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Four-dimensional microscope-integrated optical coherence tomography (4D MIOCT) guidance in a model eye subretinal surgery.

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Abstract

Purpose: Subretinal delivery of stem cells and gene therapy vectors hold great promise in clinical care. Delivery techniques are evolving. We investigate the utility of 4-dimensional (volumes over time) microscope-integrated optical coherence tomography (4D-MIOCT) to guide such maneuvers.

Methods: This investigational 4D-MIOCT system displayed heads-up cross-sectional and stereoscopic volumetric views through the microscope oculars at 3.3–10 volumes/second. In enucleated porcine eyes, we evaluated novel information available to the surgeon during subretinal: 1) injection of fluid and cell suspensions, 2) retinotomy creation, 3) insertion of membranes with a retinal pigment epithelium (RPE) cell monolayer and 4) subretinal instrument maneuvers, all in postmortem porcine eyes.

Results: Interactions between tools, membranes, retina and RPE were often not detectable on the conventional microscope view but were obvious with 4D-MIOCT. 4D-MIOCT visualization was essential to: monitor the extent of induced retinal detachment, guide the cannula tip and instrument maneuvers in the subretinal space, and identify efflux of cells into the vitreous cavity. This allowed adjustment by the surgeon to avoid complications—e.g. scissors scraping RPE.

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Conclusions: 4D-MIOCT is useful for continuous monitoring of model subretinal surgery and may help avoid technique-related complications. 4D-MIOCT can contribute to the advancement of subretinal delivery of therapies under development.

Brief Summary Statement:

4-dimensional (volumes over time) microscope-integrated optical coherence tomography (4D MIOCT) is valuable tool for guidance of model subretinal surgical maneuvers. It is an important addition to the conventional microscope that helps with precision of subretinal therapy delivery and helps avoid surgical technique-related complications.

Keywords

Subretinal surgery; gene therapy; stem cell therapy; intraoperative optical coherence tomography; four-dimensional microscope-integrated optical coherence tomography; image guided surgery

Introduction

Macular degeneration-related atrophy of the retinal pigment epithelium (RPE) remains without a definitive treatment. In the elderly, age-related macular degeneration (AMD) is a leading cause of blindness; in younger population, Stargardt disease is the most common cause of macular degeneration which also results in blindness. Common to both diseases, lesions of macular (geographic) atrophy are characterized by death of the RPE and loss of photoreceptors and visual function. Recent advances in stem-cell research and gene therapy have opened the door to several potential treatments. Subretinal (SR) implantation of RPE cells can reduce the loss of photoreceptor and the loss of visual function.¹ However, these potential treatments are still in the early phases as preclinical studies, mostly in animal models.²

Despite the developing potential therapies over the past decade, surgical technique remains a challenge. Ongoing and upcoming studies, including multiple clinical trials, rely on SR delivery of RPE cells. The new advancements in therapies have necessitated evolving improvements in the surgical technique and visualization involving the SR space which are sometimes performed with viewing of OCT before and after surgery [the new reference] or of B-scans during maneuvers and OCT volumes at pauses in surgery. We have described the ability to visualize SR structures during surgical manipulation in human surgery, using four-dimensional (4D) microscope-integrated optical coherence tomography (MIOCT).³ We described how the ability to view 3D volumes in near-real time was important in orienting the surgeon for live image guidance.⁴ In this report, we investigate the utility of 4D MIOCT to guide SR maneuvers involving SR placement of instruments and SR delivery of materials.

Methods

Fresh cadaveric porcine eyes were obtained for harvesting RPE cells for cell culture and for conducting vitreoretinal surgical maneuvers in a wet lab setting. Imaging and surgical maneuvers were performed within 4 to 12 hours of harvesting of the eyes, in order to preserve corneal clarity, avoid media opacification and optimize image quality. Fresh

porcine RPE cells were cultured for at least 10 days. Pigmented RPE cells were then harvested from culture and suspended in normal saline at 100 cell/uL. RPE monolayer was obtained by seeding the fresh RPE cells on 6.5mm Corning Transwell with 0.4 um pore polyester (PET) membrane inserts (Sigma-Aldrich, Inc., St. Louis, MO, USA). After 7–10 days, PET membranes with a confluent monolayer of pigmented RPE were harvested and trephined. Each eye was positioned under the MIOCT microscope on a styrofoam head. A 25g valved trocar cannula system was placed at the standard locations for 3-port vitrectomy, with a flat contact lens for viewing. After vitrectomy and before surgical manipulation of the retina, the MIOCT parameters were optimized.

