Report

Adaptive Optics Imaging shows Rescue of Macula Cone Photoreceptors

Advanced age-related macular degeneration and inherited macular diseases remain largely untreatable. Clinical trials using stem cell transplantation have recently commenced and mark a further step in introducing cellular therapy for these diseases.[1] Earlier techniques such as macular translocation[2] and autologous retinal pigment epithelium (RPE), namely, choroidal transplantation[3] demonstrated rescue of visual function in severe neovascular age-related macular degeneration. These studies aimed at long-term rescue of functional photoreceptors and macular anatomy.

In the past, there has not been a way to demonstrate objectively the extent of rescue and survival of individual cone photoreceptors following interventions. Rescue was implied by functional improvement in visual acuity, retinal sensitivity by microperimetry and the integrity of the photoreceptor inner/outer segment layer on optical coherence tomography (OCT) scans.[3] The emergence of high-resolution adaptive optics (AO) retinal imaging system in 1996[4] made it possible to image the human retina directly at a cellular level in vivo.

Recently, AO retinal imaging has been used to study the cone photoreceptors in macular diseases. We used an AO camera to image patients who have undergone macular translocation, a form of indirect RPE transplantation, to study the level of photoreceptor rescue and demonstrate the longevity of rescue of cone function and structure.

The study protocol was approved by the local Research Ethics Committee and complied with the tenets of the Declaration of Helsinki. Three female patients aged 67, 76, and 82 years, who had previously (>5 years) undergone macula translocation surgery for neovascular age-related macular degeneration were recruited from the Moorfields' vitreoretinal clinic. The best-corrected visual acuity was assessed with a modified Early Treatment Diabetic Retinopathy Study distance visual acuity chart at 4 meters. All patients underwent spectral domain OCT (Spectralis, Heidelberg Engineering, Heidelberg, Germany). Horizontal volume scans and infrared fundus images were used as a reference to mark the location of the area of healthy and diseased retina.

An AO en face reflectance imaging system (rtx1, Imagine Eyes, Orsay, France) with infrared flood illumination (wavelength, 850 nm) was used to image 4×4 degree areas of the macular cone photoreceptor layer through a dilated pupil. Retinal sensitivity was determined using microperimetry (MP; Nidek MP-1, Padova, Italy). Fixation was tested with a 1 degree cross-fixation target using a 200-ms duration Goldmann III stimulus. Stimulus intensity ranged over a 20-point logarithmic scale from 0 to 20 dB (400−4 asb). An automated 4-2 staircase threshold test strategy was used over a preselected 76-point grid area centered over their fovea.

Cone photoreceptor images were registered with color fundus, infrared + OCT and MP in Adobe Photoshop (CS5, version 12.0, Adobe Systems Inc, San Jose, CA) for analysis. Cone photoreceptors in the images were described by their presence and by the pattern of distribution. Manual cone counting was performed on a retinal area of 50×50-μm size using the image-processing programme, ImageJ (National Institutes of Health, Bethesda, MD). The area chosen was close to fixation, along either the horizontal or vertical meridian through the fovea.

Subjects attained and maintained best-corrected visual acuity of 0.04, 0.20, and 0.20 logarithm of the minimum angle of resolution, respectively, at 6, 7, and 8 years postoperative. The presence of cones, inner/outer segment layer, and RPE layer were clearly noted at the new position of the translocated macula in all the 3 patients (Figures 1B, C, 2B, C, and 3B,C; available at http://aaojournal.org). In the diseased areas, the retinal architecture and anatomy is disrupted with loss of the inner/outer segment and RPE layers (Figs 1D, 2D, and 3D; available at http://aaojournal.org). Cones were present throughout in cases 1 and 3, and focally at the fovea and superior to the fovea in case 2. The cone photoreceptor density for cases 1, 2, and 3 at an eccentricity of 0.18, 0.21, and 0.24 mm from the fovea were 22.8×10^3, 12.8×10^3, and 13.2×10^3 cones/mm^2, respectively. The corresponding MP retinal sensitivities for these 3 cone density sampled areas were 14, 10, and 13 dB, respectively.

Good visual function was noted throughout the healthy areas in cases 1, 2, and 3 with good retinal sensitivity on MP. No sensitivity was seen in the old diseased area (Figures 1A, 2A, and 3A; available at http://aaojournal.org).

