Real-Time Monitoring of Suprachoroidal Space (SCS) Following SCS Injection Using Ultra-High Resolution Optical Coherence Tomography in Guinea Pig Eyes

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PURPOSE. To monitor the change of suprachoroidal space (SCS) using ultra high resolution-optical coherence tomography (UHR-OCT) following SCS injection with different drug formulations.

METHODS. An amount of 10 or 20 μL of saline or indocyanine green (ICG) or triamcinolone acetonide (TA) suspension (40 or 80 mg/mL) was injected suprachoroidally into the guinea pig eye with a 30-gauge needle. Immediately after injection, the eyes were imaged by UHR-OCT from 60 minutes up to 24 hours. At each time point, the SCS area on each OCT cross-section was measured in pixels with Image J and the area change from the baselines was analyzed over time.

RESULTS. A 20-μL injection produced 130% to 200% SCS expansion compared to a 10-μL injection for saline and TA suspension (P < 0.01). After SCS injection, the time that expansion persisted was formulation dependent. Thus, expansion in response to injection of TA suspension, ICG, and saline persisted for 24 hours, 180 minutes, and 60 minutes, respectively. Moreover, ICG injection produced a significantly larger area of distribution in the SCS than the TA suspension (0.626 vs. 0.275 cm², P < 0.0001).

CONCLUSIONS. The SCS is expandable and can recover to preinjection status after injected fluid is cleared by physiological processes. The injection-induced SCS expansion is volume-dependent, and the drug/dye retained in the SCS is formulation-dependent. The current injection technique with a volume of 20 μL or less is well tolerated in guinea pig eyes.

Keywords: suprachoroidal injection, suprachoroidal space, ultraresolution OCT, triamcinolone acetonide, ICG

Posterter segment diseases of the eye are sight threatening and hard to treat due to their chronic and relapsing nature. These blinding chorioretinal diseases are a worldwide epidemic, and pose challenges to medical society and health providers. One of the challenges is the lack of a safe and reliable system to deliver and keep the therapeutics at the disease site. Presently, the most effective drug delivery method for the retina is the intravitreal delivery route. However, most therapeutics have a very short vitreous half-life and frequent injections are inevitable.1 Even large molecule drugs, such as recently Food and Drug Administration (FDA) approved antiangiogenesis monoclonal antibodies and peptides, mandate 6 to 12 intravitreal injections per year.2-4 Frequent intravitreal injections increase the risk of complications, such as vitreous hemorrhage, retinal detachment, and endophthalmitis. Frequent injections also negatively affect the patients’ quality of life.

Compared to intravitreal injection, subtenon injection is a safer alternative for the treatment of posterior segment eye diseases. The retina is made of fragile, nonregenerative sensory neurons that are prone to damage from intravitreal drug aggregates5,6 or localized high drug concentrations.7,8 Similarly, the vitreous is a delicate gel that is free of blood vessels, prone to harboring bacteria,9 and its clarity is easily disturbed by the drug or delivery system injected.10 In contrast to the vitreous and retina, the sclera consists of tightly packed collagen and its permeability does not alter by aging, cryotherapy, and laser treatment.11 Sclera also is well known for its durability from surgical manipulation as seen in scleral buckling for retinal detachment repair. However, due to the scleral barrier and drug loss to conjunctival and episcleral circulation of blood and lymphatics, subtenon drug delivery is less effective than intravitreal injection.12,13 Recently, suprachoroidal space (SCS) drug delivery has emerged as a promising ocular drug delivery route for posterior segment eye diseases.14-18 Suprachoroidal drug delivery bypasses the sclera and is more efficient than transscleral delivery in moving therapeutics to the retina. The suprachoroidal space is virtual and becomes a real space following the injection of a drug solution. Olsen et al.14 reported that suprachoroidal cannulation in pig eyes was safe and reproducible, though some adverse effects from the surgical manipulation were seen in an early study using monkey eyes. Later, Patel et al.19 demonstrated suprachoroidal injection using a custom made hollow microneedle on
enucleated eye globes of several species. With the hollow microneedle, up to 35 μL of suspension was delivered consistently. However, spatiotemporal change of the SCS in a living eye following a suprachoroidal injection has not yet been reported. In addition, ocular safety data associated with suprachoroidal injection are scarce, especially long-term data. The current study was undertaken to evaluate suprachoroidal space change over time following a single SCS injection using a custom-designed optical coherence tomography (OCT). This custom-built ultra-high resolution OCT instrument (UHR-OCT) with an axial resolution of approximately 3 μm or less, has the ability to image retina, choroid, and even sclera. In the current study, the guinea pig eye model was elected because the guinea pig has a lens-to-vitreous ratio close to the human eye, and a thin sclera for easy observation of the suprachoroidal space.