Our 4D MIOCT system images live surgery and displays the data as feedback to the surgeon in near real time. As previously described, this custom 4D MIOCT system consists of an ultrafast swept source OCT system operating at 100-kHz A-scan rate. Mechanically, it integrates into a commercial operating microscope (Leica Microsystems, Inc., Buffalo Grove, IL, USA). It uses custom graphic processing unit software in order to acquire, process, and render volumes in real time. Via a custom microscope-integrated heads-up display, it provides stereoscopic visualization of OCT volumes through the microscope oculars.³ The volumetric frame rate varies between 3.3 and 10 volumes per second depending on field of view and A-scan sampling density. Imaging parameters are controlled by an imager who operates the MIOCT system and tracks the surgeon's view. The volumetric rendering perspective is controlled by the surgeon using a foot pedal. OCT volumes are also recorded continuously during surgery and reviewed after the surgery.

4D MIOCT imaging for surgeon guidance was performed for several surgical maneuvers. Using either a subretinal *injection dual bore needle* with 41g (0.1mm) tip (D.O.R.C. International B.V., Zuidland, The Netherlands) or a subretinal *PolyTip cannula* with 38g (0.12mm) soft tip (MedOne Surgical, Inc., Sarasota, FL, USA), we injected normal saline solution through the retina to create hydrodissection-induced subretinal blebs. We used a stainless steel *NanoFil (NF33BL-2) needle* with 33g blunt tip (World Precision Instruments, Inc., Sarasota, FL, USA) in order to inject a suspension of pigmented RPE cells into the subretinal space. For subretinal implantation of membranes, we first created a large retinotomy (2mm) using 25g Pinnacle 360° vertical scissors (Synergetics, Inc., O'Fallon, MO, USA). Then, we created a sclerotomy (3.5mm) and inserted the membrane through the large retinotomy into the subretinal space using 23g Pinnacle 360° fine tip Eckardt forceps (Synergetics, Inc., O'Fallon, MO, USA). Membranes imaged included a polyethylene membrane, a polyester (PET) membrane, and a PET membrane with RPE monolayer.

Results

Hydrodissection using normal saline

High quality 4D MIOCT images of the retina were visible to the surgeon while operating. When either subretinal cannula was advanced in the field of view, the soft tip was seen as an *en face* shadow on the summed voxel projection image (SVP). As it approached the retina, the tip was not rendered in the volumetric image due to thresholding of the volumes and low reflectance from the tip, but was visible on B-scans. Guided by SVP and the B-scan data, the operating surgeon visualized the position of the tip relative to the retina. 4D MIOCT

successfully visualized the effect of the tip when it pressed on the retina, induced a fold or deformation in the retina, touched RPE, and broke through Bruch's membrane. On 4D MIOCT, the operating surgeon monitored the positioning of the tip against the surface of the retina. This position was used as a cue for the surgeon and assistant to initiate the injection and induce hydrodissection in the subretinal space. The formed subretinal bleb was visualized as an elevation on volumetric rendering (Figure 1, Video). The operating surgeon monitored the expansion of the subretinal bleb in the planar dimensions and its growth in the axial dimension. By gauging the elevation of the bleb on 4D MIOCT, the operating surgeon assessed the need for further fluid injection, depending on whether the bleb was to be used for injection of RPE suspension or for RPE monolayer on PET.

Injection of RPE suspension

The metallic needle used for injecting RPE suspension was highly reflective and cast a blocking shadow on the underlying tissue (Figure 2, Video). Guided by the volumetric 4D MIOCT data, the surgeon advanced the needle through the initial retinotomy hole on the retinal bleb, into the subretinal space. 4D MIOCT visualization of delivery of the RPE suspension was essential to identify complications. The efflux of pigmented RPE cells was visualized in the vitreous cavity. Interactions between the tip and the native RPE or Bruch's membrane inside the bleb was not detected on the conventional microscope, but obvious on 4D MIOCT.

Insertion of membranes

Polyethylene and PET membranes were successfully visualized above and under retina (Figure 3). Folds and deformations of the membranes were identified on 4D MIOCT. RPE cells on PET were successfully visualized on B-scans and volumetric rendering. 4D MIOCT allowed the surgeon to distinguish the side of PET on which RPE cells were located, and to determine whether the transparent PET membrane needed to be flipped prior to insertion. On the conventional microscope view, PET membrane under the retina was visible only through the opening of the retinotomy, while on 4D MIOCT it was visualized through the retinotomy opening and under elevated retinal tissue (Figure 3). On 4D MIOCT, the surgeon could view the forceps under the retina, their position with respect to native RPE or subretinal PET membranes and their opening and closing (Figure 4). This allowed the operating surgeon to adjust the surgical maneuvers in order to avoid complications such as forceps or membrane touching RPE or PET-related retinal deformation.

