Università degli Studi di Ferrara

DOCTORAL COURSE IN
ADVANCED THERAPIES AND EXPERIMENTAL PHARMACOLOGY

CYCLE XXXVIII

Coordinator Full Professor KATIA VARANI

Outcomes of Descemet Membrane Epiretinal Graft for Refractory Macular Hole Closure

Scientific/Disciplinary Sector (SDS) MED/30

Candidate
Dr. ADAMO GINEVRA GIOVANNA

Supervisor
Associate Professor
PARMEGGIANI FRANCESCO

Year 2022-2025

INDEX

PART I

ABSTRACT

INTRODUCTION

- *ANATOMY AND PHYSIOLOGY OF THE MACULA*
- *FULL-THICKNESS MACULAR HOLES*
  - *Etiology and pathophysiology*
  - *Epidemiology and risk factors*
  - *Classification*
  - *Clinical presentation and diagnosis*
  - *Surgical management*
- *REFRACTORY AND RECURRENT MACULAR HOLES*
- *SURGICAL ALTERNATIVES FOR REFRACTORY MACULAR HOLE*
  - *ILM-based techniques*
  - *Autologous tissue grafts*
  - *Amniotic membrane transplantation*
  - *Rationale for alternative grafts*
- *DESCEMET MEMBRANE AS A RETINAL SCAFFOLD*

PART II

PURPOSE

MATERIALS AND METHODS

RESULTS

DISCUSSION AND CONCLUSIONS

BIBLIOGRAPHY

Abbreviations and Acronyms

FTMH = full-thickness macular hole; MH = macular hole; ILM = internal limiting membrane; PVD = posterior vitreous detachment; OCT = optical coherence tomography; ERM = epiretinal membrane; ELM = external limiting membrane; EZ = ellipsoid zone; VMA = vitreomacular adhesion; VMT = vitreomacular traction; RPE = retinal pigment epithelium; PPV = pars plana vitrectomy; BCVA = best-corrected visual acuity; ART = autologous neurosensory retinal transplantation; AM = amniotic membrane; DM = Descemet membrane; DMEK = Descemet membrane endothelial keratoplasty; PFCL = perfluorocarbon liquid.

PART I

ABSTRACT

Purpose: To report the visual and anatomical outcomes of Descemet membrane epiretinal graft for refractory full-thickness macular holes (FTMH).

Methods: This interventional case series included patients with refractory macular hole (MH) who previously underwent standard pars plana vitrectomy with internal limiting membrane peeling for repair of MH with no success. Donor corneas unsuitable for keratoplasty were stripped at the Eye Bank. The corneo-scleral button was stained with blue dye, punched to obtain a 2-mm Descemet membrane graft which was positioned over the MH. At the end of surgery, 20% sulfur hexafluoride gas tamponade was administered.

Results: Twelve eyes of 12 patients with a mean follow-up of 8 ± 4 months were included. The MH closure rate was 91.7%. Best-corrected visual acuity significantly improved from 1.09 ± 0.56 preoperatively to 0.46 ± 0.54 logMAR (20/63 Snellen) after surgery (P = 0.006). The external limiting membrane and ellipsoid zone recovery rates were 33.3% and 41.7%, respectively.

Conclusions: Descemet membrane epiretinal graft leads to satisfactory anatomic and visual outcomes for eyes with refractory FTMH.

INTRODUCTION

ANATOMY AND PHYSIOLOGY OF THE MACULA

The macula is a highly specialized area of the neurosensory retina, essential for central and high-resolution visual acuity. The macular area measures 5.5 mm in diameter and consists of three regions: fovea, parafovea, and perifovea, which are characterized by distinct histological and functional features.

The fovea, located at the center of the macula, is uniquely adapted for tasks involving fine spatial detail, color discrimination, and contrast sensitivity. This region contains the highest density of cone photoreceptors and lacks rod photoreceptors, reflecting its specialization for daylight and high-acuity vision. Within the fovea, the central foveola, approximately 350 μm in diameter, has no retinal blood vessels, forming the foveal avascular zone. The lack of vessels minimizes light scattering and enhances the photoreceptor signal transmission. Moreover, the absence of inner retinal layers within the foveal pit further allows incident light to pass with minimal absorption or deviation before reaching the cone outer segments, thus improving optical quality.[1,2]

A defining feature of the foveal architecture is the Müller cell cone, a specialized conical configuration of Müller glial cells that spans the full thickness of the foveal pit. These cells provide essential biomechanical support, metabolic regulation, and optical signal guidance. Additionally, Müller cells regulate extracellular ions and neurotransmitters and maintain retinal homeostasis. The unique arrangement of Müller cells within the fovea is essential for preserving both the structural integrity and the function of the foveal pit. The macular region comprises also the parafovea and the perifovea, as illustrated in Figure 1. The parafovea surrounds the fovea with an approximate width of 0.5 mm. More peripherally lies the perifovea, which is approximately 1.5 mm wide and represents the transitional zone between the macula and the peripheral retina.[2,3]

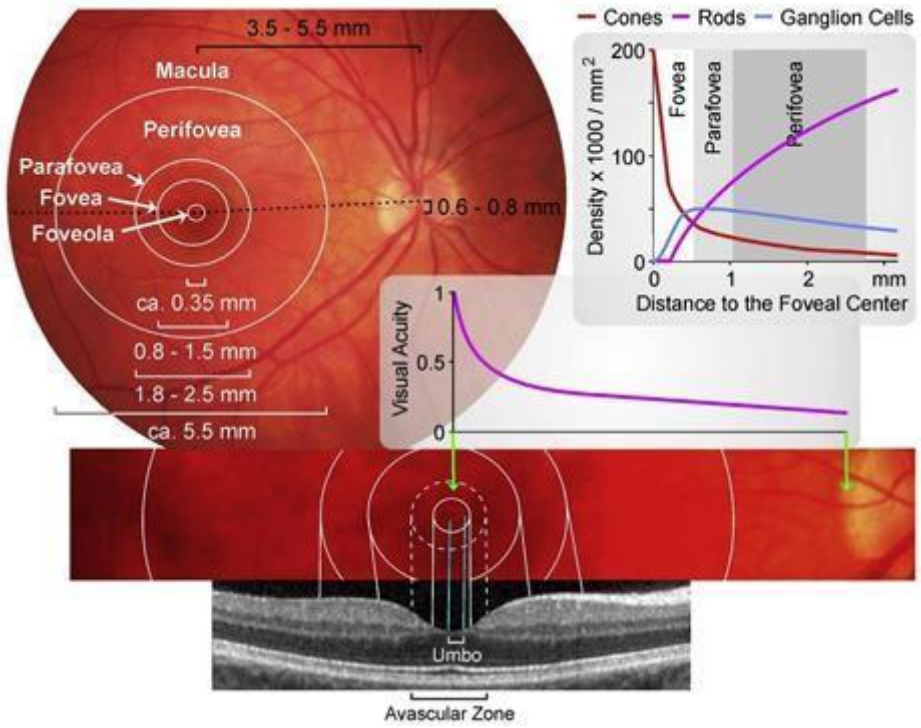

Figure 1. Anatomy of the macula (Bringmann A, Syrbe S, Görner K, Kacza J, Francke M, Wiedemann P, Reichenbach A. The primate fovea: Structure, function and development. Prog Retin Eye Res. 2018 Sep;66:49-84. doi: 10.1016/j.preteyeres.2018.03.006. Epub 2018 Mar 30. PMID: 29609042).

The vitreoretinal interface represents the dynamic junction between the posterior vitreous cortex and the internal limiting membrane (ILM). The posterior vitreous cortex is composed of tightly packed collagen fibrils, hyaluronan, and associated extracellular matrix components. It is firmly anchored to the ILM, particularly at sites of strong adhesion including the vitreous base, the optic nerve head, retinal vessels, and the macula (Figure 2).[4]

The ILM, a highly specialized basement membrane, demarcates the boundary between the vitreous body and the neurosensory retina. It is primarily formed by type IV collagen, laminin isoforms, and proteoglycans synthesized by Müller cell footplates. The ILM thickness varies across the retina, being thinnest in the macular region, a feature that has direct implications for FTMH pathogenesis and surgical manipulation.[5]

Age-related changes and vitreous liquefaction are key factors influencing macular biomechanics. Synchysis (liquefaction of the vitreous) and syneresis (collagen fibers aggregation) contribute to the formation of lacunae within the vitreous body, eventually leading to posterior vitreous detachment (PVD). An incomplete or

anomalous PVD can result in persistent vitreofoveal adhesion, generating tractional forces that increase the risk of MH formation.[6,7]

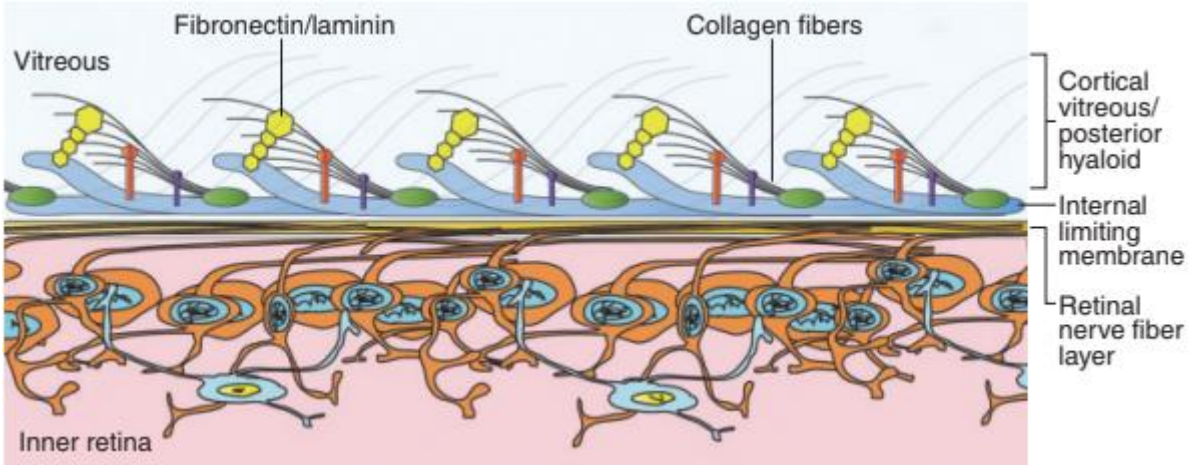

Figure 2. Illustration of the vitreoretinal attachments at the vitreoretinal interface. The posterior vitreous cortex is attached to the ILM by collagen fibers at the vitreoretinal interface. These fibers fuse with the ILM and along with macromolecules, such as laminin, fibronectin, and chondroitin anchor the vitreous cortex to the retina (Barak Y, Ihnen MA, Schaal S. Spectral domain optical coherence tomography in the diagnosis and management of vitreoretinal interface pathologies. J Ophthalmol. 2012;2012:876472. doi: 10.1155/2012/876472. Epub 2012 Jun 4. PMID: 22701779; PMCID: PMC3373197).

