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Case Report

Comparative analysis of macular microstructure in eyes treated with human amniotic membrane plug or internal limiting membrane transplant for Failed Macular Hole

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ABSTRACT.
Objective: **To document comparative analysis of macular microstructures of eyes treated with autologous internal limiting membrane (ILM) transplant and human Amniotic Membrane (hAM) plug transplant for failed macular holes (FMH).**
Materials and Methods: **Six patients who underwent successful surgeries for FMH were evaluated. The first three patients had undergone autologous ILM transplant, the others had undergone hAM plug transplant. They were examined using Adaptive Optics (AO) at baseline and at 6 months after surgery; OCT and OCT-Angiography were performed at 6 months. All images were evaluated morphologically; AO images were also analysed using the internal software.**
Results: **Regarding the AO analysis in ILM-patients, the average cone density inside the lesion was 7684.44 ± 362.96 and the total spacing was 10.86 ± 0.97. In hAM-patients, the average cone density inside the plug area was 10197.9 ± 326.62 and the total spacing was 10.6 ± 0.72. It was not possible to analyse cone density in the area outside the lesion on any patients. All patients were also evaluated morphologically. OCT analysis in ILM-patients showed a subverted anatomical situation, while in hAM-patients revealed the regrowth of tissue layers and a resumption of foveal depression. OCT-Angiography analysis revealed an enlargement of the FAZ in ILM-patients and a fairly normal appearance and size of the FAZ in hAM-patients, in comparison to the fellow eye.**
Conclusions: **Our findings require validation with a longer follow-up in a larger quantity of patients, but already suggest important differences in the cellular mechanism that underlies the tissue remodelling in these two techniques.**

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Introduction

Failed macular hole (FMH) is a full-thickness defect of the fovea that fails to close despite the surgical intervention; its treatment is challenging for vitreoretinal surgeons. The modern approach to manage these pathologies is to insert alternative tissues into the macular holes, to promote their anatomical closure and improve their visual acuity. The use of the human amniotic membrane (hAM) plug has been proven effective in closing FMH, demonstrating complete anatomical success and remarkable visual improvement, even in complex cases (Caporossi et al. 2019, 2019, 2019; Rizzo et al. 2019). Autologous internal limiting membrane (ILM) transplant has also had great success, with both anatomical and functional satisfying results (Morizane et al.,).

New high-resolution imaging techniques provide numerous structural details, nevertheless, the changes that occur at the cellular level are not fully known. Adaptive optics (AO) provides high-resolution retinal imaging that enables investigating changes at the cellular level before and after surgery. To date, to the best of our knowledge, there are no reports in the literature of AO imaging in patients treated with ILM transplant, and there is no comparative analysis of microstructural outcomes of different surgical techniques for the treatment of FMH with AO imaging.

In this study, we report six cases of six patients affected by FMH treated with ILM transplant or hAM transplant, and we perform a comparative analysis of macular microstructures via OCT, Adaptive Optics and OCT-Angiography at the 6-month follow-up.

Materials and Methods

This retrospective study included six patients affected by FMH treated with a hAM plug transplant and an autologous ILM transplant by the same expert vitreoretinal surgeon (T.C.) at the Eye Clinic of Careggi University Hospital (Florence, Italy). Written informed consent for participation was obtained from all patients and the study is adhered to the principles outline in the Declaration of Helsinki. All patients previously underwent pars plana vitrectomy (PPV) with ILM peeling and 20% sulphur hexafluoride (SF6) with macular hole closure failure; none of them suffered from other different retinal diseases. Five patients out of 6 were female; the mean age was 67.7 years. All patients underwent a comprehensive ophthalmological examination at baseline and 2 weeks, 1, 3 and 6 months after the surgery. AO imaging (AO flood-illuminated technology – rtx1TM; Imagine Eyes, Orsay, France) was performed at baseline and the 6-month follow-up; OCT (AngioVue Optovue, Fremont, CA, USA and Nidek Mirante Nidek Co. Ltd., Gamagori, Japan) and OCT-angiography were performed at 6 months; OCT-A was performed also in the fellow eye.

