Uveo-scleral outflow pathways after ultrasonic cyclocoagulation in refractory glaucoma: an anterior segment optical coherence tomography and in vivo confocal study

Sub-title

Ultrasonic cyclocoagulation induces anatomical modifications of the sclera, represented by intra-stromal hypo-reflective spaces, which could be involved in the aqueous humor drainage through the uveo-scleral pathways

Rodolfo Mastropasqua, MD,1* Luca Agnifili, MD, PhD,2§* Vincenzo Fasanella, MD,2 Lisa Toto, MD, PhD,2 Lorenza Brescia, MD,[2] Silvio Di Staso, MD,[3] Emanuele Doronzo, MD,[2] and Giorgio Marchini, MD [1]

[1] Ophthalmology Unit, Department of Neurological, Neuropsychological, Morphological and Movement Sciences, University of Verona, Verona, Italy; [2] Ophthalmology Clinic, Department of Medicine and Ageing Science, University G. d'Annunzio of Chieti-Pescara, Chieti, Italy; [3] Ophthalmic Clinic, Department of Surgical Science, University of L'Aquila, L'Aquila, Italy.

[*] RM and LA contributed equally to this work and share primary authorship

[§] Corresponding Author:
Luca Agnifili
Address:
Ophthalmology Clinic, Via dei Vestini snc, 66100 Chieti, Italy
Fax: +39-0871-358794
Telephone: +39-0871-358410
E-mail: l.agnifili@unich.it

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Abstract

Aims: To evaluate, using anterior segment-optical coherence tomography (AS-OCT) and *in vivo* confocal microscopy (IVCM), the uveo-scleral aqueous humor outflow pathway modifications after ultrasonic circular cyclo-coagulation (UCCC).

Methods: Forty-four patients with refractory glaucoma underwent 4- or 6-second UCCC (Group 1, 24 eyes, and Group 2, 20 eyes). AS-OCT and IVCM were performed at baseline and after one month, to evaluate the sclera and conjunctiva. The main outcomes were: mean intra-scleral hyporeflective spaces area (MIHSA: mm^2) at AS-OCT, mean density and area of conjunctival microcysts (MMD: cysts/mm^2; MMA: μm^2) at IVCM. The relations between MIHSA, MMA and MMD with IOP were analyzed.

Results: At baseline the mean IOP was 26.91±2.82 mmHg in Group 1, and 27.55±4.07 in Group 2. Intra-scleral hyporeflective spaces and conjunctival microcysts were observed in both groups, without significant differences in MIHSA, MMA, and MMD. After one month, UCCC was successful in 63.63% of patients (41.6 % in Group 1, and 80 % in Group 2). IOP significantly reduced to 18.83±3.22 and 17.15±2.72 mmHg, respectively ($p<0.001$). MIHSA showed a twofold and threefold increase in Group 1 and 2 ($p<0.05$), with a statistically significant difference between groups ($p<0.05$). MMA and MMD increased in both Groups ($p<0.05$), with values significantly higher in Group 2 ($p<0.05$). Significant relations were found between MIHSA and IOP in both groups ($p<0.01$).

Conclusions: UCCC induced anatomical modifications of the sclera and conjunctiva, which suggested that the enhancement of the trans-scleral AH outflow, is one of the possible mechanisms exploited by ultrasounds to reduce IOP.

Introduction

In glaucoma refractory to conventional treatments the ciliary body ablation is still considered as a last resort treatment to reduce the intra-ocular pressure (IOP). Several ablation procedures have been proposed over the years,[1-5] with the trans-scleral photocoagulation remaining the most used approach.[3]

In the last decades of the 20th century, high-intensity focused ultrasound (HIFU) was also proposed as a cyclo-destructive procedure (Sonocare, Inc., Ridgewood, NJ).[6-12] The major advantage of using high-operating frequency ultrasounds is to focus on a well-defined volume, at the desired depth, thus limiting damage to neighboring tissues. Despite the good IOP lowering efficacy,[11,12] the procedure was abandoned because of its excessive complexity.

To overcome these limitations, a modified HIFU technique (ultrasonic circular cyclo-coagulation (UCCC)) was recently proposed.[13-18] The procedure appears safe, with an IOP reduction from 26 to 36% at 12 months.[14,15,18]

The aqueous humor (AH) inflow reduction following the thermic necrosis of ciliary epithelium seem to play the main role in the final IOP lowering.[16] However, also an increase of the suprachoroidal and trans-scleral (TS) AH outflow, have been hypothesized.[13-17] The aim of our study was to investigate the modifications of the uveo-scleral outflow pathway after UCCC in refractory glaucoma, by using anterior segment-optical coherence tomography (AS-OCT) and *in vivo* confocal microscopy (IVCM).