FULL-THICKNESS MACULAR HOLES

A full-thickness macular hole (FTMH) is a retinal defect that extends through the entire neurosensory retinal layer at the foveal center, from the ILM to the retinal pigment epithelium (Figure 3). Affected patients complain of central visual field loss, distorted vision (metamorphopsia) and reading difficulties. Diagnosis is primarily based on optical coherence tomography (OCT), which provides detailed cross-sectional imaging of the fovea.

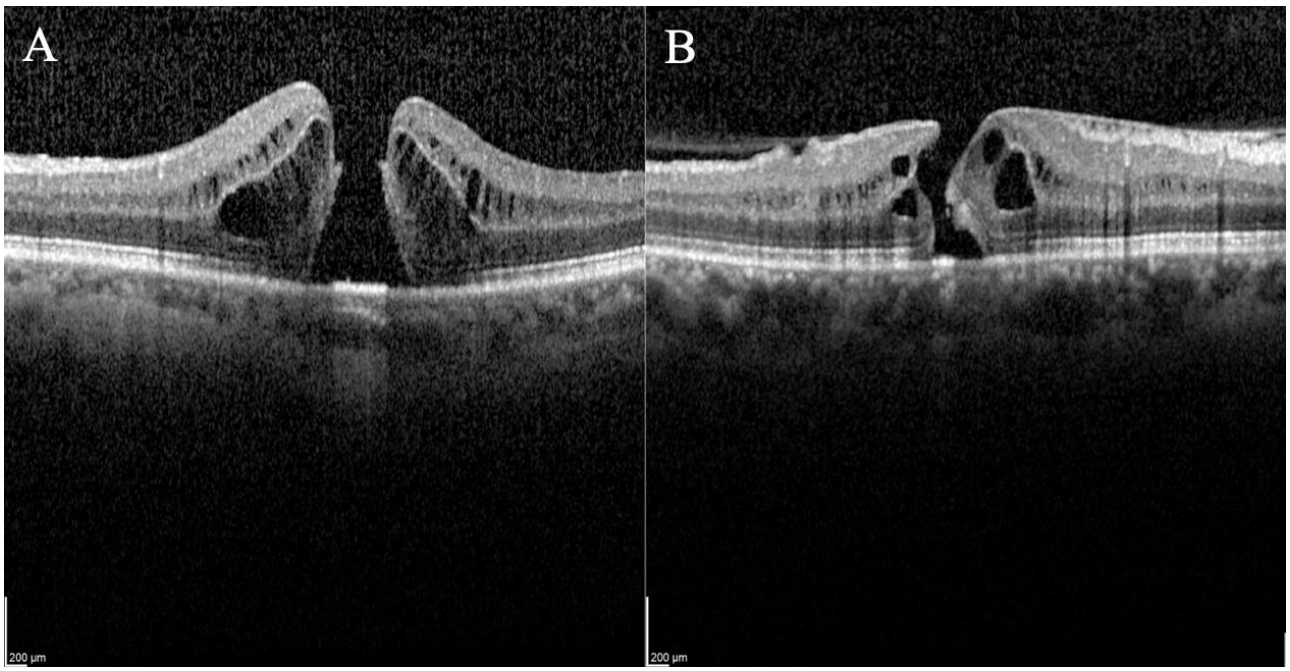

Figure 3. OCT scans from two patients with full-thickness macular holes. A-B. OCT scans showing full-thickness neuroepithelial defects.

Etiology and pathophysiology

Full-thickness macular hole formation is a multifactorial process resulting from the combined effects of tractional mechanisms acting upon the fovea and the intrinsic retinal vulnerability. Anteroposterior traction is related to persistent vitreofoveal adhesion of the posterior vitreous cortex during partial or anomalous PVD, while tangential traction arises from ILM rigidity or epiretinal membrane (ERM) contraction. Experimental and clinical data indicate that anteroposterior traction initiates early foveal deformation, while tangential forces contribute to hole enlargement and chronic evolution of the hole.[8]

Müller cell dysfunction represents another critical factor. Müller cells constitute the primary biomechanical scaffold of the foveal architecture. Disruption of their characteristic cone-shaped configuration during tractional stress may compromise the structural integrity of the foveal center, amplifying susceptibility to full-thickness tissue separation. Histopathological analyses of MH specimens demonstrate Müller cell depletion, gliosis, and disruptions of the outer plexiform layer, confirming the central role of glial alterations in disease pathogenesis.[9]

The full-thickness retinal defect results in centrifugal displacement of photoreceptors from the foveal center, thinning of the outer nuclear layer, and disruption of the

external limiting membrane (ELM) and ellipsoid zone (EZ). These microstructural alterations correlate strongly with visual postoperative outcomes, emphasizing the importance of preserving or, when possible, restoring the integrity of the outer retinal microstructure through appropriate surgical techniques.[10]

Epidemiology and risk factors

The estimated annual incidence of FTMH ranges from 7.8 to 8.5 cases per 100,000 individuals, with prevalence increasing substantially with age. Epidemiologic studies consistently demonstrate a sex disparity, with female-to-male ratio of 2.2-3:1 in several cohorts. [11] This difference has been attributed to sex-specific patterns of vitreous degeneration, hormonal influences on collagen architecture, and potentially longer-lasting macular vitreoretinal adhesions.[12,13]

Age is the most significant epidemiologic determinant. Vitreous liquefaction and anomalous posterior vitreous detachment (PVD) occur more frequently in individuals over 60 years of age, explaining the peak incidence of MH observed during the sixth and seventh decades of life.[14,15]

Classification

For many years, the Gass classification was the standard in classifying FTMHs.[16] The staging was based on biomicroscopic observations and described MH formation as a four-stage process:

- **Stage 1A**: an early or “impending” MH, with persistent vitreous adhesion at the fovea, the loss of the normal foveal depression, and showing a central yellow spot;
- **Stage 1B**: the vitreous is still attached to the fovea, the serous foveolar detachment causes a visible yellow ring;
- **Stage 2**: a FTMH characterized by a pseudo-operculum and vitreous still adherent to the perifoveal region;
- **Stage 3:** a FTMH with vitreous still attached at the optic disc and absence of the Weiss ring;
- **Stage 4:** a FTMH with complete posterior vitreous detachment, often with a Weiss ring.

The introduction of the OCT, providing high-resolution and detailed imaging of retinal layers and associated biomechanical forces, has substantially refined the understanding of MH formation.

Current OCT-based classification expands upon Gass's work by defining the role of vitreofoveal traction and provides a more detailed assessment of morphological features. In this context, the International Vitreomacular Traction Study group established standardized OCT definitions for vitreomacular adhesion (VMA), vitreomacular traction (VMT), and FTMH, which are now widely adopted in both clinical research and surgical planning.[17]

The aim of the International Vitreomacular Traction Study Group was to establish an OCT-based anatomical classification system for vitreomacular interface disorders, including FTMH. Their classification encompasses VMA, VMT, and FTMH; it also provides OCT-based definitions for lamellar macular holes and pseudoholes, which are among the principal differential diagnoses of FTMH. In this classification, FTMHs are defined as a retinal defect involving a disruption of all retinal layers from the ILM to the retinal pigment epithelium (RPE), and are stratified by size, by the presence or absence of vitreomacular traction, and by etiology.

Size classification in based on the minimum linear diameter of the hole, measured at the level of the middle retinal layers on OCT (Figure 4):

- **Small**: ≤ 250 µm;
- **Medium**: > 250 µm and ≤ 400 µm;
- **Large**: > 400 µm.

Regarding the traction status, FTMHs are classified according to whether there is coexisting VMT.

Based on etiology, FTMH can be divided into primary and secondary forms. Most FTMHs are primary, formerly referred to as idiopathic, resulting from vitreous traction on the fovea from anomalous PVD (vitreomacular traction, VMT; Figure 5). Whereas secondary FTMHs result from underlying pathologic conditions, independent of VMT.[17]

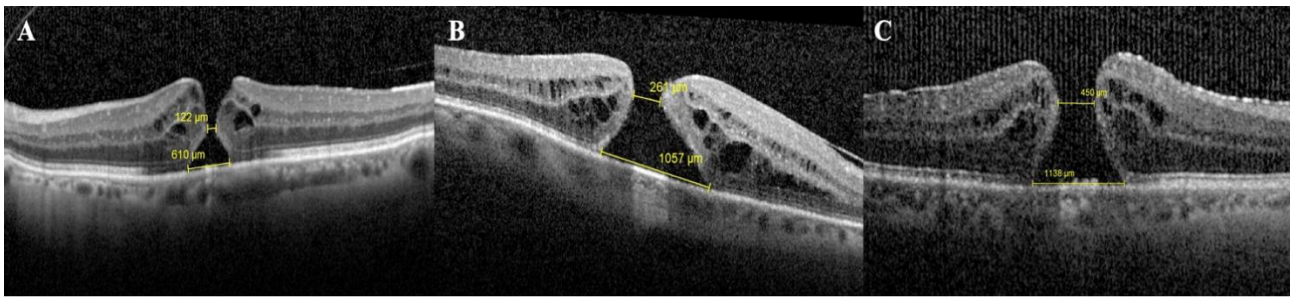

Figure 4. Classification of full-thickness macular holes based on minimum linear diameter: A: Small hole with a minimum linear diameter ≤ 250 µm. B: Medium hole with a minimum linear diameter > 250 µm and ≤ 400 µm. C: Large hole with a minimum linear diameter > 400 µm.