Adaptive optics

AO images were acquired using a commercially available flood-illuminated AO retinal camera (Rtx1, Imagine Eyes). Cones are automatically detected by software provided by the manufacturer (AO detect Mosaic b13; Imagine Eyes) that determines cone density and spatial distribution. Adaptive Optics images were acquired at 2° from the fovea along with the 4 meridians (nasal, temporal, superior and inferior). Each image was evaluated both morphologically and quantitatively, and cones were automatically detected by the software provided by the manufacturer (AOdetect Mosaic b13; Imagine Eyes). Their spatial distribution was analysed in terms of intercone spacing and local cell density. The mean value was calculated from the values in all 4 directions. Cones within the 2° central area (i.e. up to 1° from the centre of the fovea) cannot be resolved because of the limit of resolution of the device.

OCT- Angiography

OCT-A (AngioVue Optovue, Fremont, CA) scanning area was captured in 3 × 3 mm sections in affected eyes and in fellow eyes, and was centred on the fovea. OCT-A was used to morphologically analyse the foveal avascular zone (FAZ) and to compare its size and appearance to that of the healthy fellow eye.

Surgical techniques

The first 3 patients underwent 25-gauge PPV (CONSTELLATION, Alcon Surgical, Fort Worth, TX, USA) and autologous ILM transplant; a mixture of Vital Dyes (Dual Blue; DORC International, Zuidlan, Netherlands) was used to check for ILM remnants in the posterior pole. The ILM was harvested from inside the vascular arcades, starting from the edge of the previously removed ILM, in a circular fashion for approximately 1-disc diameter, and inserted into the macular hole. Fluid-air exchange was performed. Other patients underwent hAM plug transplantation involving 25-gauge PPV (CONSTELLATION, Alcon Laboratories, Fort Worth, TX, USA) with a 25-gauge chandelier endoilluminator to facilitate bimanual manoeuvres. A mixture of Vital Dyes (Dual Blue; DORC International, Zuidlan, Netherlands) was used to check for ILM remnants in the posterior pole. No extra peeling manoeuvres were necessary. The hAM came from the Eye Bank of Lucca, Italy. The hAM plug was precut with a cutaneous 1-mm punch (Disposable Biopsy Punch, Kai Medical, Solingen, Germany), based on the maximum diameter size of the macular hole measured using OCT. The plug was inserted through the trocar and, once in the vitreal chamber, gently manipulated and manoeuvered into the macular hole in the subretinal space with the chorion layer facing the retinal pigment epithelium (RPE) to position the plug. Inside the vitreous chamber, the chorion layer of the hAM plug was determined by identifying the sticky side of the plug using vitreal forceps. Fluid-air exchange was performed.

In all patients, 20% SF6 (Fluoron GmbH, Germany) was used as the endotamponade. Patients were asked to maintain a face-down position for 5 days after the surgery.

Results

Six patients affected by FMH were included in this study. The first 3 patients underwent PPV with autologous ILM transplantation and the others underwent PPV with hAM plug transplant.

Mean preoperative BCVA was 0.9 logMAR in ILM-patients and 1.0 logMAR in hAM-patients. The mean size of the maximum FMH diameter measured at SD-OCT was 806.33 ± 24.17 μm (784-832) and 762.33 ± 49.5 μm (713-812) respectively. The FMH had closed by the 2-week follow-up in all cases.

At the 6-month follow-up, mean BCVA of ILM-patients was 0.7 logMAR (20/100) and of hAM-patients was 0.6 logMAR (20/80).

