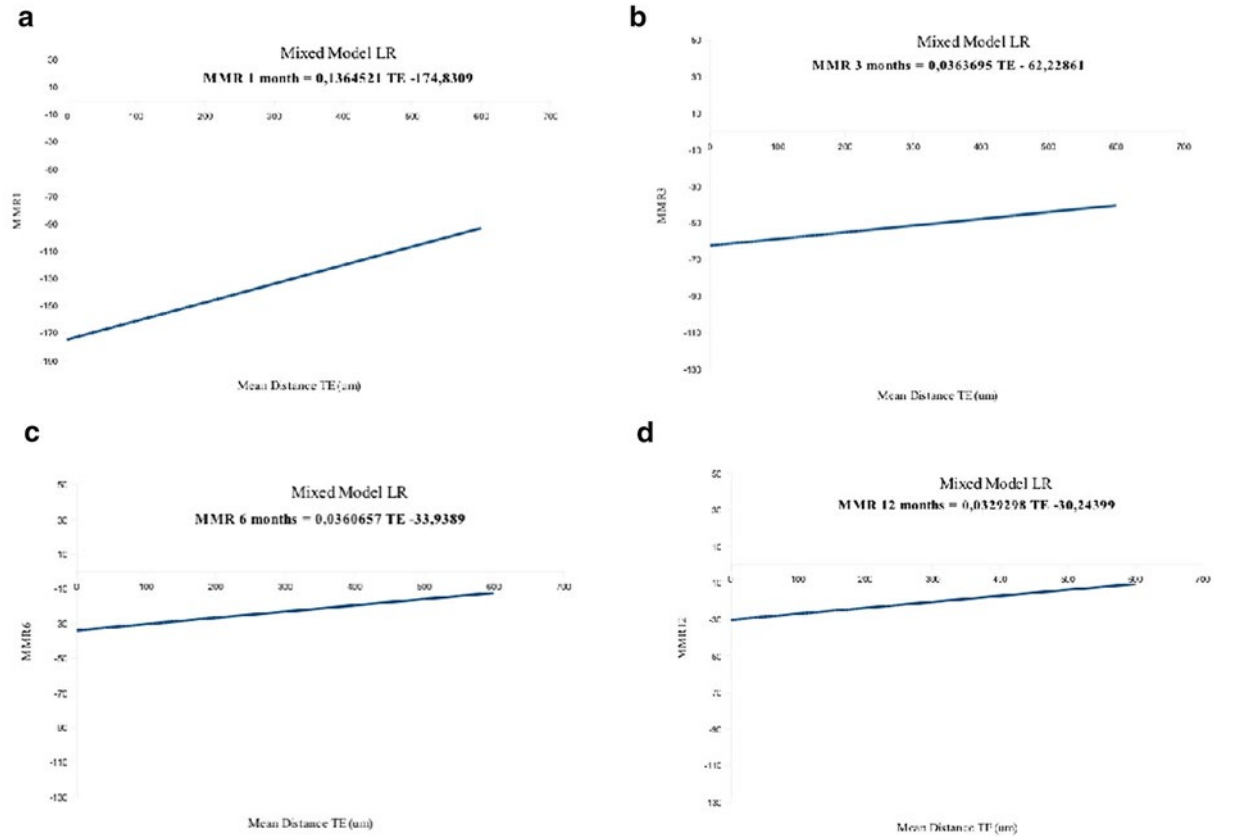

Fig. 8 A mixed-effects linear regression model was used to analyze the relationship between the mean monthly reduction (MMR) in ECD (cells/mm^2) and the distance from the tube to the endothelium (TE). **a** At 1 month, mean EC loss began at 174.8 ± 65.2 cells/mm^2 for TE = 0 µm ($p < 0.01$) and decreased progressively with increasing TE distance. **b** At 3 months, EC loss began at −62.2 ± 27.8 cells/mm^2 for TE = 0 µm ($p = 0.02$). **c** At 6 months, EC loss began at −33.9 ± 12.8 cells/mm^2 ($p < 0.01$) and reached a value close to zero when the TE distance was greater than 600 µm. **d** At 1 year, EC loss began at −30.2 ± 11.3 cells/mm^2 ($p < 0.01$) and showed the same protective distance at 600 µm

cells/mm^2) than in the group with no PAS and history of hypotony (−72.2 ± 30.5 cells/mm^2), $p = 0.01$. No significant differences were found at 3 and 6 months or at 1 year.

