Diffusion Tensor Imaging tracks repair of Retinal Pigment Epithelium (RPE) layer using Hematopoietic Stem Cells in mice

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Introduction: Embryonic stem cells have the ability to differentiate into all lineages, whereas adult hematopoietic stem cells (HSCs) are restricted in their capacity to differentiate. However, the HSCs can be programmed to differentiate into specific tissue by transducing them with vectors expressing specific genes. The purpose of this study is to restore visual function in mice impaired by retinal degeneration (*rd11*) by using HSCs to repair the retinal pigment epithelium (RPE) layer. HSCs were infected with lentiviral vectors expressing RPE-specific gene RPE65, leading to their differentiation into RPE cells thereby repairing this layer of the retina[1]. We used diffusion tensor imaging (DTI) to successfully observe differences between retinas of mice treated with genetically modified HSC or control cells. Ongoing research in this line aims to establish the use of HSCs in restoring visual function and using DTI as a non-invasive tool to track progress of stem cells.

Methods: All animal protocols were in accordance to University of Florida Institutional Animal Care and Use Committee (IACUC) guidelines. We compared three groups for this study. (1) The mice in Group 1 are from a strain that spontaneously develop retinal degeneration *(rd11)* that received by intravitreous injection, 5,000 purified c-kit+/Sca-1+ bone marrow-derived HSCs that were transduced with lentiviral vector expressing the RPE65 gene. (2) Group 2 are rd/rd mice that received by systemic (intravenous) injection of 15,000 purified c-kit+/Sca-1+ bone marrow-derived HSCs transduced with lentiviral vector expressing the RPE65 gene. This was to test the route of administration, to see if systemic route differed in its effect from local injection (i.e. at the site of injury). (3) Group 3 are rd/rd animals that received by intravitreous injection, 10,000 purified c-kit+/Sca-1+ bone marrow-derived HSCs transduced with lentiviral vector expressing the LacZ gene (as a control for transduction). All DTI processing was done using fanDTasia software developed at UF [2].

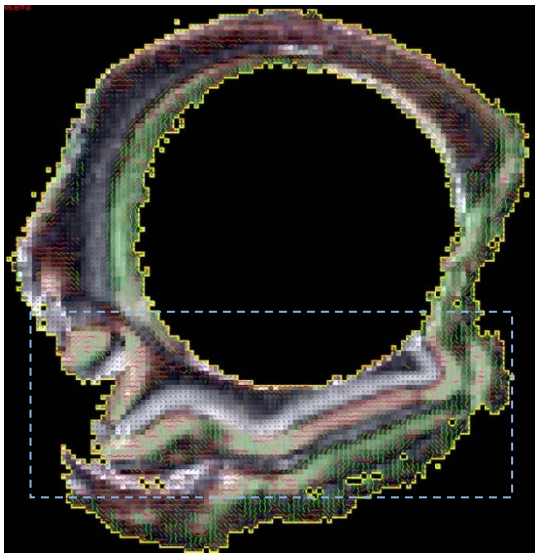

Fig 1: DTI of eye from Group 2, which is a representative of a restored RPE layer. The dotted box outlines the retina.

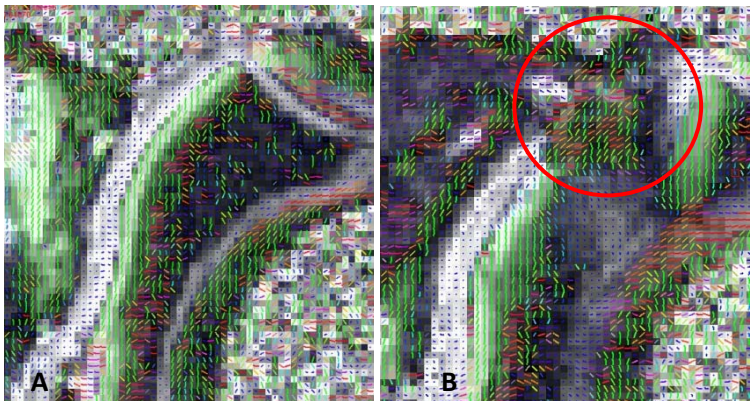

Fig 2: DTI detects differences between a normal retina (A) compared to a retina with depleted RPE layer (B) shown by the red circle.

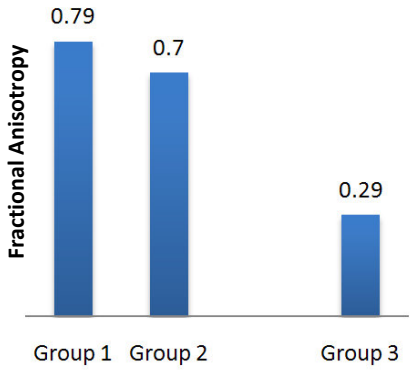

Fig 3: Comparison of FA in the retinas of repaired RPE layer (Groups 1, 2) as compared to ablated RPE layer (Group 3)

Results: Fig.1 is the DTI of an eye from group 2 having a repaired RPE layer. The dotted box shows the organized structure of a retina. A healthy retina has strong boundaries between its layers and consequently the FA in the retina is significantly higher than in the surrounding anatomy. Fig. 2 compares normal (A) and degenerate (B) sections in the same eye from Group 3. In this case, the degenerate RPE layer (denoted by the red circle) is clearly detected by DTI. Fig 3 is a comparison of the FA in the retinas of Groups 1, 2 and 3. Group 3 has a significantly lower FA than groups 1 and 2, thereby detecting loss of organized structure in the RPE layer.

Conclusions: Hematopoietic stem cells expressing the gene RPE65 were successful in differentiating into RPE cells and can repopulate the RPE layer in *rd11* mice. Diffusion tensor imaging successfully detected differences between repaired and unrepaired RPE layers in mice. The FA in repaired retinas was observed to be higher due to the organized structure of the RPE layer in the retina. The FA is markedly lower in degenerate retinas due to the disintegration of the RPE layer. Ongoing experiments in this study aim to establish the use of HSCs to restore visual function.

References: [1] N. Sengupta et al, 'Regulation of Adult Hematopoietic Stem Cells Fate for Enhanced Tissue-specific Repair', American Society of Gene & Cell Therapy, vol. 17 no. 9, 1594–1604 sep. 2009 [2] A. Barmpoutis and B. C. Vemuri, "A unified framework for estimating diffusion tensors of any order with symmetric positive-definite constraints", In Proceedings of ISBI10: IEEE Int'l Symposium on Biomedical Imaging, 2010