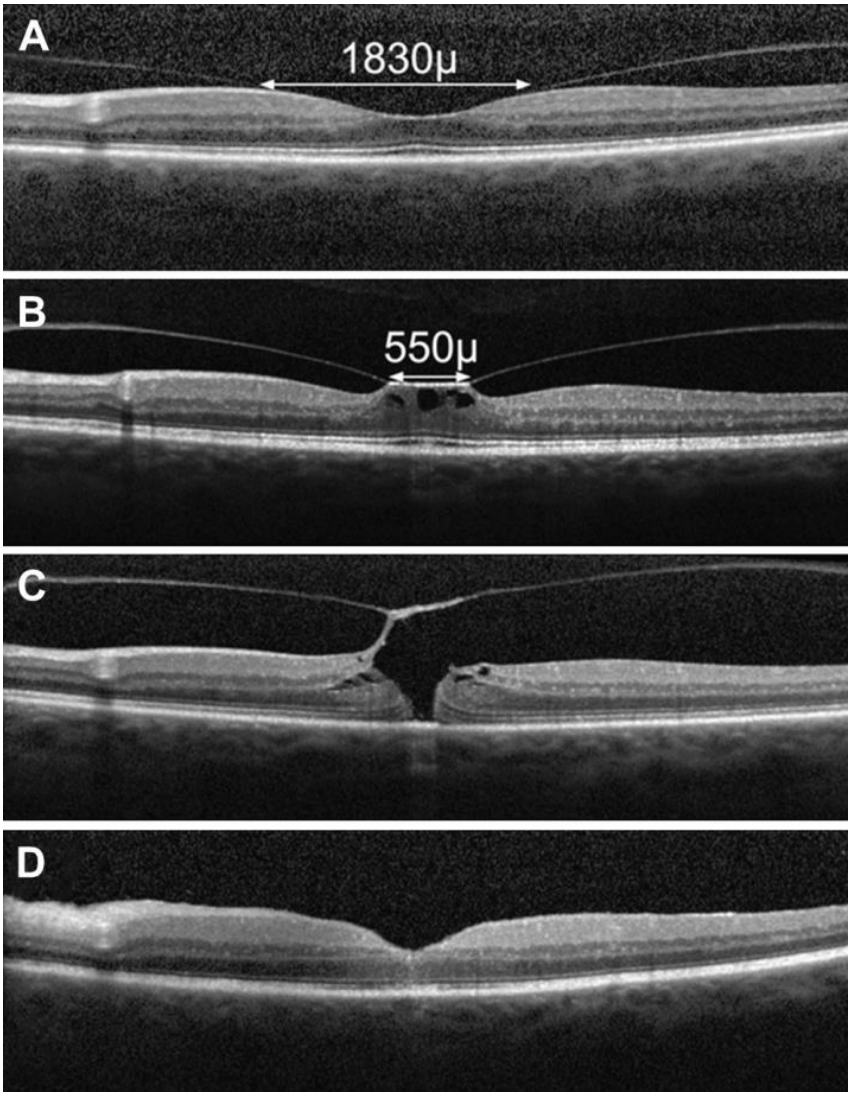

Figure 5. Optical coherence tomography images showing progression of vitreomacular pathologic features. **A**, At presentation, the patient had broad, isolated vitreomacular adhesion (VMA) with the region of attachment having a diameter of 1830 mm. **B**, One year later, she demonstrated mild

distortion. The diagnosis was focal, isolated vitreomacular traction (VMT) with a diameter of attachment of 550 mm. Note the change in contour of the inner fovea with cystoid spaces in the inner retinal tissue. **C**, Three months later, the patient demonstrated a primary macular hole. The inner flap of retina was adherent to the vitreous, there were cystoid spaces in the retina, and there was a slight upturn of the inner margins of the hole. **D**, One month after surgical repair that included vitrectomy, membranectomy, and fluid-gas exchange, the macular hole was closed anatomically (Duker JS, Kaiser PK, Binder S, de Smet MD, Gaudric A, Reichel E, Sadda SR, Sebag J, Spaide RF, Stalmans P. The International Vitreomacular Traction Study Group classification of vitreomacular adhesion, traction, and macular hole. Ophthalmology. 2013 Dec;120(12):2611-2619. doi: 10.1016/j.ophtha.2013.07.042. Epub 2013 Sep 17. PMID: 24053995).

High myopia has emerged as an important risk factor for the development of secondary FTMH (note that some eyes with high myopia demonstrate FTMH with VMT as a precursor), particularly in East Asian populations where the prevalence of myopia is rapidly increasing.[18] High myopic eyes present a distinct biomechanical environment characterized by posterior staphyloma, scleral thinning, and chorioretinal atrophy, which generate additional tractional forces on the fovea, including posterior pole expansion, oblique distortion of the foveal architecture, and mechanical stress on the ILM and inner retinal layers. Myopic MHs consequently tend to be larger, more chronic, and more frequently accompanied by foveoschisis or macular detachment, contributing to poorer surgical prognosis and a higher recurrence rates.[19] The compromised outer retina in these eyes further limits the potential for postoperative restoration of ELM and EZ integrity. Despite advances in surgical techniques, closure rates for high-myopic MHs remain consistently lower than those observed in idiopathic cases, highlighting the need for alternative surgical approaches.[20]

Tangential traction caused by ERMs can also contribute to macular hole formation. ERMs are detected in a significant proportion of FTMH cases, with prevalence estimates ranging from 8% to 40% depending on patient population and imaging modality.[11] Additional secondary causes include the following: blunt trauma, lightning strike, macular schisis, macular telangiectasia type 2,wet macular degeneration treated with anti-vascular endothelial growth factor therapy, microaneurysm, prior intraocular surgery (e.g. vitrectomy).[17]

Clinical presentation and diagnosis

The clinical diagnosis of FTMH is based on history and clinical exam. Central vision loss, central scotoma, or metamorphopsia (distortion of the central vision), may be reported. However, a subset of patients may remain asymptomatic, particularly in early or small lesions. Visual acuity is often substantially reduced, frequently around 20/200. Diagnosis typically involves assessment of visual acuity, direct ophthalmoscopy or slit-lamp biomicroscopy, and OCT. OCT is an high-resolution imaging system allowing for both cross-sectional and three-dimensional evaluation of the macula and has become the gold standard in the diagnosis, management, and follow-up of MHs.[21]

Although OCT remains the main imaging technique for FTMH, fundus autofluorescence (FAF) is also important. FAF can provide a precise evaluation of the size of the macular hole preoperatively and allows assessment of retinal pigment epithelium (RPE) atrophy during follow-up, providing additional prognostic information and guiding surgical planning and postoperative monitoring. [22]

Surgical management

FTMH was largely regarded as untreatable cause of centrale visual loss until 1991, when Kelly and Wendel revolutionized MH management by publishing the first clinical outcomes of patients undergoing pars plana vitrectomy (PPV). In their series, they reported anatomical closure in 58% of eyes, and 73% of those with successful closure experienced a gain of at least two lines in best-corrected visual acuity (BCVA). On the other hand, 15% of the cohort developed complications, such as increase in the size of the macular hole, mottling of the RPE, or vascular occlusion. The surgical procedure involved posterior hyaloid removal, peeling of epiretinal membranes, total air-fluid exchange, and injection of a non-expansible concentration of sulfur hexafluoride (SF_6) gas to provide temporary tamponade. Patients were instructed to maintain strict occiput-up position for at least one week following surgery.[23]

Gas tamponade was proven to be crucial for maintaining temporary apposition of the hole edges. To date, sulfur hexafluoride (SF_6) and perfluoropropane (C_3F_8) are utilized. The primary role of gas tamponade is to isolate the borders of the MH from intraretinal and subretinal fluid, thereby allowing the healing process to occur.

Although increasingly debated, face-down positioning continues to be routinely recommended in many centers, with different timing indications, particularly for large or chronic macular holes, based on evidence suggesting improved anatomical closure.[24,25]

In the decades that followed, the introduction of ILM peeling by Eckardt and colleagues markedly improved MH closure rates by relieving tangential traction and promoting gliotic activity at the hole edges.[26]

ILM peeling, together with the use of gas tamponade and postoperative face-down positioning, has made it possible to improve upon the outcomes reported by Kelly et al. in 1991. In a 2005 randomized clinical study comparing patients with FTMH who underwent ILM removal with those who did not, hole closure was achieved in 92.3% of patients in the peeling group, whereas persistent holes were observed in 68% of those treated with vitrectomy alone. The study demonstrated also a clear functional advantage associated with peeling, as patients in the peeling group gained an average of 3.7 lines of visual acuity, compared with 1.5 lines in the control group who did not undergo ILM removal.[27] Recent studies reported high primary anatomical closure rates in small FTMHs that underwent PPV combined with ILM peeling. The “SMALL” multicenter study reported a closure rate of 98% in the ILM-peel group for small idiopathic macular holes (≤ 250 μm), compared with 85% in the no-peel group ($p < 0.0001$).[28] Furthermore, the ILM is known to serve as a scaffold for epiretinal membrane formation through complex interactions among retinal glial cells, hyalocytes, fibroblasts, and myofibroblasts. Consequently, the removal of the ILM reduces the incidence of postoperative epiretinal membrane development, a recognized complication following MH surgery.[29,30]

Various techniques in combination with ILM peeling, such as ILM flaps, have been reported to enhance the success rates of FTMH surgery.

In 2010, Michalewska et al., introduced the inverted ILM flap technique. [31] The original technique is based on ILM peeling centripetally up to the MH rim and folding it upside-down on top of the MH (“Cover” technique) while one of the most widespread variation is based on folding multiple ILM layers within the MH defect (a variant called “Fill” technique). [32]

The superiority of the inverted ILM flap over standard ILM peeling has been debated over the years. In macular holes measuring 400 μm or less, both techniques have

shown excellent outcomes, with no statistically significant anatomical or functional differences. [33] Therefore, both represent valid options for the treatment of small and medium-sized holes (minimum linear diameter 400 μm or less). Furthermore, in medium-sized holes, better macular sensitivity has been observed in patients treated with ILM peeling compared with those who underwent placement of an inverted ILM flap over the macular defect.[34]

Nevertheless, a systematic review of inverted ILM-flap for large holes (>400 μm), clearly supported the use of the inverted ILM flap technique, which provided a closure rate of 95% of cases and improved the BCVA in 75%.[35] Also Shen et al. have shown better anatomical outcomes in patients with large MHs (minimum linear diameter greater than 400 μm) treated with the inverted flap technique than those treated with ILM peeling: the hole closure rate was higher in the inverted flap group, as was visual acuity three months after surgery. However, at six month postoperatively, BCVA did not differ significantly between the two groups.[36]

More recently, a comparative cohort study indicated a 90.8% primary closure rate following the inverted flap technique versus 59.6% with standard ILM peeling in large macular holes ($p < 0.001$).[37]

Although ILM peeling is highly effective in the surgical treatment of macular holes, the optimal extent of peeling remains unclear. A recent study showed that a larger extent of the ILM peeling leads to a greater tangential movement, possibly improving the MH closure rate.[38]

According to the International Vitreomacular Traction Study, small and medium full-thickness macular holes exhibit a high likelihood of achieving anatomical closure following PPV, ILM peeling and gas tamponade, with a documented primary closure rates of 90–95%. In contrast, the management of large-sized, chronic, refractory (persistent or recurrent) MHs, as well as those associated with retinal detachment, continue to pose significant challenges for vitreoretinal surgeons. Several surgical techniques have been proposed to improve the success rate of this complex entity, however there is not enough evidence from which to draw a firm conclusion regarding the best surgical option.[39]

REFRACTORY AND RECURRENT MACULAR HOLES

Refractory MHs include two categories:

- **Refractory (or persistent) MHs**: failure of anatomical closure following primary surgery;

- **Recurrent MHs**: reopening of a macular hole after initial successful closure.