DISCUSSION

This 1-year follow-up study aimed to analyze postoperative endothelial cell changes in patients after glaucoma surgery with the PRESERFLO implant and the relationship that these changes may have with the tube's position in the AC, among other risk factors.

We found that after PRESERFLO, ECL begins soon after the surgery and continues over time, following the same pattern of ongoing ECL previously described for long-tube shunts [10]. Phacoemulsification and trabeculectomy both have a well-described effect on ECD, with reductions occurring in the immediate postoperative period that tend to stabilize over time. Additionally, in the current study, a closer position from the tube to the endothelium was found to accelerate endothelial cell loss, with a greater effect in the immediate postoperative period that decreased over time. It was also found that patients who underwent combined surgery experienced higher rates of ECL at 1

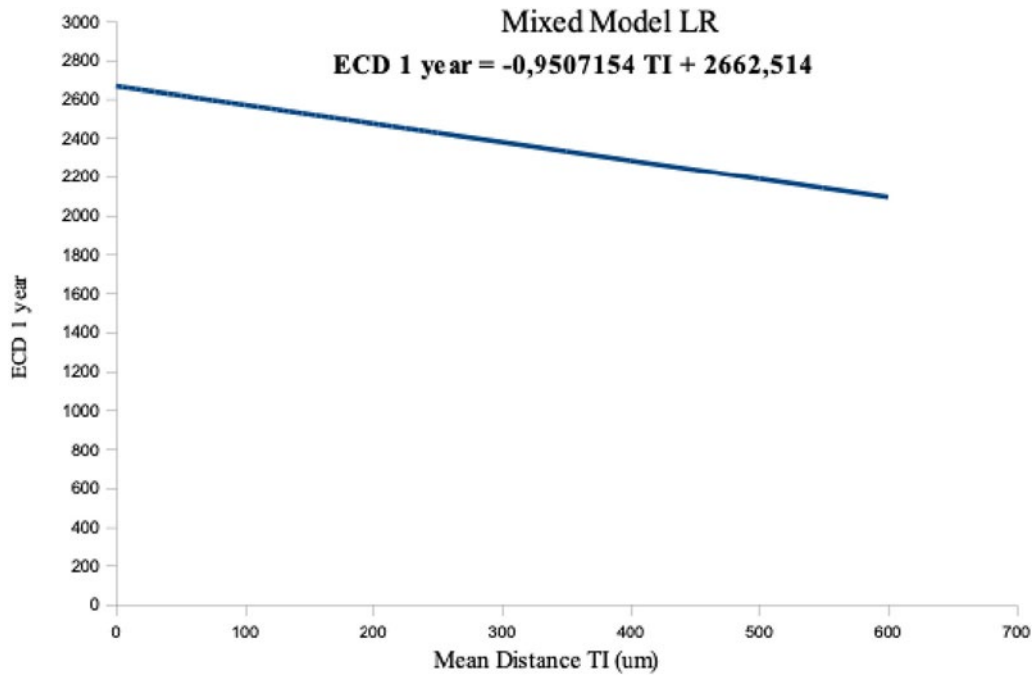

Fig. 9 A mixed-effects linear regression model was used to analyze the relationship between endothelial cell density (ECD, cells/mm^2) at 1 year and the mean distance from the tube to the iris (TI). The model showed an inverse linear relationship ($p < 0.01$): the greater the TI distance, the lower the ECD

month and lower rates at 1 year than after standalone procedures, suggesting that once ECL has stabilized after phacoemulsification, other factors related to the implant continue to influence endothelial cell loss.