The 6-months OCT analysis in hAM-patients revealed a resumption of the foveal depression, an intense hyper-reflectivity at the level of the external retina due to the persistence of the heterologous tissue and tissue ingrowth above the hAM plug (Fig. 1), . In ILM-patients, we observed the closure of the macular hole with the reconstitution of a flat profile and proliferation of glial tissue inside the fovea (foveal fibroplasia) (Fig. 2) as described in the literature. (Lee et al. 2018). SD-OCT also revealed an ellipsoid zone (EZ) external limiting membrane (ELM) interruption in the subfoveal zone. A dissociated optic nerve fibre layer (DONFL) was also evident. At the OCT-Angiography morphological analysis, hAM-patients' images showed the reconstitution of a fairly normal architecture of parafoveal vascular plexuses, with a fairly normal appearance and size of the Foveal Avascular Zone (FAZ), compared to the FAZ of fellow-healthy eyes. OCT-Angiography images of ILM-patients showed an enlargement of the FAZ and a mild congestion of the deep capillary plexus (DCP), compared to the FAZ of the fellow eye (Fig 3, 4)

Regarding the AO morphological analysis, in hAM-patients the plug was found round-edged and well-defined, with a defocus effect at the interface with the peripheral retina, due to variation in the orientation of the external retinal margin over the plug. Outside the margin of the plug, we detected hyper-reflective dots; similar fine structures were apparent in the hAM plug. The integrated software (AO Detect Mosaic) identified these hyper-reflective dots and interpreted them as photoreceptors. Some artefacts

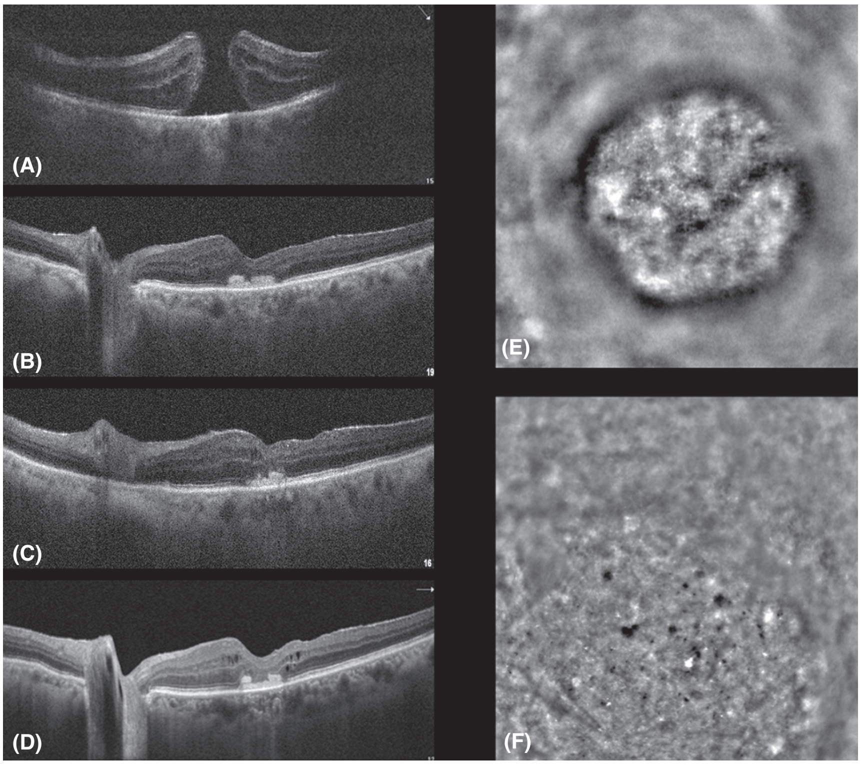

Fig. 1. OCT of a hAM-patient showing failed macular hole (A) and hole closure after hAM plug transplant at the 1-month, 3-month and 6-month follow up (B-C-D). AO of the same patient at baseline and at the 6- month follow-up (E-F)