Reported persistence rates range between 4% and 10%, whereas recurrence rates range from 2% to 10%, depending on MH size, surgical technique, and patient characteristics.[40]

Clinically, refractory MHs tend to be larger, exhibit greater chronicity, and occur more frequently in highly myopic eyes. Postsurgical reopening may result from progressive retinal atrophy, ERM contraction, or the development of new tractional forces.

Several factors can predict the failure of primary vitrectomy:

- **Hole size**

The size of the macular hole is a critical prognostic factor for surgical outcomes. Holes are generally classified as large when the minimum linear diameter exceeds 400 μm, but closure rates vary significantly within this category, highlighting the need to subdivide large hole into prognostically distinct subgroups. Reported closure rates are 98% for holes measuring 400-477 μm, 91% for 478-558 μm, 94% for 559-649, and 76% for 650-1416 μm, with lower rates in the largest holes reflecting greater surgical complexity and higher rates of persistent defects.[41,42]

A large minimum linear diameter (>500 μm) remains the most significant predictor of unsuccessful macular hole closure following primary surgery.[43]

- **Chronicity**

Chronic MHs are characterized by gliotic margins, photoreceptors dropout, and increased rigidity of the ILM, all of which impede the centripetal displacement of retinal tissue necessary for closure. Holes persisting for more than 6–12 months show significantly reduced closure rates and poorer visual recovery.

- **High Myopia**

Myopic MHs frequently coexist with foveoschisis, posterior staphyloma, or retinal thinning. These morphological alterations substantially diminish the efficacy of conventional surgical techniques.[19]

- **Outer retinal integrity**

Preoperative absence or disruption of the ELM and EZ adjacent to the macular hole strongly predicts limited functional recovery, even when anatomical closure is achieved. Restoration of these layers is considered a major determinant of visual prognosis.[44,45]

- **Intraretinal Cystic Changes**

Extensive intraretinal cystic degeneration reflects advanced retinal tissue compromise and reduced reparative potential. Additional factors that may contribute to MHs closure failure include inadequate postoperative positioning and persistent traction from residual ILM or ERM.

OCT has become essential for assessing surgical prognosis in MHs. Preoperative OCT parameters may provide clinically valuable information in predicting anatomical closure patterns in MH surgery. Traditional indices such as macular hole index (MHI), (THI), Hole form factor (HFF) and diameter hole index (DHI) showed significant associations with closure types. Furthermore, not only anatomical closure but also the postoperative restoration of outer retinal layers, especially the integrity of the EZ and ELM, is critical for achieving favorable visual outcomes. Shimozono et al. reported eyes with preoperative ELM or EZ disruption adjacent to the macular hole exhibiting poorer anatomical restoration and reduced visual recovery. [45,46] These biomarkers allow categorization of MHs into prognostic groups and guide the selection of advanced surgical techniques, including grafting methods, for refractory cases.[47]

SURGICAL ALTERNATIVES FOR REFRACTORY MACULAR HOLES

Persistent or recurrent MHs remain a significant challenge for vitreoretinal surgeons, despite major advances in surgical technique. When standard PPV with ILM peeling does not achieve anatomical closure, several innovative techniques have been developed to enhance closure rates, promote retinal remodeling, and optimize functional outcomes.

ILM-based techniques

Free ILM flaps

Free ILM flap transplantation has been introduced as an alternative for large or refractory MHs, particularly when the ILM adjacent to the fovea is unavailable due to prior peeling. In this technique, a free-floating fragment of ILM, harvested from an area near the vascular arcades, is positioned within or over the macular defect to stimulate glial proliferation and facilitate closure.[48]

Although effective, free ILM flaps present several technical limitations, including challenges in maintaining correct flap orientation, a tendency for the flap to fold, displace, or migrate during fluid–air exchange, and the need for precise and highly controlled bimanual manipulation.

Closure rates range between 85% and 95%; however, anatomical variability and surgeon-dependent techniques limit widespread standardization.

Inverted flap techniques

The inverted ILM flap technique, first described by Michalewska et al., involves peeling the perifoveal ILM while maintaining a hinged remnant that is inverted and positioned into or over the MH. This technique has demonstrated particular efficacy in the management of large macular holes (>400 μm) and those associated with high myopia. Advantages include the preservation of a ILM attachment point, which minimizes the risk of flap displacement, and an enhanced restoration of the ELM and EZ.[31]

Closure rates with the inverted ILM flap approach frequently exceed 95%, especially for large idiopathic MHs. Nonetheless, residual discontinuities in the outer retinal layers are common, and the technique is limited by the availability of sufficient residual ILM tissue in eyes that have undergone prior ILM peeling.

Pedunculated flaps

Pedunculate or temporal inverted flaps preserve a broader ILM attachment, allowing easier intraoperative manipulation. These techniques reduce the risk of flap displacement and provide more stable coverage of the MH, representing an evolution of the inverted flap concept and offering improved reproducibility and predictability.[49]

Despite their benefits, ILM flaps techniques have limitations, as they cannot be used

when ILM is completely absent from previous surgery, and if the flap is positioned intraretinally or within the hole it may impede photoreceptor migration. These limitations motivate the search for alternative scaffolds for refractory MHs.

Autologous tissue grafts

Autologous tissue grafts represent an additional class of surgical strategies for refractory MHs management. These grafts provide structural support by filling the foveal defect and promoting tissue bridging and gliosis.

Autologous lens capsule transplantation

Both anterior or posterior autologous lens capsules have been utilized as a scaffold to facilitate the closure persistent MHs. The lens capsule is elastic, transparent, and easily obtainable during cataract surgery or following posterior capsulotomy.[50]

Advantages include: accessibility, biocompatibility, ability to act as a mechanical template for gliosis. Nevertheless, disadvantages are notable: intraoperative difficult manipulation due to the capsule's elasticity, risk of incorrect orientation, variable integration with retinal tissue, postoperative visual outcomes often inferior to those achieved with ILM flaps or other grafts. Reported postoperative BCVA is frequently limited by incomplete restoration of ELM/EZ layers.

Autologous retinal transplantation (ART)

Autologous neurosensory retinal transplantation (ART), introduced by Grewal et al., involves harvesting a full-thickness retinal patch from the midperiphery and positioning it into the MH.[51]

Multiple series report closure rates ranging from 87% to 100%; however, the technique carries substantial challenges: retinal trauma at the donor site, risk of inducing scotomas, need for complex bimanual dissection, risk of graft dislocation, uncertain functional integration of transplanted photoreceptors. While ART can be effective in achieving closure of very large or highly refractory macular holes, the impact on visual recovery remains variable, as structural integration of the graft often requires several months and may remain incomplete.

Autologous grafts are particularly advantageous when local tissue is insufficient. Nevertheless, their use is limited by procedural complexity, donor site morbidity, and

potentially worse restoration of outer retinal architecture due to the invasive nature of graft harvesting.

Amniotic Membrane Transplantation

Human amniotic membrane (AM), widely employed in ocular surface reconstruction, has been adapted to retinal surgery as a scaffold for refractory MHs. AM possesses a thick basement membrane rich in collagen types IV and VII, fibronectin, and laminins, providing a biologically favorable substrate for tissue repair.[52]

AM is biocompatible, anti-inflammatory, and anti-fibrotic. It can be placed either subretinally, beneath the MH edges, or epiretinally, overlying the retinal surface. Studies have reported closure rates ranging from 90% to 100% for refractory holes, including highly myopic cases.[53] However, subretinal placement of the membrane may impede photoreceptor layer reapproximation, potentially limiting functional recovery. Despite its advantages, the use of amniotic membrane (AM) as a retinal scaffold is associated with limitations:

- challenges in correctly orienting the AM due to the asymmetry of its stromal and epithelial surfaces;
- potential folding or detachment during fluid–air exchange;
- risk of photoreceptor or RPE damage during subretinal placement;
- delayed or incomplete restoration of the ELM and EZ.

Furthermore, the relatively greater thickness of AM compared with ILM or Descemet membrane (DM) may also impede a precise anatomical alignment of the outer retinal layers.

Rationale for alternative grafts

Gliosis represents a primary mechanism underlying MH closure following surgery. Müller glial cells proliferate and migrate centripetally to bridge the tissue defect created by the hole. When the edges of the MH lack adequate support, this reparative process becomes insufficient, particularly in large or chronic holes. A graft placed over or within the MH provides a structural *scaffold* that facilitates:

- centripetal migration of Müller cells;
- extracellular matrix deposition;

- mechanical stabilization of the tissue defect;
- promotion of centripetal retinal remodeling.

Histologic analyses have demonstrated that transplanted scaffolds guide glial reorganization, creating a matrix that supports reconstitution of the outer retinal layers, including the ELM and EZ.[54]

While many grafting techniques achieve high closure rates, functional recovery depends heavily on:

- restoration of ELM and EZ;
- absence of trauma to outer retinal layers;
- scaffold transparency and thinness;
- capacity to permit photoreceptor migration.

Comparisons among techniques suggest:

- ELM/EZ restoration tends to be highest with epiretinal or thin grafts (ILM, DM);
- subretinal placement frequently compromises photoreceptor reorganization;
- autologous retina closes holes but does not necessarily integrate functionally.

Therefore, an optimal graft should exhibit the following characteristics:

- Biocompatibility: minimal induction of inflammation or fibrosis;
- Transparency: to prevent obstruction of the visual axis;
- Thinness: to allow close apposition and potential restoration of photoreceptors;
- Structural stability: to resist dislocation during surgery;
- Ease of manipulation: particularly during fluid–air exchange;
- Ability to support glial proliferation: providing a substrate for Müller cell activity.

This rationale sets the foundation for the use of Descemet membrane as a novel grafting material.