There are widespread data on the effects of trabeculectomy with mitomycin C (MMC), phacoemulsification, and tube-shunt implantation for comparison with the results of this study on the PRESERFLO MicroShunt. Within the first 3 months after trabeculectomy with MMC 0.2 mg/ml, an ECL of 13.9% and a mean cell loss of −265 cells/mm^2 were observed [11], higher than the 2.3% ECL and −150 cells/mm^2 found after PRESERFLO implantation. In contrast, ECL after trabeculectomy with and without MMC trabeculectomy appears to occur in the immediate postoperative period. One study reported 3- and 12-month ECL of 9.5% and 10%, respectively, indicating no significant ongoing ECL after the first few months (MMC 0.2 mg/ml) [12]. Another study with a 3-month follow-up of trabeculectomy without MMC reported an ECL of 4.6%, of which only 1.2% occurred between months 1 and 3 [13]. The percentage of ECD decrease found with PRESERFLO for the total population of the current study revealed ongoing EC decrease, with 7.4% loss at 1 year.

On the other hand, routine phacoemulsification has been reported to be associated with an ECL of 7.6 to 9.5% within the first 2 weeks after surgery, stabilizing over time [14, 15] and leading to an ECL of 7.3% reported at 12 months [16]. In contrast, comparison between groups of combined versus standalone PRESERFLO in the current study showed greater ECL in the combined group in the first month (7.7% and 0%, respectively) but comparable ECL at 12 months (5.5% and 7.5%). These results suggest initial ECL associated with phacoemulsification in the immediate postoperative period followed by sustained loss most likely associated with the presence of the tube in the AC, which is greater than the gradual physiological decline in the ECD of the normal adult cornea (estimated 0.6% per year) [17]. The MMR in ECD in these two groups also exhibited a greater