Material and methods

Patient's enrollment

This was an interventional, case control study. Written informed consent was obtained from all patients before enrollment. The research adhered to the tenets of the Declaration of Helsinki and our institutional review board (Department of Medicine and Ageing Science of the University "G. d'Annunzio" of Chieti-Pescara, Chieti, Italy) approved the project.
Forty-four consecutive Caucasian patients scheduled to undergo UCCC for refractory glaucoma, were enrolled. Before enrollment patients underwent best correct visual acuity (BCVA), slit lamp biomicroscopy, Goldmann applanation tonometry (three measurements at 09:00, 12:00, and 16:00), fundus examination, and visual field (VF) test (30-2 test, full-threshold, HFA II 750; Carl Zeiss Meditec, Inc., Dublin, CA, USA).
Inclusion criteria were: age ≥18 years, refractory glaucoma with at least one previous incisional glaucoma surgery, uncontrolled IOP (>22 mmHg) under maximal tolerated medical therapy and VF damage progression.
Exclusion criteria were: history of topical or systemic therapy that could modify AH hydro-dynamics, ciliary body trauma, surgical or laser procedure in the last two months, previous ciliary body ablation, retinal detachment, and pregnancy. If both eyes were eligible, the eye with the higher IOP or the more advanced glaucoma stage was included in the study.
Ten consecutive patients with medically controlled primary open-angle glaucoma (POAG), who did not undergo filtration surgery, were used as controls. Inclusion criteria were: mean IOP lower than 20 mmHg with medical therapy unmodified in the last 6 months, and no history of VF progression during the last 12 months.
After enrollment patients scheduled to surgery underwent ultrasound biomicroscopy (UBM; Aviso; Quantel Medical, Clermont-Ferrand, France), AS-OCT (RTVue XR Avanti; Optovue, Inc, Fremont, California, USA), and laser scanning IVCM (HRT III Rostock Cornea Module; Heidelberg Engineering GmbH, Dossenheim, Germany).

UCCC was considered successful when a one-third reduction in preoperative IOP was obtained at the last follow-up, with or without anti-glaucoma treatment.

The surgical procedure and the technical characteristics of the HIFU were previously described. [14] Briefly, a 30 mm diameter ring containing six active piezoelectric elements was inserted in the upper part of a polymer-made coupling cone, previously placed in direct contact with the ocular surface, and filled with saline solution. The transducers are elliptic cylinder shaped segments of a 10.2-mm radius cylinder with a 4.5-mm width and a 7-mm length (active surface area of 35 mm^2), distributed along the circumference of the ring. HIFU probes are commercialized in three different ring diameters (11, 12, and 13 mm), which allow to fit most ocular sizes. UBM was used to select the right diameter of the probe, as previously described.[14]

Treatment consists in the sequential activation of each transducer lasting 4 or 6 seconds. Patients randomly received (by computer generated random numbers) a 4-second (Group 1) or a 6-second (Group 2) exposure time.

Postoperative treatment included topical dexamethasone and tobramycin (Tobradex; Alcon Laboratories, Inc., Fort Worth, TX, USA) given for two weeks three times a day. Preoperative hypotensive medications were maintained during the follow-up.

Follow-up visits included safety checks at day one and seven with BCVA determination, tonometry, slit lamp biomicroscopy and fundus examination. At day 30, mean IOP, VF, AS-OCT, and IVCM were repeated. For controls, follow-up was scheduled at day 30, with AS-OCT, IVCM and IOP determination.

AS-OCT and IVCM examinations

AS-OCT and IVCM were performed to analyze the scleral and conjunctival features at the sites of insonification, before (day -1) and thirty days after the UCCC.

To avoid in the field of analysis the sites of previous surgeries we considered the transducers taking contact with the superior-temporal or superior-nasal sectors. To comply

with this intention we marked the conjunctiva overlying the presumed site of transducer contact at 10 or 2 o'clock, delineating a 6x8 mm area (48 mm^2), at least 3 mm away from the site of the previous surgery. To be sure that transducer will match with the marked conjunctival area, also the coupling cone and the probe were marked at 2 or 10 o'clock. The AS-OCT and IVCM were performed within the marked area. A set of reference photographs was acquired at baseline, to analyze the same area after one month.