DESCEMET MEMBRANE AS A RETINAL SCAFFOLD

The evolution of corneal surgery has progressed from full-thickness keratoplasty to increasingly selective procedures, culminating in the development of Descemet Membrane Endothelial Keratoplasty (DMEK) in 2006. DMEK involves transplantation of the Descemet membrane and endothelial layer, enabling lower rejection rates and faster postoperative recovery.[55,56] However, high-quality endothelial tissue is essential for successful corneal transplantation, a substantial proportion of tissues are excluded due to insufficient endothelial cell density, and are more frequently derived from older donors, highlighting the influence of donor age on endothelial integrity. Current eye-bank criteria remain stringent, resulting in the exclusion of up to 50% of donor corneas.[57,58]

These limitations prompted us to investigate an alternative clinical application, specifically the treatment of FTMH. The acellular and mechanically stable Descemet membrane (DM) provides an attractive biological scaffold, supporting glial migration and proliferation, which may facilitate macular hole closure and restoration of retinal microarchitecture.[59] The absence of cellular components minimizes immunogenicity and inflammation, enhancing its suitability for intraocular transplantation. Owing to its biocompatibility and adhesion to neurosensory retina, DM may serve as a promising substrate for FTMH repair, potentially complementing or replacing established techniques using the ILM, lens capsule, or human AM. DM may offer several practical advantages, including transparency, thinness to minimize interference with outer retinal architecture, elasticity for controlled manipulation without excessive folding, and ease of staining for intraoperative visualization. Unlike AM, DM lacks an adhesive stromal surface, reducing graft orientation difficulties, and, compared with retinal autografts, avoids donor-site trauma and graft-related morbidity.

PART II

PURPOSE

The purpose of this study was to evaluate the use of Descemet's membrane as a novel biological scaffold in the surgical management of refractory FTMHs, focusing on the anatomical effectiveness and safety of the surgical technique.

MATERIAL AND METHODS

This retrospective study included consecutive patients who underwent Descemet membrane epiretinal graft for refractory MH at the Ophthalmology Unit of the Sant'Anna University Hospital (Ferrara, Italy) or at Ospedali Privati Forlì "Villa Igea" (Forlì, Italy) between September 2023 and September 2024. Informed consent was obtained from all patients, the study adhered to the tenets of the 2013 Declaration of Helsinki and was approved by the local Institutional Review Board.

All included patients had either a refractory MH, defined as failure of hole closure after primary surgery, or a recurrent MH, defined as reopening after initial successful closure, and had previously undergone standard MH surgery via pars plana vitrectomy and ILM peeling.

All patients received a complete ophthalmological examination including macular OCT before surgery and postoperatively during the follow-up period. The MH minimum diameter was measured with OCT as the diameter at the narrowest point between the hole edges through the foveal center, with measurements performed at the level of the middle retinal layers. Since highly myopic eyes were included, OCT measurements were corrected for axial length by linear scaling.[60] The basal MH diameter was defined as the diameter between hole edges at the retinal pigment epithelium level. The pattern of MH closure was classified as U-shape, V-shape, or flap closure (MH closure by the Descemet membrane epiretinal graft only).[61] The continuity of the EZ and ELM was assessed using OCT images. For each eye, these layers were classified simply as either "intact" or "disrupted", without further grading of disruption severity. Evaluations were performed independently by two experienced retinal specialists, and any discrepancies were resolved by consensus. Postoperative

restoration of the ELM and EZ was defined as homogenous reflectivity within the respective layers in the foveal region.

For Descemet membrane epiretinal graft, human donor corneas unsuitable for corneal transplantation due to low endothelial cell count were obtained by eye banks. Prior to shipping of the tissue, the Descemet membrane was manually stripped by an experienced eye bank technician leaving a small peripheral hinge. The peeled tissue was then repositioned back on the corneo-scleral button.

The surgical technique is summarized as follows: 25-gauge pars plana vitrectomy was performed with a chandelier endo-illuminator to facilitate bimanual maneuvers. After the injection of triamcinolone acetonide, the vitreous base was shaved. Blue dye was used to assess the presence of ILM remnants in the macular region. The donor corneo-scleral button was positioned on a trephination block and the endothelial layer was decellularized using a polyvinyl alcohol sponge. After staining the tissue with MembraneBlue-Dual (Trypan Blue 0.15% + Brilliant Blue G 0.025%; D.O.R.C., the Netherlands), a dermal punch was used to cut a circular graft of Descemet membrane with a 2-mm diameter, a size selected according to the surgeon's experience to ensure adequate coverage of the macular defect and safe intraocular handling. The Descemet membrane graft was inserted through one of the cannulas using vitreoretinal forceps and gently placed over the MH (Figure 6).

In case of poor attachment of the graft to the inner retinal surface, a small bubble of perfluorocarbon liquid (PFCL) was injected over the graft to stabilize it. Fluid-air exchange was performed by passive aspiration with a backflush needle over the disk. To avoid turbulence, infusion air pressure was set at 20 mmHg. In case of decentration of the graft during this phase, the tip of the backflush needle was used to gently reposition the Descemet graft over the MH. At the end of surgery, air was replaced with 20% sulfur hexafluoride gas (Figure 6). After surgery, patients were instructed to maintain a face down position for three days. Follow-up ophthalmological examinations were performed at 1 day, 1 week, 1, 3, 6 and 12 months after surgery. Statistical analysis was performed using R and RStudio software. The Wilcoxon test was used to compare continuous variables before and after surgery. A P-value of less than 0.05 was considered statistically significant.

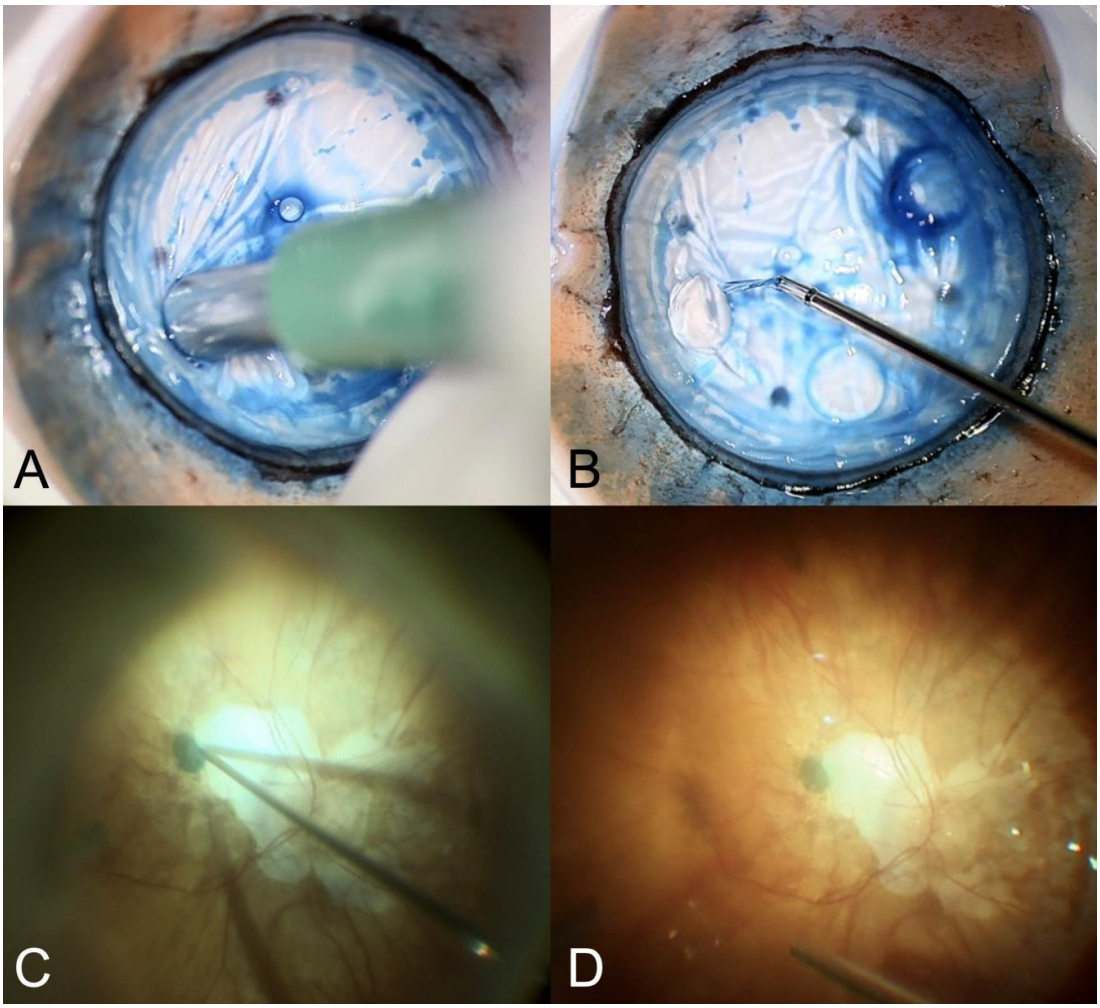

Figure 6. Intraoperative photographs showing the surgical procedure for Descemet membrane epiretinal graft; (**A**) the donor corneo-scleral button is punched with a 2-mm circular dermal punch; (**B**) the Descemet graft is grasped with end-gripping vitreoretinal forceps; (**C**) the graft is positioned on the inner retinal surface over the macular hole; (**D**) the graft is in position after fluid-air exchange.

RESULTS

A Total of 12 eyes of 12 patients with refractory MH were included. Baseline clinical and demographic parameters are reported in Table 1. Mean axial length was 26.1 ± 3.1 mm (range, 23.1–31.7 mm), with 4 patients (33.3%) having an axial length >26 mm. The mean minimum and basal MH diameters were 523 ± 129 and 1136 ± 756 µm, respectively. All patients were pseudophakic and had previously undergone pars plana vitrectomy with ILM peeling. The mean time interval between primary surgery and repeat vitrectomy with Descemet membrane epiretinal graft was 4 ± 5 months

(range, 1–17 months). Surgery was uneventful in all cases; PFCL was employed in 4 patients (33.3%), in the remaining 8 ones (66.7%), the Descemet graft attached via gas tamponade alone. The mean follow-up after Descemet membrane epiretinal graft was 8 ± 4 months (range 3-12 months). Complete data were available for all 12 eyes at 3 months, for 10 eyes at 6 months, and for 6 eyes at 12 months. A complete closure of the MH was obtained in 11/12 patients (91.7%) (Figure 2, Figure 3). Mean BCVA significantly improved from 1.09 ± 0.56 (20/250 Snellen) to 0.47 ± 0.56 logMAR (20/63 Snellen) at 3 months, 0.49 ± 0.62 logMAR (20/63 Snellen) at 6 months, and 0.29 ± 0.19 logMAR (20/40 Snellen) at 12 months (all $P < 0.05$). At the last follow-up available, which represents the final visit for each patient and could occur before 12 months in some cases, mean BCVA was 0.46 ± 0.54 logMAR (20/63 Snellen), with 7 patients (58.3%) achieving a BCVA ≥ 20/40.