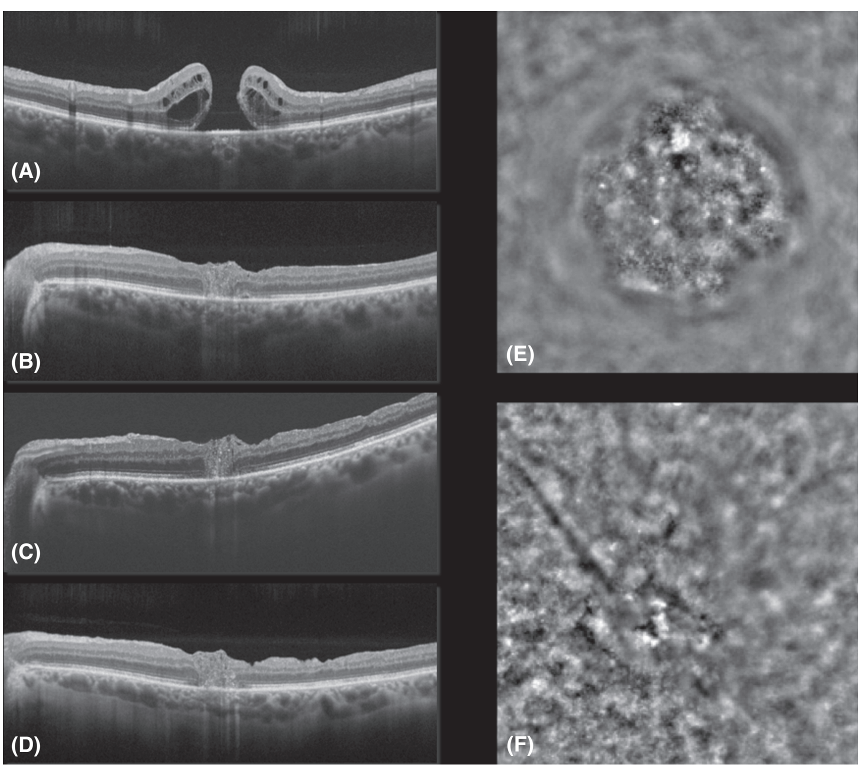

Fig. 2. OCT of an ILM-patient showing failed macular hole (A) and hole closure after autologous ILM transplant at the 1-month, 3-month and 6-month follow up (B-C-D). AO of the same patient at baseline and at the 6-month follow-up (E-F)

were observed in the fovea due to the irregularity of the implant surface. Larger hypo-reflective dots were also found in the centre and on the edge of the plug that were not recognized as photoreceptors by the software (Fig. 5, 6). ILM-patients presented a smaller central area with less fined margins; photoreceptors were not easily visually recognizable in central areas (Fig. 5). The average cone density inside the lesion of ILM-patients was 7684.44 ± 362.96 and the total spacing was 10.86 ± 0.97. In hAM-patients, the average cone density inside the plug area was 10197.9 ± 326.62 and the total spacing was 10.6 ± 0.72.

Discussion

SD-OCT imaging in hAM-patients showed a resumption of the foveal depression, a tissue ingrowth above the plug, and formation of layers mimicking the normal external retina, in compliance with recent results in the literature (Caporossi et al. 2020). In ILM-patients, a flat profile, which is completely different from the normal foveal depression and foveal fibroplasia was observed, as described in the literature (Lee et al. 2018).

Currently, there are no studies in the literature that use AO to evaluate the outcome of FMH surgery after ILM transplant. In Caporossi et al (Caporossi et al. 2020), AO-FIO has been used to investigate the outcomes of hAM transplant. AO-FIO technology has been used to analyse the status of photoreceptors after idiopathic macular hole surgery only by Markan (Markan et al. 2019). In our study, using AO-FIO, we have found hyper-reflective dots in the hAM-treated patients, which were interpreted as photoreceptors by the integrated software (Fig. 6). Some larger hypo-reflective dots were found in the centre on the edge of the plug, perhaps corresponding to RPE patches or macrophagic cells (Fig. 6). This supports the hypothesis of an initial remodelling process triggered by the presence of the hAM plug. In patients treated with ILM, the photoreceptors were less visually recognizable within the lesion and the average density value calculated by the software was reduced, compared to the hAM-treated patients (Fig. 5). ILM transplantation induces a glial proliferation process, as shown at