The success of cellular therapies for macular diseases will ultimately depend on the extent and longevity of rescue of photoreceptors. In this study, we have demonstrated that it is possible to image the rescued photoreceptors 6 to 8 years after treatment. To quantify the extent of photoreceptor rescue we compared the cone photoreceptor densities of the 3 cases with appropriate, published, age-matched normative data[5] at equivalent eccentricities. For cases 1, 2, and 3 the paired cone counts and age matched normals were 22.8×10^3 and $52.6\pm4.4\times10^3$; 12.8×10^3, and 46.5 to $50.2\pm2.1\times10^3$; and 13.2×10^3 and 46.5 to $50.2\pm2.1\times10^3$ cones/mm^2, respectively. This results in cone densities of approximately 2.3 times less for case 1 and 3.5 to 3.8 times less for cases 2 and 3, but still consistent with acuities of 0.04, 0.20, and 0.20 logarithm of the minimum angle of resolution, respectively. These counts show the rescue of photoreceptors for significant lengths of time, but at reduced levels. Being able to quantify the actual rescue of photoreceptors and correlating this with function for future cellular therapies will be crucial in defining minimal levels of useful rescue.

This observational case series demonstrates survival of cone photoreceptors, using in vivo AO retinal imaging in cases of indirect RPE transplantation in macular disease. It suggests that cone imaging may be useful for monitoring future therapies and quantifying photoreceptor rescue relative to function. Given the recent onset of clinical trials in cellular therapies for macular disease, it is timely to show that this type of imaging is feasible and provides useful information about outcomes.

Manickam Nick Muthiah, MRCOphth, MEd[1,2,3]
Pearse A. Keane, MD, FRCOphth[1,2,3]
Joe Zhong, PhD[2]
Carlos Gias, PhD[3]
Gurmit Uppal, MD, FRCOphth[2]
Peter J. Coffey, PhD[3]
Lyndon da Cruz, FRCOphth, PhD[1,2,3]

[1]*National Institute for Health Research, Biomedical Research Centre for Ophthalmology, London, United Kingdom;* [2]*Moorfields Eye Hospital, London, United Kingdom;* [3]*UCL Institute of Ophthalmology, London, United Kingdom*

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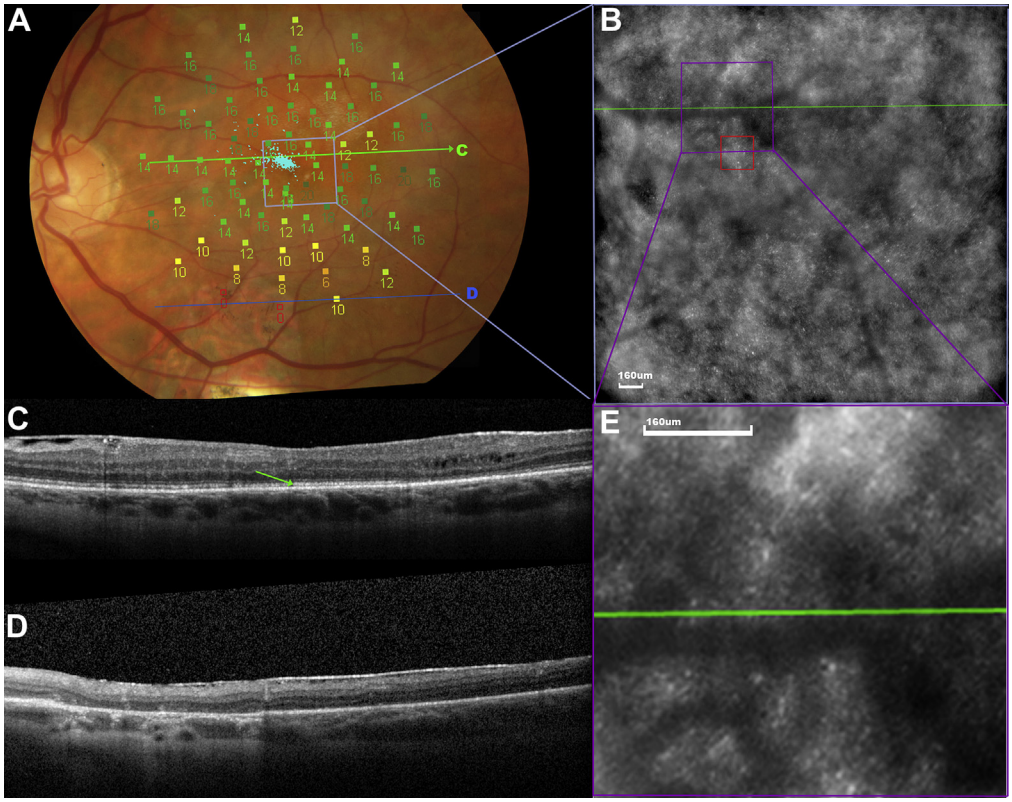