Characteristic	All patients (n = 12)	No high myopia (n = 8)	High myopia (n = 4)
Women, n (%)	6 (50)	2 (25)	4 (100)
Age (y), mean ± SD	67.7 ± 9.9	68.1 ± 6.5	66.1 ± 16.1
Axial length (mm), mean ± SD	26.1 ± 3.1	24.1 ± 0.8	30.1 ± 1.6
MH minimum diameter (µm), mean ± SD	523 ± 129	526 ± 155	515 ± 63
MH basal diameter (µm), mean ± SD	1136 ± 756	1015 ± 247	1376 ± 1356
Baseline BCVA (logMAR), mean ± SD	1.09 ± 0.56	1.05 ± 0.52	1.16 ± 0.71

SD: standard deviation; MH: macular hole.

Table 1. Characteristics of patients undergoing Descemet membrane epiretinal graft.

Macular OCT imaging showed a U-shape MH closure in 5 patients (41.7%), V-shape closure in 6 patients (50.0%), while flap closure was not observed in any of the patients. Restoration of the ELM and EZ was observed in 4 (33.3%) and 5 (41.7%) patients, respectively.

The Descemet graft was visible at macular OCT as a thin hyperreflective line (Figure 7, Figure 8); the appearance of the graft did not change over the follow- up time. Besides surgical failure in 1 patient who experienced dislocation of the graft and persistence of the MH, no other intraoperative or postoperative complications occurred. A second surgery was proposed to the patient who failed, but she declined further treatment.

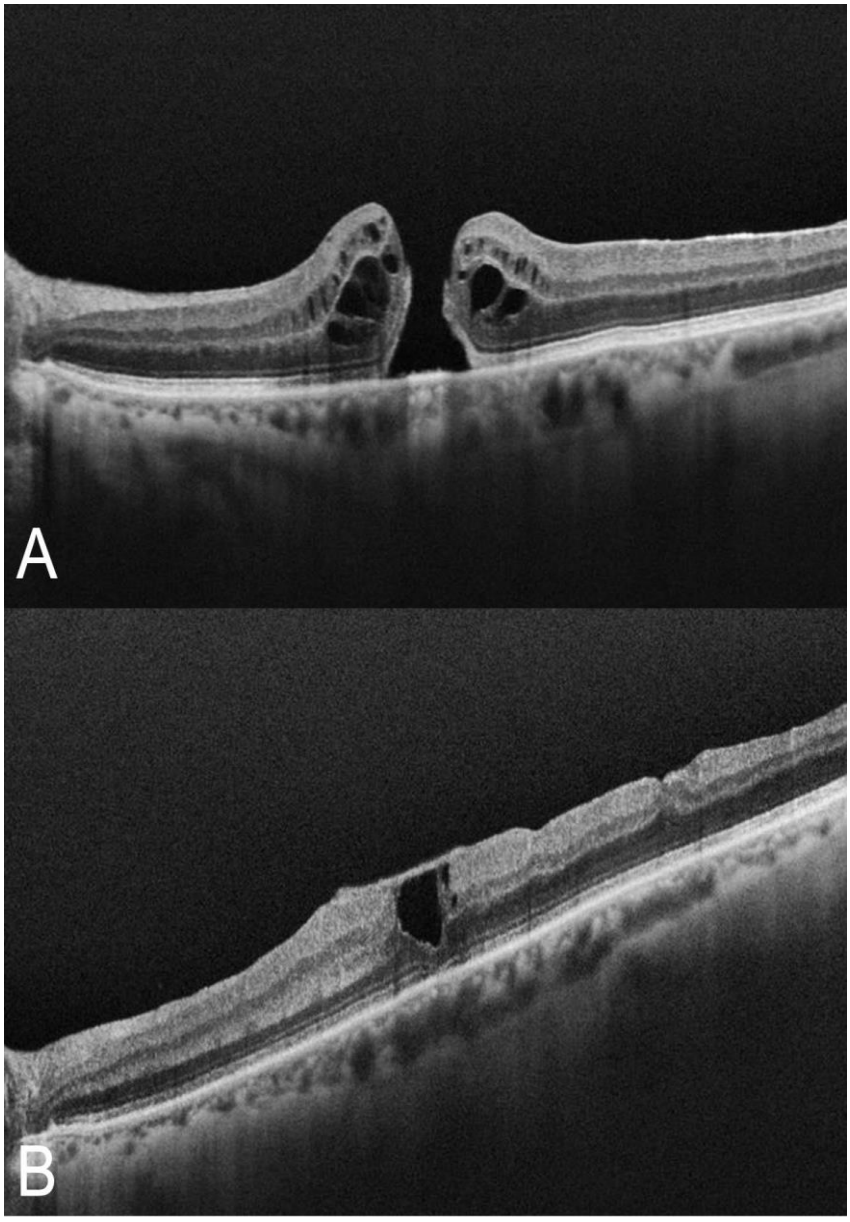

Figure 7. OCT showing a representative case who underwent Descemet membrane epiretinal graft.

A, Refractory full-thickness macular hole before surgery, vision was 20/100. **B**, Closure of the hole after surgery, vision improved to 20/25. The Descemet graft is visible as a thin hyperreflective line bridging the foveal depression.

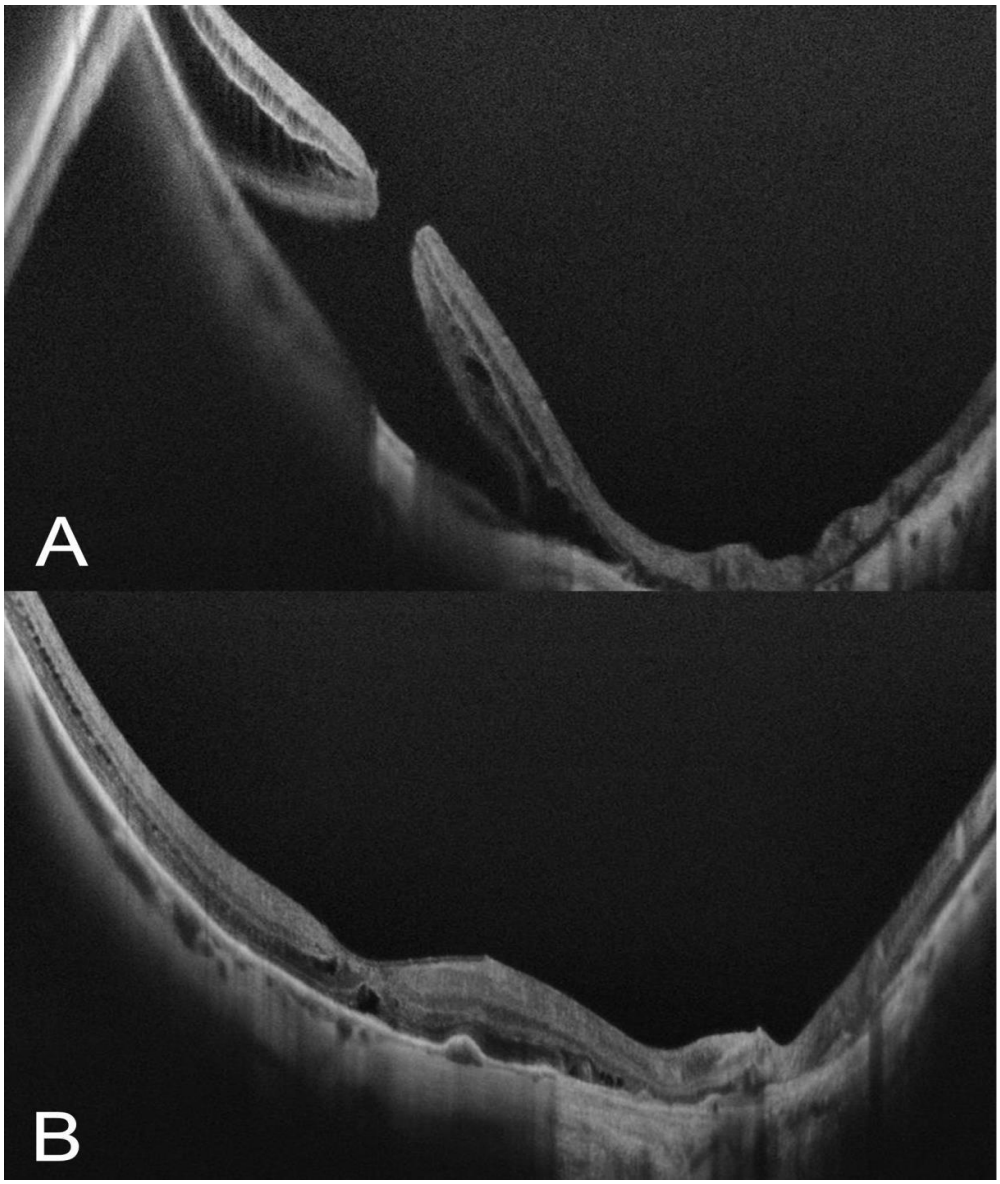

Figure 8. OCT showing a representative case who underwent Descemet membrane epiretinal graft. **A**, Refractory full-thickness macular hole in an eye with high myopia before surgery, vision was 20/200. **B**, Closure of the hole after surgery, vision improved to 20/50. The Descemet graft is visible as a thin hyperreflective line on the inner retinal surface.

DISCUSSION AND CONCLUSIONS

In the era of lamellar keratoplasty, the routine preparation of corneal grafts for endothelial disease includes isolating the Descemet membrane and endothelium, resulting in a transparent graft that spontaneously rolls itself when stripped off from the donor cornea.