In patients who underwent only one filtration surgery, we analyzed the superior-temporal sector; in patients who received more than one surgery, we analyzed the available unmanipulated sector.

For the AS-OCT, vertical cross sectional scans were acquired to identify hypo-reflective spaces (HS) within the sclera. The scan selection was set in the automatic mode, to have automatic standard video brightness and contrast. A single operator (LB) performed all examinations, and selected three high quality images (from 10 randomly selected images), which were evaluated and results averaged by a second operator (ED). The mean intra-scleral hyporeflective spaces area (MIHSA, mm^2) was calculated.

HS were defined as intra-stromal areas having a lower degree of reflectivity compared to the surrounding sclera. To distinguish between HS and physiological variations of the scleral homogeneity (and reflectivity), we considered only spaces with well-defined edges and with a mean gray value lower than the 50% of the mean gray value of the surrounding sclera (Fig.1). The reflectivity of normal sclera and HS was determined with Image J (http://rsb.info.nih.gov/nih-image). To calculate the reflectivity of the normal sclera, we selected three highly homogeneous stromal areas (100x100 μm) without evidence of HS, in each of the three selected images. To be representative of the entire sclera, the three areas were selected in three different regions of the middle-outer sclera, where HS are normally rare.

The software determines the average gray value of the selected area, and this value corresponds to the sum of gray values of all pixels divided by the number of pixels. The mean value of the three areas was finally considered. All HS present in the image were analyzed; the average gray value was determined within the entire area of each space. IVCM was performed as previously described.[19] Sequential images (400x400 μm) derived from automatic scans and manual frames were acquired at the intermediate layer of the epithelium. A single operator (VF) performed all examinations and selected eight high quality images (from 40 randomly selected images), which were evaluated and the results averaged by a second IVCM operator (LT). IVCM was aimed at identifying epithelial microcysts, defined as round or oval-shaped optically clear structures, occasionally filled with amorphous material and roundish hyperreflective elements. The mean microcyst density (MMD, cysts/mm^2) and area (MMA, μm^2) were calculated. Microcyst density was calculated using the analysis software of the instrument, whereas microcyst area using ImageJ. The AS-OCT and IVCM operators were masked for the patient surgical history and status.

MIHSA, MMD and MMA at day 30, were the primary outcomes; the IOP reduction was the secondary outcome. The relations between MIHSA, MMD, and MMA and IOP at month one were investigated.

Statistical analysis

Analysis was performed using SPSS V.21.0 Software (SPSS Inc.; Chicago, Illinois, USA). Student t and χ^2 tests were used to evaluate age, IOP, gender differences, where appropriate, between Groups, and Groups and controls. All data are reported as the mean ± standard deviation (SD) or frequency and percentage, as appropriate. Differences were considered statistically significant if the p-value was less than 0.05. One-Way ANOVA with Dunnett's correction was used to evaluate the differences in MIHSA, MMD, MMA, and IOP

between Groups, and Groups and controls. Spearman's Correlation Analysis was used to investigate the relations between MIHSA, MMD, and MMA and IOP.

Results

No significant differences were found between Group 1 and 2 for demographic and clinical data (Table 1). Thirty patients (68 %) were insonified with a 12 mm diameter probe, whereas twelve (28 %) and two (4 %) with a 13 and 11 mm probe, respectively. Twenty-four patients were treated with the 4-second dose (Group 1) and twenty with the 6-second dose (Group 2). UCCC was completed in all patients and none of them received a second treatment during the study period.

Baseline IOP was not significantly different between Groups; at day one, IOP significantly reduced in Group 1, and at day seven in both Groups ($p<0.001$). At month one, IOP significantly reduced in both Groups ($p<0.001$), with Group 2 showing values significantly lower compared to Group 1 ($p=0.001$); in controls, IOP and mean number of medications did not significantly change compared to baseline (Table 2). The overall success rate of UCCC was 63.63% (28/44), with a higher rate in Group 2 (80%; 16/20) compared to Group 1 (41.66%; 10/24) respectively ($p<0.05$)). All successful cases were classified as qualified success, and the number of medication significantly reduced compared to baseline in both groups ($p<0.05$) (Table 2).

Overall, the procedure was safe without significant differences between groups for all reported complications (Table 3). Group 1 and 2 did not show significant baseline differences in MD (dB±SD) (-12.34±1.48, and -13.88±1.64, respectively) and BCVA (log MAR±SD) (0.92±0.15, and 0.93±0.18). MD and BCVA did not significantly change at month 1 (-12.84±1.74 and -13.61±1.54; 0.90±0.16 and 0.91±0.17). During the follow-up period none of the patients received systemic or topical therapy.