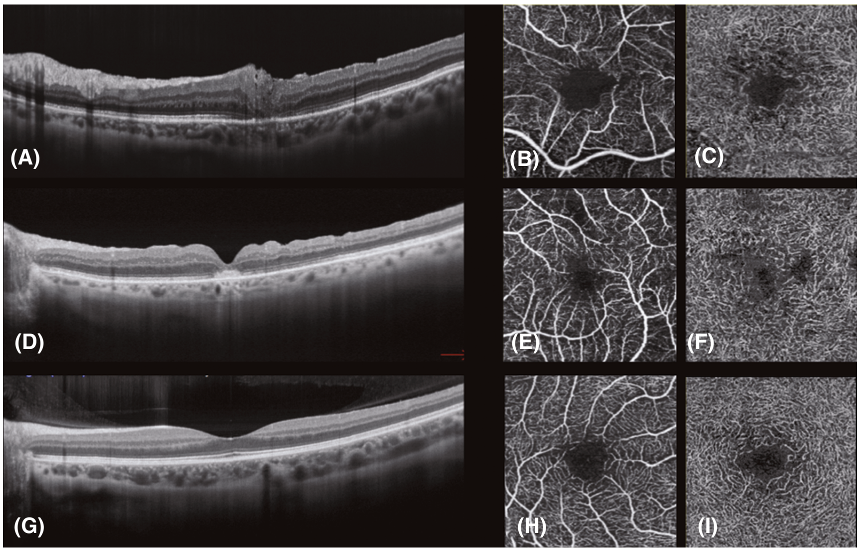

Fig. 3. OCT of an ILM-patient (A) and OCT-Angiography of the same patient (B-C) showing an enlargement of the FAZ and mild congestion of the deep capillary plexus. OCT of an hAM patient (D) and OCT-Angiography of the same patient showing a fairly normal size and appearance of the FAZ (E-F). OCT and OCT-Angiography of the fellow-healthy eye of the same patient (G-H-I)