**Materials and Methods**

**Study Design**

We hypothesize that the injection-induced change of the SCS may be different between the injection of a solution and the injection of a suspension. We also hypothesize that physiochemical properties of a solution may have a role in the injection-induced SCS change. Therefore, we used indocyanine green (ICG) and saline as solution models and triamcinolone acetonide (TA) as a suspension model. Indocyanine green is poorly absorbable in mammalian biological systems and is useful in imaging due to its easy visualization. For the injection volume determination, we used the intravitreal injection volume for rabbit eye as a reference in which 50 μL can be safely administered without paracentesis. The vitreous volume of guinea pig is approximately 300 μL, which is 1/5 of rabbit vitreous volume (1500 μL). Accordingly, 10 μL was used as a starting volume for suprachoroidal injection in guinea pig eyes. To evaluate if a larger volume is feasible, an additional 5 μL was added to each subsequent escalation until we saw backflow from the injection site.

**Suprachoroidal Injection Procedure.** Handling of animals was in accordance with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Visual Research. The body weights of the guinea pigs were between 500 and 600 g. All guinea pigs were anesthetized by an intraperitoneal injection of xylazine (4 mg/kg) and 2% pentobarbital sodium (20 mg/kg). The 0.5% proparacaine ophthalmic drops (Alcon, Fort Worth, TX, USA) were used as topical anesthetic and pupils were dilated with 0.5% tropicamide before anesthesia. Under the direct view of a surgical microscope (F18; Leica, Wetzlar, Germany), a superonasal conjunctival peritomy was performed with a radial cut posteriorly along the superior rectus muscle to expose the sclera. A 30-gauge hypodermic needle was fitted to a 6-inch extension tubing (Extension set 15.2 cm ID 0.8 mm OD 1.6 mm; EAGLE LABS; Rancho Cucamonga, CA, USA) which then was connected to a 250 μL Hamilton syringe. The Hamilton syringe was loaded into a syringe dispenser (PB-600-1 Repeating Dispenser; Hamilton Company, Reno, NV, USA) with which each actuation delivers 1/50 of the total capacity of that syringe. The injection was made 5 to 6 mm behind the limbus at the globe meridian of 1 o’clock for the right eye and 11 o’clock for the left eye. With the needle bevel facing the scleral surface at a 15° angle, the needle was inserted into the suprachoroidal space. After the injection, the needle was kept in place for approximately 30 seconds before removing. After the procedure, the conjunctiva and Tenon were restored by an 80 suture and ofloxacin eye ointment was applied after the last examination.

**IOP Measurement**

The IOP was obtained by an ophthalmic tonometer (Tono-Pen; AVIA, Reichert Technologies, Depew, NY, USA) before and after the SCS injection. The IOP measurement was stopped when IOP returned to the baseline level.

**Quantitating Surface Area of Suprachoroidal ICG and TA**

Due to the thin sclera of guinea pig eyes, the area of ICG and TA diffusion within the suprachoroidal space after injection was measurable through the sclera. For area quantitation, ICG or TA was injected temporally 3 mm away from the limbus for the purpose of easy observation. Immediately after each injection, the globe was enucleated and a photograph, including a ruler, was acquired through a surgical microscopic imaging system. Image J software (version 1.45, the National Institutes of Health [NIH], Bethesda, MD, USA, available in the public domain at http://rsbweb.nih.gov/ij/download.html) was used to measure the green area (for ICG) or white area (for TA). If one image could not cover the whole area, a second image was taken for relaying the measurement. The highlighted “region” was returned in pixels using the “measure” function.

**Electroretinography (ERG) Examination.** Scotopic and photopic ERG was performed at baseline and 21 days after injection. Pups were diluted with 0.5% tropicamide and phenylephrine eye drops, then dark-adapted for 30 minutes before the scotopic ERGs. After the scotopic ERGs recording, 10 minutes of light adaptation was performed before single-flash then 30-Hz flash ERG recordings. The ERGs were recorded with a commercial system (Ganzfeld Q422C; Roland Consult, Brandenburg, Germany) as described previously.