AS-OCT (Sclera)

Baseline

In all patients the sclera presented a relatively homogeneous and hyperreflective aspect with scattered intra-stromal HS (Fig. 2, A and C; Fig. 3, A). MIHSA was not significantly different between Group 1 and 2, and between Groups and controls (Table 4).

One month

MIHSA significantly increased in both groups ($p<0.05$) with values higher in Group 2 compared to Group 1 ($p<0.05$) (Table 4). Intra-scleral spaces appeared hyporeflective compared to the surrounding stroma, with different size and features. They were generally located in the middle and inner layers of the stroma, frequently surrounded by additional smaller spaces (Fig. 2, B and D). In one case, a deep hyporeflective area was identified in the supra-choroidal space (Fig. 4). In controls, MISHA did not significantly change compared to baseline (Table 4) (Fig. 3, B).

IVCM (Conjunctiva)

Baseline

Epithelial microcysts were found in all patients, with MMD and MMA not significantly different between Group 1 and 2, and between Groups and controls ($p>0.05$) (Table 4) (Figs. 5 and 6, A).

One month

MMD and MMA significantly increased in both groups ($p<0.05$) with values higher in Group 2 ($p<0.05$) (Figs. 5 and 6, B); in controls, MMD and MMA did not significantly differ compared to baseline (Table 4).

A strong significant correlation was found between MIHSA ($p=0.001$; $r=-0.967$) and IOP, in both groups.

Discussion

UCCC is a cyclo-destructive procedure, which reduces the AH inflow by inducing a thermal necrosis of the ciliary epithelium.[16] Additionally, a stimulation of the suprachoroidal and TS portions of the uveo-scleral outflow pathway has been also documented.[13-17]

One month after UCCC we observed a marked increase of the intra-scleral HS, which strongly correlated with the IOP reduction.

We hypothesize that the increase of HS was consequence of a scleral fiber delamination: in fact, a partial heating of supra-choroidal space, sclera, and conjunctiva during procedure is hypothesizable. The preliminary results of a thermal infrared imaging study (our unpublished data, 2015) supported this supposition: we observed a significant increase of the ocular surface temperature at the site of insonification, immediately after UCCC. Thus, the transducer produces a thermic halo (which is 1.89 mm^3) with a temperature gradient from the ciliary body to the outer tissues. This mechanism may also account for the higher MIHSA increase in Group 2, which received a prolonged duration of the insonification.

The increase of HS within the stroma may finally increase the hydraulic conductivity and the TS AH flow. This was stated by Denis and coworkers,[14] who proposed that the exposure to ultrasound leads microarchitecture changes in the uveo-scleral tract.

The effects of ultrasounds on the sclera have been reported also *in vitro,* where the insonification of scleral samples induced a significant enhancement of the drug permeability (Investigation on cavitation contribution in scleral permeability enhancement. 13th International symposium of therapeutic ultrasounds; may 12-15, 2013, Shangai, Cina). By using electron microscopy, the authors observed less organized and loosely arranged tissues after insonification.

Also histology documented areas of scleral thinning and intra-stromal spaces within the thinned treated sclera after UCCC (Eye Tech Care material). The HS seen with AS-OCT were similar to intra-stromal spaces observed in histology. Though the tissue dehydration

for the histological preparation could have affected the scleral architecture, the greater concentration of optically clear spaces at the site of insonification compared to the surrounding sclera, confirmed a scleral effect of the procedure.

The scleral thinning represents an effective procedure to enhance the TS AH outflow. Olsen et al. found that the surgical thinning of the sclera induced a disorganization of fiber bundles with widely spaced collagen fibers.[20] Despite surgery and insonification thin the sclera differently,[14] scleral thinning positively affect the TS fluid movement. Though we did not observe scleral thinning, because AS-OCT cannot clearly show this modification, it is likely that such a change was present.