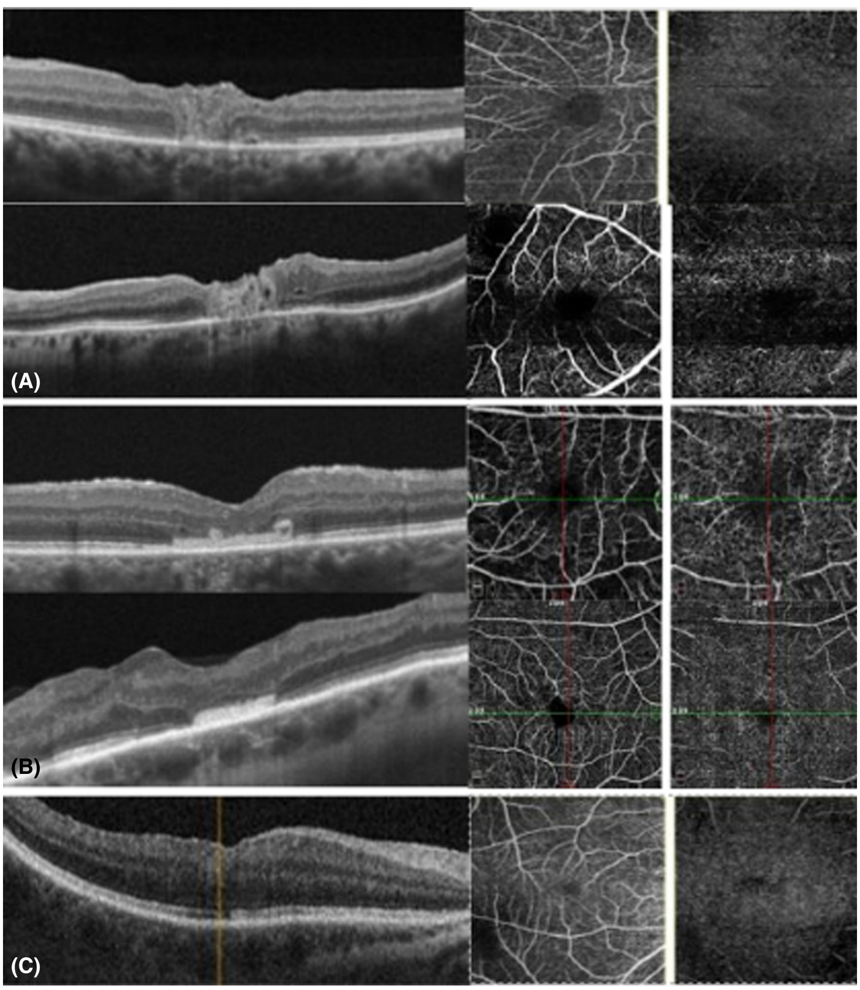

Fig. 4. OCT and OCTA images of patients treated with ILM transplant (A). OCT and OCTA images of patients treated with hAM transplant (B). OCT and OCTA images of a patient affected by macular hole (MH) and treated with conventional peeling

SD-OCT imaging (Lee et al. 2018), indicating a cicatricial healing process rather than the stimulation of cell proliferation, which could explain the reduced cone cell density observed in the present study.

As regards OCT-Angiography analysis, changes in the size of the FAZ have been documented in many pathologies and correlated with functional alterations in previous articles, often identifying them as a prognostic factor. A fairly normal appearance and size of the Foveal Avascular Zone (FAZ) and of parafoveal capillary plexuses was found in hAM-patients, according to the literature (Caporossi et al. 2020), while images of ILM-patients showed an enlargement of the FAZ and a mild congestion of the deep capillary plexus (DCP), in comparison with the fellow-healthy eyes, as previously reported in the literature (Wrzesińska et al. 2020). Moreover, there are previous studies on OCT-Angiography analysis of idiopathic macular holes operated with ILM peeling (Kim et al. 2018; Cicinelli et al. 2019 (2019).;Baba et al. 2017; Pak et al. 2017), where a remarkable reduction of FAZ is always reported. The fairly normal size of the FAZ in our 3 hAM-patients could be related to their higher photoreceptor density found at AO-FIO, and with the formation of layers and the resumption of the foveal depression found at SD-OCT imaging. On the opposite, macular microstructure in ILM-patients appeared completely subverted, with an enlarged FAZ, a reduced photoreceptor density and an alteration of the normal foveal profile with fibroplasia (Fig. 3,4,5).

In conclusion, in our study, the new high-resolution imaging techniques revealed that patients treated with hAM transplant presented morphological features at the 6-month follow-up similar to that of a healthy eye, in comparison with patients treated with ILM transplant that presented a completely subverted anatomical situation. Our findings suggest that different cellular mechanisms underlie the tissue remodelling induced by these two techniques. Further validation with a longer follow-up period and a larger sample size is required to fully characterize these differences.