**Real-Time UHR-OCT Imaging.** The SCS OCT imaging was performed with UHR-OCT configured as shown in the Table. This UHR-OCT uses a superluminescent diode (SLD; T840; SuperLum Diodes Ltd., Moscow, Russia) and was adapted onto a slit-lamp system with the installation of an 90 diopter (D) ocular lens (Volk Optical, Inc., Mentor, OH, USA) on the sample arm for guinea pig eye imaging.

The guinea pig eyes were imaged before and after SCS injection at time points of 5, 30, and 60 minutes for saline injected eyes; 5, 30, 60, and 180 minutes for ICG-injected eyes; and 5 and 30 minutes, and 1, 3, 6, and 24 hours for TA-injected eyes. To keep the eye oriented consistently at each imaging session, the guinea pigs were fixed in a custom-built restraint.

**Table. Parameters Set-Up of UHR-OCT for Guinea Pig Eye Imaging**

<table>
<thead>
<tr>
<th>Technical Details</th>
<th>Axial Resolution</th>
<th>Scan Speed</th>
<th>Center Wave Length</th>
<th>Band Width</th>
<th>Scan Width</th>
<th>Image Size of Each B-Scan</th>
</tr>
</thead>
<tbody>
<tr>
<td>UHR-OCT</td>
<td>3 μm</td>
<td>12,000 Scans/s</td>
<td>840 nm</td>
<td>100 nm</td>
<td>18 mm</td>
<td>1365 pixel (depth) × 2048 pixel (width)</td>
</tr>
</tbody>
</table>
system that limited head movement and exposed the eye toward the imaging axis. The restraint system was placed on a supporting platform during the imaging. For OCT imaging, the OCT system first identifies the optic nerve head on the computer screen through infrared light illumination then scanning starts from top to bottom within a 18 × 18 mm area (total of 32 cross-sections) along the y-axis and then from right to left along the x-axis. Therefore, there is 0.58-mm spacing between neighboring sections. For the image analysis of SCS area, six sections crossing the optic nerve head, three along the x-axis and three along the y-axis, were used to measure the area between the RPE line and the junction line of choroid and sclera. The assumption is that SCS space, either virtual before the injection or real after the injection, lies between those two lines that are well defined on the OCT images. The area between the RPE line and the junction line of choroid and sclera was measured on each cross-section using Image J. The paradigm of area measuring is shown in Figure 1.

During the measuring, the Image J “freehand drawing” tool was used to trace the RPE line starting from the optic nerve, 10 retina-thicknesses peripherally, then back toward the optic nerve along the junction of choroid and sclera, closing the tracing at optic nerve (Fig. 1). The highlighted “region” was returned in pixels using the “measure” function. For OCT images, there are 2048 pixels horizontally in 18 mm and vertically 1365 pixels in 18 mm. Two areas, a nasal area and temporal area on a section acquired along the y-axis, or superior area and inferior area on a section acquired along the x-axis, were measured on each section and the sum is the total area for the section. The same measuring paradigm was used for all identified sections at all time points.

**Statistical Analysis.** The measured areas reflecting SCS changes from the baseline were represented by number of pixels and the numbers were compared among the anatomical locations in relation to the injection site or between different injection volumes. Comparisons were performed using non-parametric statistical analysis or multivariate linear regression according to the analytical purpose and distribution of data. All statistical analyses were handled using JMP Pro 11 software (SAS Institute, Inc, Cary, NC, USA). A P value smaller than 0.05 was considered statistically significant.

**Figure 1.** The OCT image (A) demonstrates the SCS measuring paradigm. The SCS was measured from a to b and from c to d between the RPE (green arrows) and the junction of choroid and sclera (red arrows). The distance a–b = c–d = 10 RT (thickness of the retina). The OCT image (B) demonstrates the area being measured (outlined in yellow).