Figure 1. Multimodal retinal imaging of case 1. **A**, Color fundus photograph of the left eye: Grey box shows the area of adaptive optics imaging; lines C and D represent optical coherence tomography (OCT) images taken at 2 different points. Microperimetry (MP) points are shown with their values alongside. Blue points indicate fixation point as tested during MP. **B**, Adaptive optics image taken at the fovea, from the grey box within **A**, shows cone photoreceptors. **C**, An OCT scan taken right through fixation, indicated by the green line shows an intact inner segments/outer segment (IS/OS) junction as marked by the green arrow, thereby confirming the healthy area (the translocated area). **D,** An OCT scan through the old, diseased area shows disruption of both the RPE layer and IS/ OS junction. **E**, Magnified adaptive optics image from an area in **B** showing the bright mosaic pattern of cone photoreceptors.

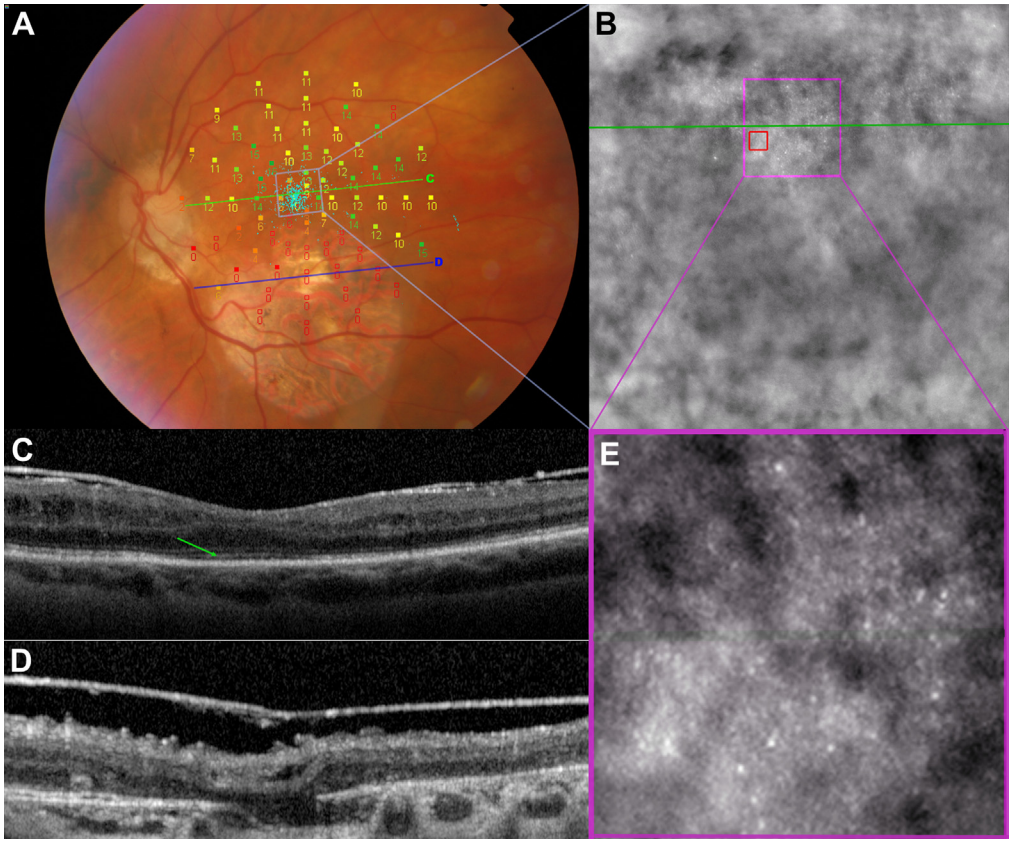

Figure 2. Multimodal retinal imaging of case 2. **A**, Color fundus photograph of the left eye: Grey box shows the area of adaptive optics imaging, lines C and D represent optical coherence tomography (OCT) images taken at the 2 points. Microperimetry (MP) points are shown with their values alongside. Blue points indicate fixation point as tested during MP. **B**, Adaptive optics (AO) image taken at the fovea, from the grey box within panel **A** showing cone mosaic photoreceptors. **C**, An OCT scan