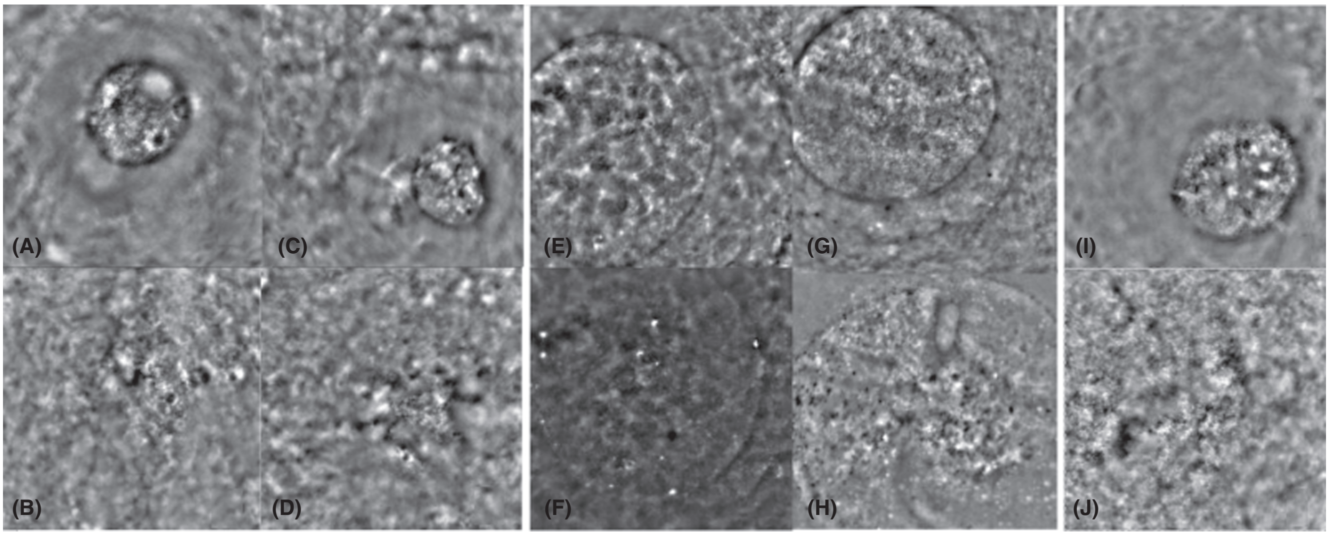

Fig. 5. Preoperative Adaptive Optics images of FMH (A,C,E,G). Six-months postoperative Adaptive Optics images of same patients treated with hAM transplant (B,D) and ILM transplant (F,H). Preoperative Adaptive Optics image of a macular hole (MH) (i); 6-months postoperative Adaptive Optics image of the same patient treated with ILM peeling (l) where irregular areas containing photoreceptors are clearly visible in the context of the lesion

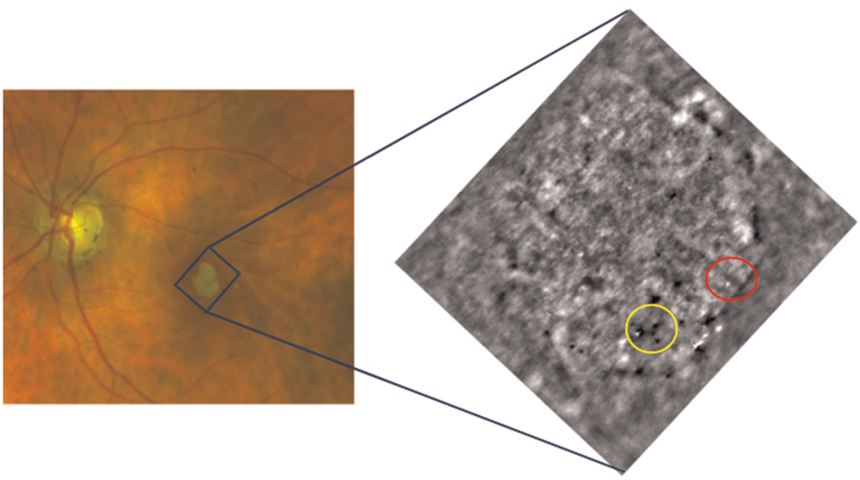

Fig. 6. Adaptive Optics image of a patient treated with hAM transplant labelled on a fundal image; hyper reflective dots are labelled with a red circle, hypo reflective dots are labelled with a yellow circle

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