**Figure 2.** The OCT horizontal cross-sections from a left eye, taken at different time points (before injection [A], 5 min after injection [B], 30 min after injection [C], and 60 min after injection [D]) following a 10-μL saline SCS injection. The right side of each cross-section corresponds to the nasal side of the eye globe.
RESULTS

Osmolality Measurement

We have previously reported saturated TA solution (without TA particles) to be 307 mOsm/kg and concurrently measured osmolality for BSS was 313 mOsm/kg. For osmolality of saline and ICG, 20 μL test solution was added into the measuring tube of a Fiske Micro-Osmometer (Model 210; Fiske Associates, Norwood, MA, USA). Saline and ICG were measured three times. The readings for saline were 303, 301, and 300 mOsm/kg and for ICG were 17, 15, and 19 mOsm/kg.

Saline SCS Injection

Two volumes of saline injection, 10 and 20 μL, were tested and six eyes were used for each volume. For 20 μL, variable degrees of backflow were observed around 15 to 20 μL (third and fourth actuation delivery with 250 μL Hamilton syringe on a syringe dispenser). The 10 μL SCS injection did not cause backflow. The SCS morphological change over time after 10 and 20 μL injection was shown in Figures 2 and 3. The space (area) between the RPE and the junction line of the choroid and sclera increases after injection from frames A to B then the space returned back to preinjection level within 60 minutes.

Figure 3. The OCT horizontal cross-sections from a left eye, taken at different time points (before injection [A], 5 min after injection [B], 30 min after injection [C], and 60 min after injection [D]) following a 20-μL saline SCS injection. The right side of each cross-section corresponds to the nasal side of the eye globe.

Figure 4. The area (between RPE and the junction line of choroid and sclera) change from baseline following SCS saline injection of 10 μL (blue line) and 20 μL (red line). Immediately after injection, the SCS space showed acute expansion then returned to baseline over a period of 60 min. The significant recovery was within the first 30 minutes. The SCS expansion was injection-volume dependent. Error bars: 1 SEM.
shown in frames C and D. Comparing Figures 2 to 3, the 20-μL injection caused wider SCS space expansion.

The saline-induced SCS change in relation to the globe topography and injection site (5–6 mm behind the limbus at the globe meridian of 1 o'clock for the right eye or 11 o'clock for the left eye) is demonstrated in Figure 4. Since the injection site was always supranasal, SCS expansion was significantly larger nasally than temporally \((P = 0.0003, \text{nonparametric for each pair using Wilcoxon})\); and similarly larger superiorly than inferiorly \((P < 0.0033, \text{Nonparametric for each pair using Wilcoxon})\). The injected saline did cross horizontal midlines, reaching the inferior half of the globes (Fig. 4). In relation to the vertical midline, the saline mostly diffused within the nasal SCS although some crossed the vertical midline into the temporal half of the globe (Fig. 4). In addition, SCS expansion was significantly greater following a 20-μL injection than that from a 10-μL injection (Fig. 5, mean peak change of area pixel \(= 75,850 \pm 48,709 \text{ vs. } 51,102 \pm 32,049, P = 0.0116, \text{nonparametric for each pair using Wilcoxon}; \) the area under time-SCS change curve for the 10 μL was 1,387,313.8 vs. 2,033,637.9 for the 20 μL).

**ICG SCS Injection**

The ICG (Akron, Inc., Decatur, IL, USA) was prepared by dissolving the dye in distilled water to a concentration of 2.5 mg/mL. A total of 20 μL of ICG was injected into the SCS of 6 eyes of 3 guinea pigs. Only 20 μL volume was tested. No

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**Figure 5.** Injection-induced SCS area change by 10 (blue) and 20 (red) μL. Error bars: 1 SEM.

**Figure 6.** The OCT horizontal cross-sections from a right eye, taken at different time points (before injection [A], 5 min after injection [B], 30 min after injection [C], and 60 min after injection [D]) following 20 μL of ICG SCS injection. Left side of each section corresponds to the nasal side of the globe. Immediately after ICG SCS injection (B), sclera (white band) seen in (A) became invisible in (B). (C) Sclera images recovered to a very limited extent (arrow). (D) A thin layer of dark area was appreciable between choroid (white speckled area) and sclera (light gray band), indicating remaining ICG in the suprachoroidal space. This was not appreciable in (B, C) due to lack of contrast from the sclera.
backflow was noted during the injection. The injection sites
and methods were the same as the saline study described
above. The SCS morphology change over time following ICG
injection is demonstrated in Figure 6. Surprisingly, the sclera
layer became invisible in the OCT images after ICG injection
(Fig. 6B). The sclera image gradually became visible again over
time but did not return to preinjection level after 180 minutes
(Fig. 6D).