taken right through fixation, indicated by the green line shows an intact inner segment/outer segment (IS/OS) junction, as marked by the green arrow, thereby confirming the healthy area (the translocated area). **D**, An OCT image through the old diseased area, indicated by the blue line, with disruption of both retinal pigment epithelium layer and IS/OS junction. **E**, Magnified AO image from an area in **B** showing the bright mosaic pattern of cone photoreceptors.

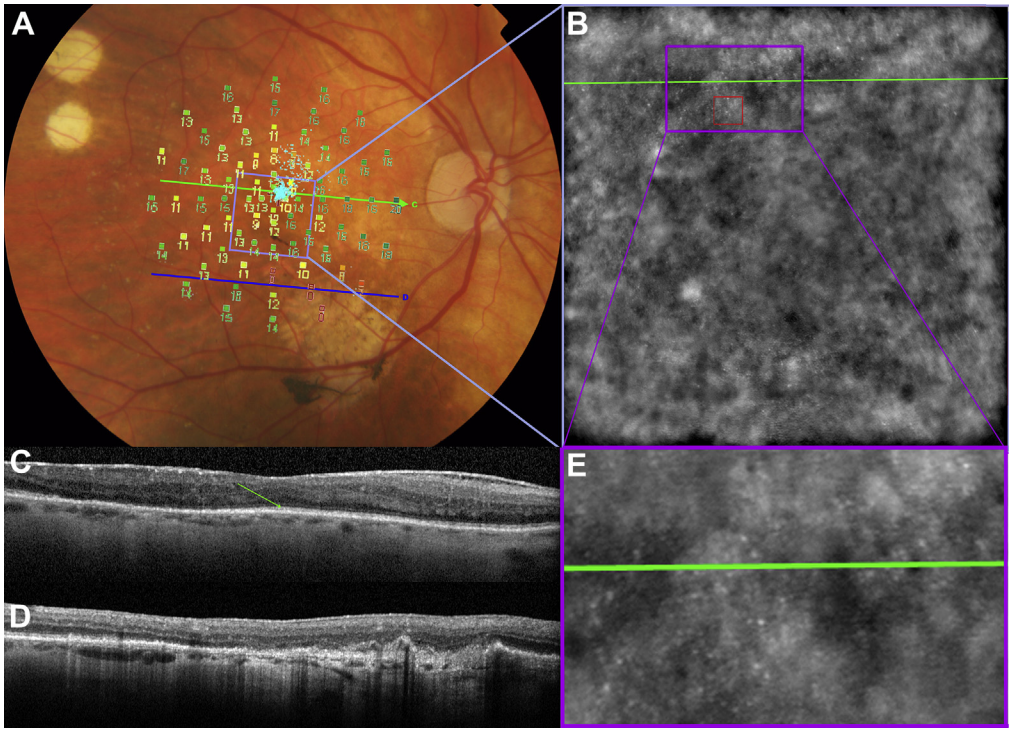

Figure 3. Multimodal retinal imaging of case 3. **A**, Color fundus photograph of the right eye: Grey box shows the area of adaptive optics (AO) imaging, lines C and D represent optical coherence tomography (OCT) images taken at the 2 points. Microperimetry (MP) points are shown with their values alongside. Blue points indicate fixation point as tested during MP. **B**, An AO image taken at the fovea, from the grey box within **A**, shows cone mosaic photoreceptors. **C**, An OCT scan taken right through fixation, indicated by the green line shows an intact inner segment/outer segment (IS/OS) junction, as marked by the green arrow, thereby confirming the healthy area (the translocated area). **D**, An OCT image through the old diseased area, indicated by the blue line, with disruption of both the retinal pigment epithelium layer and IS/OS junction. **E**, Magnified AO image from an area in **B** showing the bright mosaic pattern of cone photoreceptors.