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Three-dimensional optical coherence tomography imaging of retinal sheet implants in live rats

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ABSTRACT

Purpose: To obtain three-dimensional images from retinal transplants in live animals and evaluate the placement and structural quality of the transplants.
Methods: Donor retinal sheets were isolated from E19 fetuses of transgenic rats expressing human alkaline phosphatase (hPAP), and transplanted to the subretinal space of 19–56 days old S334ter-3 rat recipients with fast retinal degeneration (average age at surgery 32 days). A total of 143 rats were imaged 1 day to 2.8 months after surgery, using a Fourier-domain optical coherence tomography (FDOCT) system, with an axial resolution of 3.5 μm. The CCD A-line integration time was set at 200 μs for better visualization of degenerated retina. After targeting the transplant area, 139 or 199 consecutive slices were scanned. Projection images and movies of the retinal transplant area were computed and later compared with histology.
Results: OCT scans identified 137 of 141 transplants as a thickening of the degenerated retina. OCT indicated the laminar structure of the transplants and surgical defects, such as RPE/choroid damage with an accuracy rate between 83 and 99%. Three-dimensional projections showed the transplant position in the retina in relation to the optic disc. Histology of transplants by hPAP and hematoxylin–eosin staining was correlated with the OCT results.
Conclusions: Optical coherence tomography is an excellent tool to image retinal layers in a live rat. This procedure helps to evaluate the placement and quality of the transplants in the living eye.

1. Introduction

Transplantation of retinal sheets (review: Aramant and Seiler, 2004; Seiler and Aramant, 2005) aims at replacing photoreceptors and/or retinal pigment epithelium (RPE) (and other retinal cells) lost in retinal diseases such as age-related macular degeneration (AMD) or retinitis pigmentosa (RP). AMD is the leading cause of blindness among the elderly, affecting about 8 million patients in the U.S. alone (Ding et al., 2009; Jager et al., 2008), and retinitis pigmentosa is an inherited disease that affects about a million patients worldwide (Kennan et al., 2005). The inner retina remains still functional for the some time after photoreceptor loss (Humayun et al., 1999), so it may be possible to restore visual function if newly replaced retinal cells can connect with the remaining host circuitry. Using a special implantation instrument, it has been possible to gently transplant sheets of fetal retina to the subretinal space in rodent retinal degeneration models (Aramant and Seiler, 2004; Seiler and Aramant, 2005) and in human patients (Radtke et al., 2008).

The surgery in the small rat eye is however very challenging since the surgeon cannot observe where the tissue is placed. Only about 20–30% of all transplants develop a normal lamination with photoreceptor outer segments facing the host RPE, in contrast to balls of photoreceptors in rosettes (Seiler and Aramant, 1998). It is very difficult to judge the quality of transplants in fundus exams

Abbreviations: FWHM, full width at half maximum spectral width; OCT, ocular coherence tomography; NA, numerical aperture; SLD, superluminescent diode; IP, inner plexiform layer; IN, inner nuclear layer; ON, outer nuclear layer; RPE, retinal pigment epithelium.

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due to the transparency of the transplant and host retina. To save resources, it would be important to eliminate transplanted rats with surgical defects early on from the study. Therefore, we developed a systematic approach to evaluate transplants in live rats by three-dimensional ocular coherence tomography (OCT) which provides a means to analyze structures in the living eye (Jeon et al., 2008b). In contrast to a previous study (Thomas et al., 2006) that used a commercially available time domain Zeiss Stratus OCT, the OCT setup for this study used serial scans of a Fourier-domain OCT to provide a three-dimensional image of the transplant in the eye (Jeon et al., 2008a; Leitgeb et al., 2003; Zhang et al., 2004). The purpose of this study was to evaluate the accuracy of the 3D FDOCT to predict later histological results.

2. Materials and methods

2.1. Animals

For all experimental procedures, animals were treated in accordance with the NIH guidelines for the care and use of laboratory animals and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, under a protocol approved by the Institutional Animal Care and Use Committee of the University of California, Irvine. Pigmented S334ter-line-3 rats (Liu et al., 1999; Sagdullaev et al., 2003; Seiler et al., 2008a) with fast retinal degeneration were used in this study. The rats were originally produced by Xenogen Biosciences (formerly Chrysalis DNX Transgenic Sciences, Princeton, NJ), and developed and supplied with the support of the National Eye Institute by Dr. Matthew LaVail, University of California San Francisco (http://www.ucsfeye.net/mlavailRDratmodels.shtml). Recipients were the F1 generation of a cross between albino homozygous S334ter-line-3 and pigmented Copenhagen rats (Harlan, Indianapolis, IN).