Compared to the 20 µL saline SCS injection, ICG-induced
SCS changes lasted much longer (Fig. 7). The pixel area
induced by ICG injection at 60 minutes was 40,331 ± 15,025
pixels versus 15,234 ± 16,278 pixels for saline ($P < 0.0001$,
Wilcoxon test).

**TA SCS Injection.** Transton (Kunming Jida Pharmaceutical
Co., Kunming, China) was used for SCS injection. Three
formulations (volumes/doses) were each tested on 6 Guinea
pig eyes. The first formulation was 10 µL of 40 mg/mL TA
suspension; the second was 20 µL of 40 mg/mL TA suspension;
and the third was 20 µL of 80 mg/mL TA suspension. The
concentrated TA suspension was made by removing 0.5 mL of
the supernatant after centrifuging the commercial TA vial for 5
minutes at 3225.6 g. The injection paradigm was the same as
described for saline and ICG. The injection-induced SCS
changes in pixel area measured from OCT images over time
is presented in Figure 8. The TA suspension was observed for
more time points; hence, time was taken into consideration
when performing analysis. Net SCS area changes were ranked
and the ranks were used for regression analysis. Higher
volumes did cause significantly more SCS expansion (mean
area change for 10 µL was 21,450 vs. 27,509 pixels for 20 µL/
40 mg/mL and 29,946 pixels for 20 µL/80 mg/mL ($P = 0.0004$,
multivariate linear regression). As expected, time is a
significant covariate ($b = -0.16$, $P < 0.0001$). The two 20-µL
formulations did not significantly differ (Fig. 8). By calculating
the area under time-SCS change curve, the mean area for the
formulation of 10 µL/40 mg/mL was 20,693,096, while for the

**Figure 7.** A 20-µL SCS injection induced SCS change from the baseline (red line for saline and blue line for ICG). The ICG induced SCS change lasted much longer than that from saline injection. Error bars: 1 SEM.

**Figure 8.** The measured SCS area change over time for three injection formulations. The 10-µL injection-induced SCS area change was significantly lower than the two 20-µL formulations. The concentrated 20-µL formulation showed slower SCS recovery. Error bars: 1 SEM.
formulation of 20 μL/40 mg/mL it was 27,228,466, and for the formulation of 20 μL/80 mg/mL it was 42,979,935. The SCS morphology change over time following a 10-μL (40 mg/mL) and 20-μL (80 mg/mL) injection is presented in Figures 9 and 10.

Surface Area of Suprachoroidal ICG and TA

The ICG was chosen for its easy visualization through the sclera and was prepared by dissolving the dye in distilled water to a concentration of 2.5 mg/ml. A total of 20 μL of ICG was injected into the SCS of 10 eyes of five guinea pigs. Both eyes of a rabbit were used to increase sample size and reduce use of animals. For SCS TA, 20 μL of 40 mg/mL TA suspension was injected into the SCS of 12 eyes of six guinea pigs using the exact same methodology. Immediately after the SCS injection, the animal was killed and the globe was enucleated. The blue or white area was photographed and the area was measured using Image J.

The mean measured area for SCS ICG and SCS TA was 0.626 and 0.275 cm², respectively. The ICG had a significantly larger area of distribution in SCS than TA (P < 0.0001, Student's t-test, Fig. 11).

Ocular Safety Profile of SCS TA Injection. After suprachoroidal injection of TA suspension, with either formulation, no circulating aqueous humor cells were noted under slit-lamp biomicroscopy. With indirect ophthalmoscopy and fundus photography, we found that suprachoroidally injected TA was recognizable from fundus examination (Fig. 12). No subretinal or choroidal hemorrhage was noted. However, immediately after the injection the choroidal vessels located in the injected area showed variable dilation and hyperemia, which disappeared within 24 hours confirmed by indirect ophthalmoscopy.

![Figure 9](image1.png)

**Figure 9.** The OCT horizontal cross-sections from a left eye, taken at different time points (before injection [A], 5 min after injection [B], 30 min after injection [C], and 60 min after injection [D]) following a 10 μL SCS injection (TA 40 mg/mL). The right side of each cross-section corresponds to the nasal side of the globe.