The MMD and MMA increase confirmed the nature of AS-OCT findings since microcysts were proposed as signs of increased AH percolation through the sclera. Microcysts were firstly described in the bleb-wall epithelium of functioning trabeculectomy, as indicator of trans-conjunctival AH percolation.[21,22] Afterwards, they were documented also in the conjunctiva of medically treated glaucomatous patients, as hallmark of TS AH outflow. [23,24] In our study, the microcyst increase could derive from an increased AH flow due to intra-scleral spaces and scleral thinning. This hypothesis was consistent with the microcyst increase observed after glaucoma surgeries producing scleral thinning, such as supra-choroidal shunt implantation and canaloplasty.[19,25]

Though we did not investigate the suprachoroid, since AS-OCT cannot easily image this site, in one case we observed HS in the suprachoroidal region. This aspect was consistent with an UBM study, which documented suprachoroidal spaces in more than 60% of UCCC.[13]

In our study UCCC had an overall success rate of 63.6% at 1 month, in line with the 66.7% reported by Denis et al.[14] Also the higher IOP reduction in Group 2 was consistent with literature,[13,14] that reported values ranging from 22.8 to 26.4% in the 4-second dose regimen, and from 28.2 to 38.2% in the 6-second dose.

The present study has some limitations. First, 1-month findings could not represent the effect of the procedure for longer periods. However, this is the first report of a long-term prospective study. In the three and six month follow-ups, intra-scleral hyporeflective areas persisted in all successful cases, though reduced when compared to month 1. This confirms the importance of scleral changes in the final success of the procedure. ~~First~~Second, our results do not permit to ascertain the impact of the TS outflow in the final IOP reduction. Nevertheless, the strong correlation between MIHSA and IOP indicated that scleral modifications play a crucial role. ~~Second~~Third, AS-OCT doesn't allow studying ciliary body: thus, we cannot clarify whether successful and failed cases derived from more or less effective ciliary body coagulation. ~~Third~~Finally, since microcysts can be found also in healthy subjects and in some ocular surface diseases, their significance should be clarified. However, several studies indicated that in glaucoma they are hallmark of AH outflow.[19,22-25]

In summary, though the main mechanism for the IOP reduction after UCCC is a decrease of the AH inflow, the increase of the TS AH outflow seem to play a critical role in the final success. Further studies measuring the TS outflow with more accurate methodologies are mandatory to confirm our preliminary data. In addition, probe modification allowing a selective scleral insonification should be considered in the next future.

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Tables

Table 1. Demographics and clinical characteristics of the patients

	Group 1	Group 2	Controls
Patients	24	20	10
Age (Years±SD)	68.74±5.43	66.55±6.83	64.9±5.12
Gender (M/F)	11/13	10/10	6/4
Glaucoma Subtype			
POAG	14	12	10
PXG	5	5	-
PG	2	2	-
Uveitic glaucoma	1	0	-
Post-surgical glaucoma	2	1	-
Hypotensive Medications			
β-blockers	23	18	9
PGA	23	19	7
Topic CAI	23	15	2
α-agonists	20	14	3
Systemic CAI	15	16	0

Filtration surgeries (mean±SD)	1.94±0.85	2.12±0.78	NA

Group 1 = 4 seconds exposure
Group 2 = 6 seconds exposure
M = male
F = female
SD = standard deviation
CAI = carbonic anhydrase inhibitors
POAG = primary open angle glaucoma
PXG = pseudo-exfoliative glaucoma
PG = pigmentary glaucoma
PGA = prostaglandin analogs
NA = not applicable

P=NS for all parameters

Table 2. IOP and hypotensive medications

GROUPS	PARAMETERS	TIME POINTS			
		Baseline	**Day 1**	**Day 7**	**Day 30**
Group 1	**Mean IOP** (mmHg±SD)	26.91±2.82 *	19.66±5.95	15.91±5.26	18.83±3.22
	IOP reduction (%)	-	26.72	40.70	30.11
	N° of medications	2.91±0.93	-	-	2.65±1.25 †
Group 2	**Mean IOP** (mmHg±SD)	27.55±4.07 *	23.45±5.32 ‡	17.70±5.16	17.15±2.72 ‡
	IOP reduction (%)	-	15.28 ‡	36.71	38.76 ‡
	N° of medications (mean±SD)	2.84±1.03	-	-	2.25±1.25 †
Controls	**Mean IOP** (mmHg±SD)	17.14±1.98 §	-	-	17.65±1.39 ‖

SD = standard devation

IOP = intraocular pressure

* p<0.001 vs day 1, 7 and 30 for Group 1, and vs day 7 and 30 for Group 2
† p<0.05 vs baseline
‡ p=0.001 vs Group 1
§ p<0.001 vs Group 1 and 2
|| p<0.05 vs Group 1