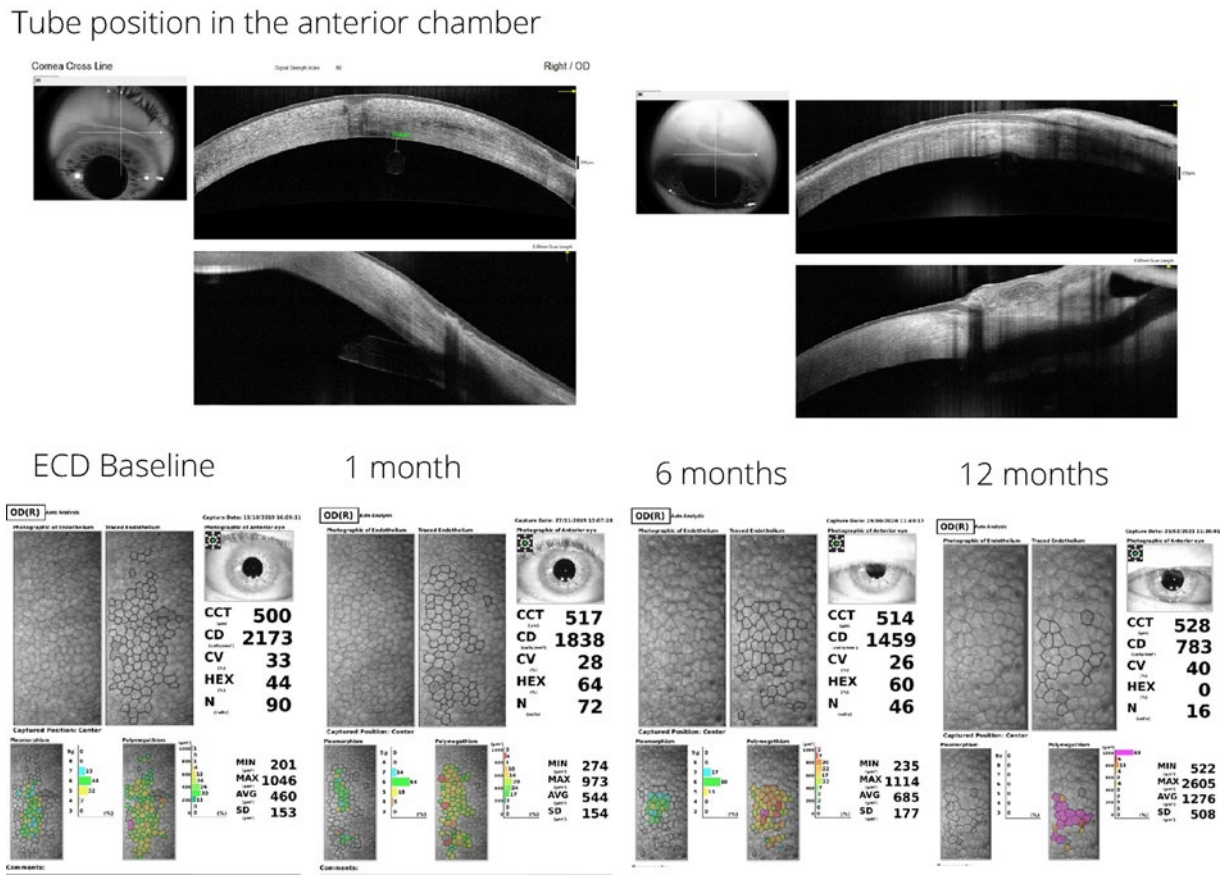

Fig. 10 Endothelial cell loss was observed in a case of a short tube–endothelium distance (176 µm), in which the tube entered the AC through the corneal stroma. The tube was repositioned when significant EC loss was noted

decrease in the first month in both the combined (-140 ± 110 cells/mm^2) and standalone (-118 ± 181 cells/mm^2) groups, whereas the opposite was observed at 1 year (higher mean monthly loss in standalone). The findings suggest the existence of other factors associated with ECL at 1 year after PRESERFLO implantation that are independent of phacoemulsification. Most likely, the presence of the tube in the AC induces sustained EC loss, similar to the Ahmed (AGV) and Baerveldt (BGI) devices, though at a slower rate according to our findings.

AC AGV tube location has been reported to be associated with a higher percentage of ECD loss at 1 year. For instance, Kim et al. [18] found a 10.5% decrease in central ECD at 12 months and Lee et al. [19] a 15.3% decrease at 1 year and an 18.6% decrease 2 years after surgery. These figures are higher than our results with the PRESERFLO device, with 7.4% ECL at 1 year. BGI implantation into the AC has also been reported to produce a high ECL at 12 months [20], at 13.1%.

For both the Ahmed and Baerveldt devices, the proximity of the tube tip to the corneal endothelium has been reported to be related to the magnitude of ECL [4, 5], which is consistent with the results of our study for PRESERFLO. Furthermore, Zhang et al. [4] compared ECL between sulcus and AC tube locations after AGV and detected higher monthly ECD loss in the AC group (29.3 cells/mm^2) than in the sulcus group (15.3 cells/mm^2). In the current study, the monthly reduction at 1 year was 14.6 cells/

mm^2, comparable to the rate of ECL after placing the tube of the AGV in the sulcus.