![Figure 10](image2.png)

**Figure 10.** The OCT horizontal cross-sections from a left eye, taken at different time points (before injection [A], 5 min after injection [B], 30 min after injection [C], and 60 min after injection [D]) following a 20 μL SCS injection (TA 80 mg/mL). The right side of each cross-section corresponds to the nasal side of the globe.
Following suprachoroidal injection, IOP was highly variable as shown in Figure 13. The IOP rose immediately following the injection and returned to baseline approximately 15 minutes later. Analysis of IOP among the three formulations did not reveal significant difference.

The ERGs were performed at week 8 following SCS TA injection on 10 eyes injected with 20 \( \mu \)L concentrated TA (80 mg/mL). We used 10 eyes without any injection as control. The b-wave amplitudes were tabulated and compared between the two groups within each type of ERG. No significant difference was found in either type of ERG (Fig. 14, \( P > 0.05, t \)-test).

**DISCUSSION**

Suprachoroidal injection is potentially a better drug delivery route than subtenon or subconjunctival injections because it bypasses the barrier of the sclera. It may be safer than intravitreal injection, which poses risks for opportunistic infection of vitreous or risks for lens and retina damage.26 The current study demonstrated that a 20-\( \mu \)L suprachoroidal injection of TA suspension, ICG, or 10 \( \mu \)L saline is feasible without short- or long-term ocular toxicity. Although IOP can spike immediately after injection, IOP returns to baseline within approximately 15 minutes. In the human eye, a similar IOP spike is seen following a 50-\( \mu \)L intravitreal injection of anti-VEGF agents or TA. In this case, the IOP spikes then drops below 30 mm Hg in 15 minutes27 and back to baseline within 30 minutes.28 Similarly, a 50-\( \mu \)L TA suprachoroidal injection in the rabbit eye caused a spike in IOP that returned to normal within 15 minutes.18 The IOP spike in the human eye may take a bit longer to return to baseline due to a thicker sclera. In the current study, such a short IOP spike seemed to be harmless to the guinea pig eye from the clinical observation and ERG examinations. No retinal toxicity was revealed in either type of ERG modality during 8 weeks of follow-up.

Upon injection in the guinea pig eyes, suprachoroidal TA was viewable from fundus examination and the choroidal vessels running over the TA had a short period of hyperemia and dilation. However, these changes disappeared within 24 hours and no adverse effects remained on retinal function.
study used a 30-gauge needle and a quantitatively controlled dispenser to deliver 5 μL by each actuation. This simple method did not show any mechanical injury to the retina. While suprachoroidal cannulation is more suitable for a large eye globe, such as the pig eye, cannulation itself may introduce mechanical damage. Compared to the custom-made hollow microneedle, the current technique seemed to have less backflow. We did not see any backflow from the 20-μL injection.

FIGURE 12. (A, C) Two different animal eyes illustrate choroidal vessel hyperemia and dilation immediately after SCS injection in the area of the TA. The hyperemia and dilation disappeared within 24 hours. (B) Image taken 1 week later than image in (A) from a same eye. (D) Image taken 2 weeks later from the same eye as image (C).

FIGURE 13. Real-time monitoring of IOP over time, following 20-μL SCS injection of different formulations. The IOP returned to baseline levels approximately 15 minutes after the injection. Error bars: 1 SEM.
injection of TA. This could be due to deeper entry into the suprachoroidal space than the hollow microneedle and incremental injection with each actuation, which provides time for equilibration of the injected volume in the suprachoroidal space. The very short hollow microneedle (700–800 \( \mu m \)) can mitigate possible choroidal or retina damage, but may be subject to more backflow of drug solution as shown during a 50-\( \mu L \) delivery into rabbit suprachoroidal space.\(^{17}\) The current injection technique was tested on guinea pig and rabbit eyes.\(^{18}\) In the human condition, the feasibility of this injection technique is unknown. Thus far, to our knowledge the only tested suprachoroidal injection technique in the human eye was microcatheter and no surgical complications were reported.\(^{26,30}\)