Table 3. UCCC related complications

	Group 1 (n=24)	Group 2 (n=20)
Conjunctival hyperemia	18	14
Sub-conjunctival hemorrhage	1	0
Punctuated epithelial keratitis	3	3
Anterior chamber inflammation	4	8
Transient hypotony	1	1
Transient hypertony	5	4
Phthisis bulbi	0	0
Anesthesia related complications	0	1

P = NS for all complications

Table 4. AS-OCT and IVCM parameters

	Group 1			Group 2			Controls		
	AS-OCT	**IVCM**		**AS-OCT**	**IVCM**		**AS-OCT**	**IVCM**	
	MIHSA (mm^2)	MMD (cysts/mm^2)	MMA (μm^2)	MIHSA (mm^2)	MMD (cysts/mm^2)	MMA (μm^2)	MIHSA (mm^2)	MMD (cysts/mm^2)	MMA (μm^2)
Baseline	0.055±0.022	9.916±4.333	1698.98±1007.511	0.054±0.037	10.545±5.763	1830.58±1132.380	0.049±0.013	10.041±3.827	1737.651±815.566
Day 30	0.102±0.044 *‡	14.375±5.173 *‡	2961.78±1256.151*‡	0.182±0.112*†‡	18.000±9.990 *†‡	3290.41±1728.301*†‡	0.050±0.010	10.151±3.747	1686.605±763.431

AS-OCT = anterior segment-optical coherence tomography
IVCM = *in vivo* confocal microscopy
MIHSA = mean intra-scleral hypo-reflective space area
MMD = mean microcysts density
MMA = mean microcysts area

* $p<0.05$ vs baseline
† $p<0.05$ vs Group 1
‡ $p<0.001$ vs controls

Figure Legends

Figure 1. Representative AS-OCT image of a patient who underwent 4-second UCCC, showing intra-stromal spaces with different reflectivity. Triple black asterisks indicate the three stromal regions (in the middle-outer scleral layers) selected to calculate the mean reflectivity of the normal sclera (mean value of 208.250, Image J). Single and double white asterisks indicate well-defined hyporeflective spaces with a very low, and low value of mean reflectivity, respectively (34.851 and 67.355, Image J). The symbol of infinity indicates an hyporeflective area without well-defined edges, which was not considered for the analysis. The right side of the image represents the limbal side.

Figure 2. A and B: Group 1. A. Baseline AS-OCT of the superior-temporal sclera in a glaucomatous patient scheduled to undergo 4-second dose UCCC. Linear or oval shaped hyporeflective spaces are evident in the middle stromal layers (arrowheads). **B.** Linear shaped hyporeflective spaces increased thirty days after UCCC in the middle-inner stroma, with widening of pre-existing spaces, and formation of new spaces (arrow). **C and D: Group 2. C.** Baseline AS-OCT of the superior-temporal sclera in a glaucomatous patient scheduled to undergo 6-second dose UCCC; sclera presented features quite similar to those observed in Group 1. **D.** Thirty days after UCCC, hyporeflective spaces markedly increased in the middle-inner stromal layers, with features of scleral delamination (arrow). The right side of each scan represents the limbal side.

Figure 3. AS-OCT of the superior temporal sclera in a control glaucomatous patient at baseline **(A)** and thirty days after UCCC **(B)**. Intra-sclera hyporeflective spaces presented features similar to those observed in patients scheduled to UCCC, and did not show

significant modifications at follow-up. The right side of each scan represents the limbal side.

Figure 4. Linear shaped suprachoroidal hyporeflective spaces (arrowheads) were observed thirty days after UCCC in a patient who underwent the 6-second dose regimen. The asterisk indicates the inner scleral edge. The right side of the image represents the limbal side.

Figure 5. In vivo confocal microscopy of the superior temporal conjunctiva in the same patient scheduled to undergo 4-second dose UCCC (Group 1). **A.** The baseline planar reconstruction shows small roundish microcysts, located at different level within the epithelium, scattered, and sometimes clustered (arrowhead). **B.** Microcysts increased density and area (arrow) thirty days after insonification. Bar represents 100 μm.

Figure 6. In vivo confocal microscopy of the superior temporal conjunctiva in the same patient scheduled to undergo 6-second dose UCCC. **A.** The baseline planar reconstruction shows features similar to those observed in Group 1. Somewhere, microcysts appear encapsulated (arrowhead) and filled with amorphous material or punctate reflective elements (asterisk). **B.** Epithelial microcysts increased density and, especially, area (arrow) thirty days after UCCC. Microcysts may appear filled with amorphous material (black arrowhead) or reflective elements, probably representing inflammatory cells (arrows). Bar represents 100 μm.