A total of 143 rats that had received embryonic day (E) 18–20 fetal retinal sheet transplants ($N = 141$) or sham surgery ($N = 2$) at the age of 19–56 days (average 31.9 ± 6.8 days) were evaluated with three-dimensional optical coherence tomography at the age of 26–116 days, 1–85 days after surgery. The donor retinal sheets were derived from transgenic hPAP (human placental alkaline phosphatase) expressing rats (Kisseberth et al., 1999). Fetal retinas were dissected free from surrounding tissues, and stored overnight in Hibernate E medium (Brainbits, IL) on ice or in the refrigerator. Some donor retinas were coated with glial-derived neurotrophic factor (GDNF) or brain-derived neurotrophic factor (BDNF) containing microspheres before transplantation (Seiler et al., 2008a) to improve the functional effect of the transplants. Immediately before transplantation, the tissue was cut to a rectangular square (about 1 mm^2) and loaded into the flat nozzle of a custom-made implantation tool (Aramant and Seiler, 2002).

For the transplant surgery, recipient rats were anesthetized with Ketamine/Xylazine (K: 37.5 mg/kg; X: 5 mg/kg i.p.), the pupils were dilated with 2% atropine, and the non-surgery eye covered with artificial tears ointment. A cut was made through sclera, choroid and retina in the periphery of the superior eyeball. The instrument nozzle was inserted into the subretinal space, advanced towards the central retina where the donor tissue was placed into the subretinal space and the nozzle withdrawn. The lesion was closed with 10-0 sutures. The transplant placement was evaluated by an eye exam following surgery. Rats with too much trauma were discontinued from the study. Rats were placed into an incubator for recovery after surgery, and surgery eyes treated with gentamycin ophthalmic ointment.

2.2. High performance Fourier-domain optical coherence tomography for retinal imaging

Fig. 1 shows a diagram of the high performance Fourier-domain ophthalmic optical coherence tomography instrument for in vivo retinal imaging designed and built at Beckman Laser Institute (Rao et al., 2008) with an axial resolution of 3.5 μm (in adult normal rat retina), sensitivity of 106 dB and imaging speed of 5K A-lines per second. However, in the retinal degenerate rats of different ages used in this study, the resolution was likely less than 3.5 μm. The axial resolution in rat retinas of different ages was not measured. Low coherence light with a center wavelength of 890 nm and FWHM bandwidth of 150 nm was protected from optical feedback using a broadband optical isolator before entering a 2×2 broadband fiber coupler based interferometer. The light from the reference arm was focused onto a reference mirror with an optical attenuator inserted into the optical path. The sample arm was modified from the patient module of a Zeiss©Stratus OCT instrument.

The system maintains sensitivity of 100 dB within 500 μm imaging depth range. The sensitivity roll-off over 2 mm depth range is 9 dB. The lateral resolution in the eye is 20 μm. Each scan resulted in 199 or 139 slices.

Two customized imaging modes were developed for the eye alignment. The first imaging mode is cross-scan mode. It includes both horizontal and vertical scans in a single scan and displays the real time processed image in two B-mode images. When the animal eye is positioned appropriately, both the horizontal and the vertical images are not tiled on the image. The second one is fast fundus imaging mode. In this fast fundus imaging mode, a bandpass filtering of K space spectrum generates a fundus projection

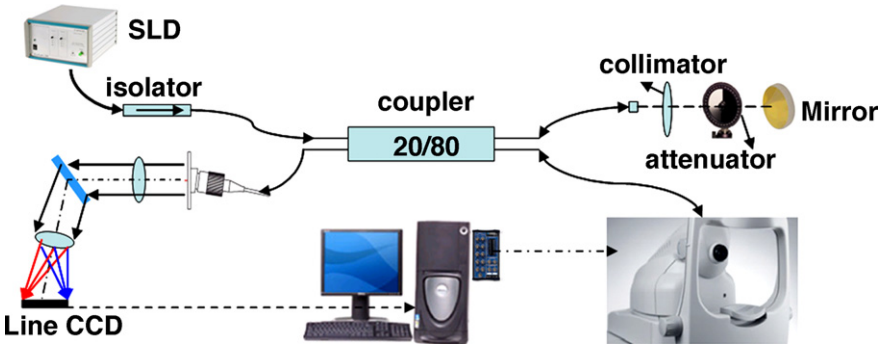

Fig. 1. OCT imaging setup. The light from a superluminescent diode (SLD) light source (with the central wavelength at 890 nm and a full width at half maximum spectral width (FWHM) of 150 nm), is protected from optical feedback using a broadband optical isolator before entering a 2×2 broadband fiber coupler based interferometer. The light from the reference arm is focused onto a reference mirror with an optical attenuator inserted into the optical path. The sample arm is modified from the patient module of a Zeiss©Stratus OCT instrument. The sample information is then transferred back through a sequence of lenses and mirrors to a line CCD camera. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)