The Descemet membrane is a true basement membrane constituted primarily by a matrix of laminins, type IV collagen, nidogens and perlecan, aligning with the structural and biochemical requirements for an effective retinal scaffold.[62] This transparent tissue has a thickness of approximately 10 μm, thinner than amniotic membrane and comparable to the ILM. Its elastic, acellular matrix provides a smooth, homogeneous surface and might serve as a scaffold to promote retinal gliosis, thus allowing the successful closure of MH. We have recently described the epiretinal transplantation of a Descemet membrane graft in two eyes with refractory MH. [59] We have reported herein the outcomes of the first series of patients treated with this new surgical technique. Anatomical closure of the hole was obtained in 91.7% of eyes. This result is in line with those obtained with other alternative techniques for refractory MH. For instance, a closure rate of 87.8% was reported by Grewal et al. after autologous retinal transplantation.[51] Morizane et al. reported a 90% closure rate using autologous ILM transplants.[48] In eyes with recurrent MH and high myopia treated with amniotic membrane transplantation, Caporossi et al. reported closure rates of 93.8% after a single intervention, and 100% after two interventions.[63]

Although closure rates among different techniques appear similar, Descemet membrane epiretinal graft may provide some advantages in terms of visual outcomes. Specifically, the Descemet graft is thin and transparent, and the epiretinal positioning allows for a more physiologic closure of the MH with restoration of the outer retinal layers (which is a well-known predictor of visual recovery), avoiding a direct trauma to photoreceptors and the RPE.[64]

On the other hand, when tissue plugs such as amniotic membrane are placed below the edge of the hole, they can act as a barrier against the re-approximation of the photoreceptor layer, resulting in EZ and ELM defects. [65] Furthermore, the insertion of a tissue plug inside the hole may result in mechanical damage to the RPE and development of parafoveal atrophy. [66] In this series, despite the relatively short follow-up period, restoration of the ELM and EZ was observed in 33.3 and 41.7 of patients,

with a mean final logMAR BCVA of 0.46. This visual result compares favorably with the BCVA reported following the insertion under the edges of the hole of amniotic membrane (0.65 logMAR by Caporossi et al.), autologous lens capsule (1.07 logMAR by Chen et al.) or autologous retinal graft (1.05 logMAR by Moysidis et al.). [65,67,68] While the epiretinal positioning may theoretically increase the risk of graft dislocation, a series of crucial surgical steps allows the successful attachment of the Descemet graft via gas tamponade. First, accurate shaving of the vitreous base should be performed to avoid egress of fluid from the hydrated peripheral vitreous during fluid-air exchange. To avoid turbulence that may dislocate the graft, fluid-air exchange should be slowly performed with an air pressure of 20 mmHg or less. At the end of this phase, in presence of a shallow layer of fluid moving towards the tip of the backflush needle, the graft may slightly dislocate tangentially, and can be gently repositioned with the tip of the cannula. In case of poor attachment to the inner retinal surface, a small bubble of PFCL can be injected to stabilize the graft in place during fluid-air exchange. Finally, once the vitreous chamber is filled with air, any additional fluid accumulating over the disc should be patiently aspirated with the backflush needle until the macula remains completely dry for at least a couple of minutes.

From a technical standpoint, Descemet membrane epiretinal graft may provide some advantages compared to other alternative techniques, resulting in a more standardized and reproducible surgery. The Descemet graft can be stained with blue and easily visualized inside the vitreous chamber. Being relatively dense, it can be placed on the retinal surface in a single-layer fashion, with less tendency to float around into the vitreous chamber or fold on itself compared to amniotic membrane and ILM flaps. Moreover, in contrast to the amniotic membrane, the graft does not have an adhesive surface, requiring less bimanual maneuvers inside the vitreous chamber to obtain the desired orientation. In this study, the graft was positioned with the convex endothelial side facing the retinal surface. The flap naturally exhibits a convex shape which allows the surgeon to correctly orient it even though it is transparent and only lightly stained with blue. Finally, the graft preparation does not require any additional invasive intraocular maneuver. By contrast, ILM flaps and autologous lens capsule require intraocular harvesting. Since our standard surgical technique for primary MH involves an ILM peeling extended over the vascular arcades, an ILM flap may not be available in case of failure of the primary surgery.[41] Similarly, the posterior capsule cannot be employed in eyes which already underwent

a posterior capsulotomy.

According to eye bank statistics, up to half of harvested corneas are deemed unsuitable for keratoplasty, with low endothelial density as the leading cause for corneal tissue discard.[57,69] Descemet membrane epiretinal grafting represents an alternative use of valuable human donor tissue that would otherwise be discarded. Other potential sources of Descemet membrane grafts include the tissue removed during descemetorrhexis in endothelial keratoplasty procedures or the donor tissue from the corneal periphery which is not transplanted during endothelial keratoplasty.

In conclusion, corneas considered unsuitable for keratoplasty can be employed for MH surgery, preventing the wastage of precious tissues from human donors. Descemet membrane epiretinal graft yields positive anatomic and visual outcomes for eyes with refractory MH.

BIBLIOGRAPHY

1. Behar-Cohen F, Gelizé E, Jonet L, Lassiaz P. Anatomie de la rétine [Anatomy of the retina]. *Med Sci* (Paris). 36(6-7):594-599 (2020).
2. Bringmann A, Syrbe S, Görner K, Kacza J, Francke M, Wiedemann P, Reichenbach A. The primate fovea: Structure, function and development. *Prog Retin Eye Res.* 66:49-84 (2018).
3. Bringmann A, Pannicke T, Grosche J, Francke M, Wiedemann P, Skatchkov SN, Osborne NN, Reichenbach A. Müller cells in the healthy and diseased retina. *Prog Retin Eye Res*. 25(4):397-424 (2006).
4. Sebag J, Wang MY, Nguyen D, Sadun AA. Vitreopapillary adhesion in macular diseases. *Trans Am Ophthalmol Soc.* 107:35-44 (2009).
5. Bu SC, Kuijer R, van der Worp RJ, Li XR, Hooymans JM, Los LI. The Ultrastructural Localization of Type II, IV, and VI Collagens at the Vitreoretinal Interface. *PLoS One*. 10(7):e0134325 (2015).
6. Bottós J, Elizalde J, Arevalo JF, Rodrigues EB, Maia M. Vitreomacular traction syndrome*. J Ophthalmic Vis Res*. 7(2):148-61 (2012).
7. Phillips JD, Hwang ES, Morgan DJ, Creveling CJ, Coats B. Structure and mechanics of the vitreoretinal interface. *J Mech Behav Biomed Mater*. 134:105399 (2022).
8. Gaudric A, Haouchine B, Massin P, Paques M, Blain P, Erginay A. Macular hole formation: new data provided by optical coherence tomography. *Arch Ophthalmol*. 117(6):744-51 (1999).
9. Ishikawa M, Sawada Y, Yoshitomi T. Structure and function of the interphotoreceptor matrix surrounding retinal photoreceptor cells. *Exp Eye Res*. 133:3-18 (2015).
10. Shimozono M, Oishi A, Hata M, Kurimoto Y. Restoration of the photoreceptor outer segment and visual outcomes after macular hole closure: spectral-domain optical coherence tomography analysis. *Graefes Arch Clin Exp Ophthalmol*. 249(10):1469-76 (2011).

11. Forsaa VA, Lindtjørn B, Kvaløy JT, Frøystein T, Krohn J. Epidemiology and morphology of full-thickness macular holes. *Acta Ophthalmol.* 96(4):397-404 (2018).
12. Inokuchi N, Ikeda T, Nakamura K, Morishita S, Fukumoto M, Kida T, Oku H. Vitreous estrogen levels in patients with an idiopathic macular hole. *Clin Ophthalmol.* 9:549-52 (2015).
13. Hayashi K, Sato T, Manabe SI, Hirata A. Sex-Related Differences in the Progression of Posterior Vitreous Detachment with Age. *Ophthalmol Retina.* 3(3):237-243 (2019).
14. Gass JD. Idiopathic senile macular hole. Its early stages and pathogenesis. *Arch Ophthalmol.* 106(5):629-39 (1988).
15. La Cour M, Friis J. Macular holes: classification, epidemiology, natural history and treatment. *Acta Ophthalmol Scand*. 80(6):579-87 (2002).
16. Gass JD. Reappraisal of biomicroscopic classification of stages of development of a macular hole. Am J Ophthalmol. 119(6):752-9 (1995).
17. Duker JS, Kaiser PK, Binder S, de Smet MD, Gaudric A, Reichel E, Sadda SR, Sebag J, Spaide RF, Stalmans P. The International Vitreomacular Traction Study Group classification of vitreomacular adhesion, traction, and macular hole. *Ophthalmology*. 120(12):2611-2619 (2013).
18. Cho SD, Lee EK, Yoon CK, Park UC. Long-term surgical outcome of high myopic full-thickness macular hole without retinoschisis. *BMC Ophthalmol.* 25(1):485 (2025).
19. Ikuno Y. OVERVIEW OF THE COMPLICATIONS OF HIGH MYOPIA. *Retina.* 37(12):2347-2351 (2017).
20. Yue H, Liu C, Zhang Y, Zhang L, Gao Z, Ma T, Zhang X. Etiologies and clinical characteristics of macular hole: An 8-year, single-center, retrospective study. *Medicine* (Baltimore). 103(32):e37878 (2024).
21. Hassenstein A, Scholz F, Richard G. OCT bei Makulaforamen [OCT in macular holes]. *Ophthalmologe.* 101(8):777-84 (2004).
22. Teke MY, Cakar-Ozdal P, Sen E, Elgin U, Nalcacıoglu-Yuksekkaya P, Ozturk F. Fundus autofluorescence imaging of patients with idiopathic macular hole. Int J Ophthalmol. 2013 Oct 18;6(5):685-9. doi: 10.3980/j.issn.2222-3959.2013.05.26. PMID: 24195050; PMCID: PMC3808922.

23. Kelly NE, Wendel RT. Vitreous surgery for idiopathic macular holes. Results of a pilot study. *Arch Ophthalmol*. 109(5):654-9 (1991).
24. Ye T, Yu JG, Liao L, Liu L, Xia T, Yang LL. Macular hole surgery recovery with and without face-down posturing: a meta-analysis of randomized controlled trials. *BMC Ophthalmol*. 19(1):265 (2019).
25. Cundy O, Lange CA, Bunce C, Bainbridge JW, Solebo AL. Face-down positioning or posturing after macular hole surgery. *Cochrane Database Syst Rev*. 11(11):CD008228 (2023).
26. Eckardt C, Eckardt U, Groos S, Luciano L, Reale E. Entfernung der Membrana limitans interna bei Makulalöchern. Klinische und morphologische Befunde [Removal of the internal limiting membrane in macular holes. Clinical and morphological findings]. *Ophthalmologe.* 94(8):545-51 (1997).
27. Kwok AK, Lai TY, Wong VW. Idiopathic macular hole surgery in Chinese patients: a randomised study to compare indocyanine green-assisted internal limiting membrane peeling with no internal limiting membrane peeling. *Hong Kong Med J.* 11(4):259-66 (2005).
28. Marolo P, Caselgrandi P, Fallico M, Parisi G, Borrelli E, Ricardi F, Gelormini F, Ceroni L, Reibaldi M; SMALL Study Group. Vitrectomy in Small idiopathic MAcuLar hoLe (SMALL) study: Internal limiting membrane peeling versus no peeling. *Acta Ophthalmol.* 103(3):e156-e164 (2025).
29. Ożóg MK, Nowak-Wąs M, Rokicki W. Pathophysiology and clinical aspects of epiretinal membrane - review. *Front Med* (Lausanne). 10:1121270 (2023).
30. Azuma K, Ueta T, Eguchi S, Aihara M. EFFECTS OF INTERNAL LIMITING MEMBRANE PEELING COMBINED WITH REMOVAL OF IDIOPATHIC EPIRETINAL MEMBRANE: A Systematic Review of Literature and Meta-Analysis. *Retina.* 37(10):1813-1819 (2017).
31. Michalewska Z, Michalewski J, Adelman RA, Nawrocki J. Inverted internal limiting membrane flap technique for large macular holes*. Ophthalmology*. 117(10):2018-25 (2010).
32. Lai CC, Chen YP, Wang NK, Chuang LH, Liu L, Chen KJ, Hwang YS, Wu WC, Chen TL (2015) Vitrectomy with internal limiting membrane repositioning and autologous blood for macular hole retinal detachment in highly myopic eyes. Ophthalmology 122: 1889–98