From an anatomical standpoint, suprachoroidal region represents a virtual space.\(^{31}\) It becomes a real space when fluid accumulates between the choroid and sclera as seen in choroidal detachment\(^{32}\) or suprachoroidal hemorrhage.\(^{33}\) The dynamics of SCS expansion and recovery following an SCS injection has not yet been reported. The current study used UHR-OCT that is able to show all layers of the eye wall, including the sclera. The UHR-OCT demonstrated that expansion of the SCS is directly related to the volume injected; larger volumes cause more expansion. The multiple OCT cross-sections at multiple time points clearly delineate this relationship by the pixel area change from the baseline. The SCS expansion induced by a 20-\( \mu L \) injection can be 130% to 200% larger than the expansion from a 10-\( \mu L \) injection. The current study also revealed that different drug formulations are likely to have different clearance kinetics from the SCS. A 20-\( \mu L \) injection of saline was almost cleared and the SCS returned to baseline configuration within 60 minutes. In contrast, the same volume of ICG showed less than 50% clearance at 60 minutes and there still was a substantial amount of ICG remaining in the SCS 180 minutes after the injection (Fig. 7). Compared to saline and ICG, TA suspension stayed in the SCS longest. Fluid injected into the SCS could be cleared through choroidal capillaries that have fenestrations connecting with the surrounding tissue fluid. Saline is an isotonic solution that can easily be absorbed into systemic circulation or diffuse to the episcleral surface via physiological pathways. Although ICG used in the current study has a much lower osmolality, it is more viscous than saline and is known for its poor absorption into mucous membranes. Therefore, ICG stayed longer in the SCS than saline. Compared to ICG, TA suspension stayed even longer in the SCS, especially the concentrated TA (80 mg/mL) formulation (Fig. 8). This indicates that suspension formulations may be a better option than solution formulations for SCS sustained drug delivery. The particles of a suspension cannot be cleared through diffusion or absorption before they degrade. Patel et al.\(^{17}\) demonstrated that 10-\( \mu m \) diameter particles (FluoSpheres; Invitrogen, Carlsbad, CA, USA) could be found in the choroid and SCS two months after the initial suspension injection. We also have demonstrated that a 2-mg TA suspension suprachoroidal injection in the rabbit eye can provide at least 2 months of therapeutic drug levels to the retina and the therapeutic effect sustained longer than a 20-mg TA subtenon injection.\(^{18}\) When TA suspension is injected, the fluid in the suspension travels further than the TA particles and gets absorbed quickly (Fig. 10). Therefore, when measuring the area from the scleral surface, the mean white area was significantly smaller than the blue-green area following the same 20-\( \mu L \) ICG SCS injection. Due to the thicker human sclera, TA in SCS was not viewable; however, Tetz et al.\(^{34}\) demonstrated that following suprachoroidal TA and bevacizumab the best corrected visual acuity (BCVA) remained stable over a postoperative period of 6 months in eyes previously not responsive to the intravitreal bevacizumab therapy, indicating sustained therapeutic effect from SCS TA.\(^{34}\)

The ICG has a light-absorbing spectrum of 600 to 900 nm, slightly varying depending on concentration and dilution medium.\(^{35}\) The current UHR-OCT uses a light source of 840 nm, falling in the light-absorbing range of ICG. Hence, the OCT light source was absorbed by the ICG and no deeper tissue (sclera) was visible immediately after SCS injection. Over time,
the ICG in the SCS was diluted by dynamic fluid and deeper tissues (sclera) gradually appeared in the imaging. It should be noted that this phenomena is different from uniform dark area in the retina or subretinal space seen on OCT cross-section of the human eye. The dark areas on human retina OCT are due to accumulated fluid that is uniform with no refractive index change or scattering of light when the OCT light source penetrates. In that case, the deeper ocular tissues are visible on OCT.

In summary, UHR-OCT can reveal SCS configuration changes over time following suprachoroidal injection of saline, ICG, or TA suspension. The injection-induced SCS expansion was volume-dependent; larger volumes caused further expansion. After SCS injection, the time the therapeutics remained in the SCS was formulation-dependent. Suspensions tended to stay longer in the SCS. The SCS is expandable and can recover to preinjection status after suprachoroidal fluid is cleared by physiological processes. In addition, the SCS injection technique used in the current study seems well tolerated by guinea pig eyes and no adverse effect was observed.

Acknowledgments

Supported by Grant No.31271022 from the National Natural Science Foundation of China (Haidian District, Beijing, China). The authors alone are responsible for the content and writing of the paper.

Disclosure: B. Gu, None; J. Liu, None; X. Li, None; Q. Ma, None; M. Shen, None; L. Cheng, None

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