Discussion

We have reported the value of 4D MIOCT in a variety of surgical applications: in human vitreoretinal surgery, in pediatric patients, and in anterior segment procedures to guide transplantation of corneal tissue in humans.⁴ We also reported the value of MIOCT feedback in surgical education and enhancement of the performance of ophthalmologists in training.

This report focuses on the ability of 4D MIOCT to visualize the maneuvers in near real time and provide feedback and guidance to the operating surgeon. Emerging therapies such as stem cell or gene therapies suggest an increasing need for subretinal procedures. Unlike

conventional drug treatments, retinal stem cell or gene therapy is a complex biological treatment whose efficacy could depend on a multitude of factors including optimized therapy formulation/vector delivery, high quality and appropriate quantity of therapy and disease state and timing of intervention. Equally complex can be the surgical delivery of therapy to the sub-retinal space, during which the surgeon must minimize trauma and maximize cell or vector delivery. A major surgical challenge for the surgeon is tissue depth assessment; specific considerations must be taken into an account to optimize therapy delivery at appropriate tissue depth and to limit complications that ensue from lack of knowledge about depth during surgical maneuvers. Intraoperative 4D MIOCT allows for precise administration of therapeutics and accurate control over instrument location. In subretinal fluid injection maneuver, it allows for visualization of primary subretinal bleb, accurate quantity delivery⁵ and secondary injection of cells. It helps to identify and thus avoid fluid escape to sub-RPE space, and fluid or cell reflux into vitreous cavity. In subretinal graft insertion maneuver as shown here, it allows for correct RPE graft orientation, and orientation of instrument/graft inside the bleb and helps avoid RPE cells and material misconfiguration, escape or fold in the graft. In all of these maneuvers, it allows for minimal retinotomy sizing, avoids stress on retina and RPE touch.

With the use of 4D MIOCT, the surgical techniques would improve and rate of complications would decrease. In turn, lower complication rates would increase the odds of success of surgical techniques and treatment options that rely on implantation of RPE cells or subretinal injection of gene therapy vectors. In order to prove the value of adding 4D MIOCT to the conventional microscope, further data may be obtained from imaging of surgical maneuvers in human clinical or animal trials.

Limitations of the intraoperative OCT systems include small OCT field of view and shadowing and blocking by intraoperative instruments or failure to image instrument tips because of material, size, surface or alignment relative to OCT scanning. In this study subretinal visualization of RPE cells was also not possible under the highly reflective postmortem porcine retinal tissue (which becomes white over time after death).

In conclusion, 4D MIOCT visualization by the surgeon is valuable for monitoring of model subretinal maneuvers. It is an important addition to the conventional microscope view that may help with precision of the delivery of therapy and may help avoid surgical technique-related complications. Use of 4D MIOCT in model eye surgery will be a valuable tool for surgeons to train on new maneuvers for emerging treatments that involve manipulation of the subretinal space. Such MIOCT efforts can contribute to the advancement of subretinal delivery of therapies under development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1 -

4D MIOCT visualization of subretinal injection of saline. Prior to injection, (*top row*) still frames from (*left to right*) volumetric image, B-scan, and en face OCT show the interaction between the porcine retina and the subretinal cannula (*red arrows*). Note artifacts due to (1) blocking from cannula shadow (*dark blue arrow*) and (2) reflections from BIOM-lens (*light blue arrow*). Time-lapse frames (*bottom row with B-scan inset*) represent the near real time visualization of bleb (*yellow arrows*) creation and progression (*left to right*) throughout the injection. Note that the bleb extends out of the plane of the B-scan (white lines) but is readily appreciated on the progressive volumetric imaging. [see Video 1]

Figure 2 -

4D MIOCT visualization of the injection of pigmented RPE cells in suspension into the existing balanced salt solution subretinal bleb in a porcine eye using 33g metal cannula (yellow arrows). The retina is dimpled by the cannula and efflux of cells into the vitreous cavity is seen (red arrows) on B-scan (inset, upper left) and in the volume. The 3D volume can be rotated (green arrow) and viewed from any angle during the injection. [see Video 1]

Figure 3 -

Conventional microscope view (A) with simultaneous 4D MIOCT video excerpts (B: volumetric view, C: B-scan) show PET membrane (green arrow) with RPE cells (A,B: tan color) heaped up (A-C: white arrow) before insertion. Vessel pattern is noticeable beneath PET (A,B). From the MIOCT views, the surgeon can appreciate that the tip of PET is bent on the surface of retina and is pressing against and lifting up retina (B,C: blue arrow). This is not appreciated in A.

Figure 4 -
Fine tip Eckardt forceps in motion under the retina: open (left) and closed (right) grasping and slightly elevating the RPE layer. This was not visible to the surgeon from the conventional microscope view.