On the other hand, using a linear mixed model to analyze the central and peripheral ECD in relation to BGI tube–cornea distance, Tan et al. [5] found that a tube position closer to the endothelium accelerated EC loss: the shorter the distance, the greater the loss. Using the same statistical tool to analyze the effect of the PRESERFLO tube location on ECD, we found that a shorter TE distance was associated with greater loss of endothelial cells in the immediate postoperative period, beginning at −174.8 cells/mm^2 for a TE distance of 0 μm and following an inverse linear relationship as the TE distance increased. At 6 and 12 months, the rate of EC loss for the same tube position was lower, and tubes located further than 600 μm from the endothelium showed a very low rate of ECL, close to zero. Tan et al. [5] reported a central loss of 6.2% at 1 year for a tube–cornea distance of 1.1 mm. In the current study, the percentage of central ECD was 17.9% for a TE distance < 200 μm, 10.5% for a distance between 201 and 500 μm, and 1.1% when the distance was greater than 500 μm. Nevertheless, as there might be differences in the AS-OCT devices used and in the measurement methodology, it is not easy to directly compare figures from different studies. In addition, it would be interesting to measure TE distances of all the different implants at the same tube length in the AC for a more consistent comparison between studies.

Moreover, the increase in ECL over time in eyes implanted with long-tube shunts has been related not only to tube–endothelial contact but also to some degree of chronic inflammation, which may further compromise the corneal endothelium and increase the risk of corneal failure [21].

Another factor that might influence the ECL rate is tube displacement in the AC after implantation. Tan et al. [5] proposed that the position of the tube of a BGI placed "free" into the AC tends to move closer to the endothelium, explaining the higher loss found in their study when the tube was "free" compared to a trans-iridial position, which maintains the tube in a more stable position. In a previous study published by our group [22], the distance from the PRESERFLO tube to the endothelium remained stable from postoperative day 1 to the third month, suggesting that the cause of ECL is not movement of the tube, at least in the current adult population of this study, but is rather the position with respect to the cornea.

On the other hand, the corneal endothelium closest to the tip of the tube has been reported to show the greatest decrease in ECD [20], which suggests that the flow of aqueous humor through the tip of the tube near the corneal endothelium causes cell damage. In general, higher resistance to flow through the tube and therefore lower flow of aqueous solution may be a protective factor. The smaller dimensions of the PRESERFLO tube compared to the tube of an AGV or BGI increases resistance to flow 325 times (1.3 mmHg/μl/min vs. 0.004 mmHg/μl/min, respectively). The corresponding decrease in aqueous humor flow through the tip of the tube might explain the lower rate of ECL found for PRESERFLO in comparison to AGV or BGI.

The percentage of endothelial cell loss reported for the main competitor, the XEN gel stent, appears to be comparable to PRESERFLO. A short-term report (3 months) [23] showed a loss of 2.1% of ECD with XEN, versus 2.3% found for PRESERFLO in the current study, in both cases lower than trabeculectomy (10%) according to the author's findings. In a longer follow-up study (2 years) of the XEN gel stent [24], a 15.4% ECD loss was reported, versus 7.4% for PRESERFLO at 1 year. The yearly ECD loss found for PRESERFLO appears to be consistent with the ECD loss reported for XEN.

The AC depth and the presence of PAS after hypotony were associated with ECD, whereby a higher ACD resulted in a higher ECD and lower mean reduction in ECD at 3 months. The presence of PAS in the first month also correlated with a higher reduction in ECD.

One of the limitations of this study was the sample size. A larger number of patients would have allowed us to assemble a wider group for the tubes located at < 200 μm, a situation not very frequent and probably related to the surgeon's learning curve. Another weakness of this study is that only the central ECD was measured and not the ECD at the area closest to the tip of the implant.

CONCLUSIONS

In conclusion, PRESERFLO implantation into the AC is associated with ECD loss that began in the immediate postoperative period, with ongoing loss of endothelial cells over time, though at slower rates, at least up to 1 year postoperatively. A closer position of the tube to the endothelium is related to a higher loss of ECD. A TE distance greater than 600 μm appears to be a protective factor for endothelial cell preservation.

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Compliance with Ethics Guidelines. This is an observational, prospective study. It was performed in accordance with the tenets of the Helsinki Declaration of 1964. All the patients gave their informed consent for data collection and further publication of the study outcomes prior to surgery.

Data Availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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