33. Lee SM, Lee JW, Lee JE, Choi HY, Lee JS, Byon I. Efficacy of inverted inner limiting membrane flap technique for macular holes of ≤400 μm: A systematic review and meta-analysis. *PLoS One.* 19(4):e0302481 (2024).
34. Ventre L, Fallico M, Longo A, Parisi G, Russo A, Bonfiglio V, Marolo P, Caselgrandi P, Avitabile T, Borrelli E, Reibaldi M. CONVENTIONAL INTERNAL LIMITING MEMBRANE PEELING VERSUS INVERTED FLAP FOR SMALL-TO-MEDIUM IDIOPATHIC MACULAR HOLE: A Randomized Trial. *Retina.* 42(12):2251-2257 (2022).
35. Gu C, Qiu Q. Inverted internal limiting membrane flap technique for large macular holes: a systematic review and single-arm meta-analysis. *Graefes Arch Clin Exp Ophthalmol.* 256(6):1041-1049 (2018).
36. Shen Y, Lin X, Zhang L, Wu M. Comparative efficacy evaluation of inverted internal limiting membrane flap technique and internal limiting membrane peeling in large macular holes: a systematic review and meta-analysis. *BMC Ophthalmol.* 20(1):14 (2020).
37. Dera AU, Stoll D, Schoeneberger V, Walckling M, Brockmann C, Fuchsluger TA, Schaub F. Anatomical and functional results after vitrectomy with conventional ILM peeling versus inverted ILM flap technique in large full-thickness macular holes. *Int J Retina Vitreous.* 9(1):68 (2023).
38. Pellegrini M, Adamo G, Vivarelli C, Bizzarri A, Mondin R, Talli PM, Nasini F, Giannaccare G, Mura M. MACULAR HOLE SURGERY AND RETINAL TECTONICS: The Impact of Internal Limiting Membrane Peeling Size on Tangential Retinal Displacement. *Retina.* 45(1):23-29 (2025).
39. Ittarat M, Somkijrungroj T, Chansangpetch S, Pongsachareonnont P. Literature Review of Surgical Treatment in Idiopathic Full-Thickness Macular Hole. *Clin Ophthalmol.* 14:2171-2183 (2020).
40. Valldeperas X, Wong D. Is it worth reoperating on macular holes? *Ophthalmology.* 115(1):158-63 (2008).
41. Duker JS, Kaiser PK, Binder S, de Smet MD, Gaudric A, Reichel E, Sadda SR, Sebag J, Spaide RF, Stalmans P. The International Vitreomacular Traction Study Group classification of vitreomacular adhesion, traction, and macular hole. *Ophthalmology.* 120(12):2611-2619 (2013).

42. Ch'ng SW, Patton N, Ahmed M, Ivanova T, Baumann C, Charles S, Jalil A. The Manchester Large Macular Hole Study: Is it Time to Reclassify Large Macular Holes? *Am J Ophthalmol.* 195:36-42 (2018).
43. Rossi T, Bacherini D, Caporossi T, Telani S, Iannetta D, Rizzo S, Moysidis SN, Koulisis N, Mahmoud TH, Ripandelli G. Macular hole closure patterns: an updated classification. *Graefes Arch Clin Exp Ophthalmol.* 258(12):2629-2638 (2020).
44. Ooka E, Mitamura Y, Baba T, Kitahashi M, Oshitari T, Yamamoto S. Foveal microstructure on spectral-domain optical coherence tomographic images and visual function after macular hole surgery. *Am J Ophthalmol.* 152(2):283-290.e1 (2011).
45. Shimozono M, Oishi A, Hata M, Kurimoto Y. Restoration of the photoreceptor outer segment and visual outcomes after macular hole closure: spectral-domain optical coherence tomography analysis. *Graefes Arch Clin Exp Ophthalmol*. 249(10):1469-76 (2011).
46. Ozal E, Ermis S, Gul C, Aksoy BK, Karapapak M, Ozal SA. Predicting anatomical success and retinal restoration following macular hole surgery using optical coherence tomography parameters. *BMC Ophthalmol*. 25(1):390 (2025).
47. Bajdik B, Vajas A, Kemenes G, Fodor M, Surányi É, Takács L. Prediction of long-term visual outcome of idiopathic full-thickness macular hole surgery using optical coherence tomography parameters that estimate potential preoperative photoreceptor damage. *Graefes Arch Clin Exp Ophthalmol.* 262(10):3181-3189 (2024).
48. Morizane Y, Shiraga F, Kimura S, Hosokawa M, Shiode Y, Kawata T, Hosogi M, Shirakata Y, Okanouchi T. Autologous transplantation of the internal limiting membrane for refractory macular holes*. Am J Ophthalmol.* 157(4):861-869.e1 (2014).
49. Kuriyama S, Hayashi H, Jingami Y, Kuramoto N, Akita J, Matsumoto M. Efficacy of inverted internal limiting membrane flap technique for the treatment of macular hole in high myopia. *Am J Ophthalmol.* 156(1):125-131.e1 (2013).
50. Chen SN, Yang CM. LENS CAPSULAR FLAP TRANSPLANTATION IN THE MANAGEMENT OF REFRACTORY MACULAR HOLE FROM MULTIPLE ETIOLOGIES. *Retina*. 36(1):163-70 (2016).

51. Grewal DS, Charles S, Parolini B, Kadonosono K, Mahmoud TH. Autologous Retinal Transplant for Refractory Macular Holes: Multicenter International Collaborative Study Group. *Ophthalmology.* 126(10):1399-1408 (2019).
52. Jirsova K, Jones GLA. Amniotic membrane in ophthalmology: properties, preparation, storage and indications for grafting-a review. *Cell Tissue Bank*. 18(2):193-204 (2017)
53. Caporossi T, Tartaro R, Bacherini D, Pacini B, De Angelis L, Governatori L, Di Leo L, Oliverio L, Rizzo S. Applications of the Amniotic Membrane in Vitreoretinal Surgery*. J Clin Med*. 9(8):2675 (2020).
54. Shiode Y, Morizane Y, Matoba R, Hirano M, Doi S, Toshima S, Takahashi K, Araki R, Kanzaki Y, Hosogi M, Yonezawa T, Yoshida A, Shiraga F. The Role of Inverted Internal Limiting Membrane Flap in Macular Hole Closure. *Invest Ophthalmol Vis Sci*. 58(11):4847-4855 (2017).
55. Melles GR, Ong TS, Ververs B, van der Wees J. Descemet membrane endothelial keratoplasty (DMEK). *Cornea.* 25(8):987-90 (2006).
56. Wilhelm TI, Gauché L, Böhringer D, Maier P, Heinzelmann S, Glegola M, Kammrath Betancor P, Reinhard T. Ten-year outcomes after DMEK, DSAEK, and PK: insights on graft survival, endothelial cell density loss, rejection and visual acuity. *Sci Rep.* 15(1):1249 (2025).
57. Wykrota AA, Weinstein I, Hamon L, Daas L, Flockerzi E, Suffo S, Seitz B. Approval rates for corneal donation and the origin of donor tissue for transplantation at a university-based tertiary referral center with corneal subspecialization hosting a LIONS Eye Bank*. BMC Ophthalmol*. 2022 Jan 10;22(1):17. Erratum in: *BMC Ophthalmol.* 22(1):34 (2022).
58. Schön F, Gericke A, Bu JB, Apel M, Poplawski A, Schuster AK, Pfeiffer N, Wasielica-Poslednik J. How to Predict the Suitability for Corneal Donorship? *J Clin Med.* 10(15):3426 (2021).
59. Pellegrini M, Mura M, Yu AC, Spena R, Ruzza A, Ponzin D, Busin M, Bovone C. Descemet Membrane Epiretinal Graft for Refractory Full-Thickness Macular Hole*. Ophthalmol Retina.* 8(6):611-613 (2024).
60. Scoles D, Mahmoud TH. Inaccurate Measurements Confound the Study of Myopic Macular Hole. *Ophthalmol Retina.* 6(2):95-96 (2022).

61. Imai M, Iijima H, Gotoh T, Tsukahara S. Optical coherence tomography 495 of successfully repaired idiopathic macular holes. *Am J Ophthalmol.* 128:496 621–627 (1999).
62. de Oliveira RC, Wilson SE. Descemet's membrane development, structure, function and regeneration. *Exp Eye Res.* 197:108090 (2020).
63. Caporossi T, Pacini B, De Angelis L, et al. HUMAN AMNIOTIC MEMBRANE TO CLOSE RECURRENT, HIGH MYOPIC MACULAR HOLES IN PATHOLOGIC MYOPIA WITH AXIAL LENGTH OF ≥30 mm. *Retina.* 40(10):1946-1954 (2020).
64. Theodossiadis PG, Grigoropoulos VG, Theodossiadis GP. The significance of the external limiting membrane in the recovery of photoreceptor layer after successful macular hole closure: a study by spectral domain optical coherence tomography. *Ophthalmologica.* 225(3):176-84 (2011).
65. Park JH, Lee SM, Park SW, et al. Comparative analysis of large macular hole surgery using an internal limiting membrane insertion versus inverted flap technique. *Br J Ophthalmol* 103: 245–250 (2019).
66. Tsai DC, Huang YH, Chen SJ. Parafoveal atrophy after human amniotic membrane graft for macular hole in patients with high myopia. *Br J Ophthalmol.* 105:1002e1010 (2021).
67. Caporossi T, Pacini B, Bacherini D, et al. Human amniotic membrane plug to promote failed macular hole closure. *Sci Rep*. 10(1):18264 (2020).
68. Moysidis SN, Koulisis N, Adrean SD, et al. Autologous Retinal Transplantation for Primary and Refractory Macular Holes and Macular Hole Retinal Detachments: The Global Consortium. *Ophthalmology.* 128(5):672-685 (2021).
69. Gavrilov JC, Borderie VM, Laroche L, Delbosc B. Influencing factors on the suitability of organ-cultured corneas. *Eye* (Lond). 24(7):1227-33 (2010).