Viability of human fetal retina/RPE sheets after 4 days cold storage

Magdalene J. Seiler1,2, Gabriel Nistor3,1, Norman D. Radtke4, Robert B. Aramant1

1. Department of Anatomy and Neurobiology; University of California at Irvine, CA; 2 current address: Department of Physical Medicine & Rehabilitation, University of California at Irvine, CA; 3 current address: California Stem Cell Inc., Irvine, CA; 4 Retina Vitreous Resource Center, Louisville, KY

PURPOSE
● To determine viability of human retinal sheets with its RPE after prolonged cold-storage, including shipping in temperature-controlled containers

Background

Transplantation of retinal sheets

● Fetal retinal sheets can be easily implanted into the subretinal space and restore a degenerating retina

● Retinal sheet transplants have shown to improve visual responses in four different rodent retinal degeneration models

● In a phase II clinical trial, sheet transplants of retina together with its RPE improved vision as tested by ETDRS in 7 of 10 patients after one year (Radtke et al., 2008)

● Evidence of tissue viability after prolonged cold storage would widen the applicability of this procedure for clinical trials. The results would also be important for development of future trials with hESC-derived tissue.

Methods

1. Tissue preparation

Permission to use fetal tissue for research was obtained from the Western Institution Review Board, Norton Healthcare Research Office, and the IRB of the University of California, Irvine. Retinas together with its RPE was dissected from fetal eyes (enrolled at 1 day (d) after abortion). Eyes were placed in cold PBS containing 1× Penicillin-Streptomycin (Gibco) and 0.05% trypsin (Gibco) immediately after harvest. Eyes were treated with dispase (Col. Res. Inc.) for 15 minutes at 37°C. Dissected tissue was cut into pieces (ranging in size between 2 to 7 mm). Some pieces were fixed immediately in 4% paraformaldehyde (PFA), and the remaining pieces soaked up in flat plastic molds in immersion medium in shipping tube (described below).

2. Temperature monitoring

The temperature in the container was measured continuously by a temperature probe.

3. Histology and TUNEL staining (determining cell viability)

Tissues were immersion-fixed in 4% paraformaldehyde, dehydrated in 30% sucrose, and frozen in OCT. 10 µm cryostat sections (3 sections/whole eye) were stained by an in situ cell death detection kit (Roche) based on fluorescent TUNEL staining (Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling). Each staining set contained DAPI-labeled positive controls and negative controls (omission of reagents). Sections were counterstained with DAPI (blue). To 28 fluorescent images/patient were taken on a Nikon FxA microscope in 2008.

4. Cell counting

The number of TUNEL-stained (+) cells per mm² and % TUNEL-stained cells (green) of total cells (blue) was counted in 10 µm cryostat cross-sections, using Diapositive Instruments GFP software, by counting in separate channels.

REFERENCES


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Gabriel Nistor: Code N (No Commercial Relationship);
Norman D. Radtke: Code N (No Commercial Relationship);
Robert Aramant FARVO: Ocular Transplantation LLC; Code E (Employment), Code P (Patent);