Spontaneous closure of small full-thickness macular holes: Presumed role of Müller cells

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ABSTRACT.

Purpose: To document with spectral domain optical coherence tomography the formation and spontaneous closure of small full-thickness macular holes and to propose the active role of Müller cells in macular hole closure.

Methods: A retrospective case series of five patients with spontaneous closure of macular holes is reviewed. In one patient, foveal images were recorded over a period of 18 months.

Results: In a 66-year-old man, vitreofoveal traction caused a detachment of the inner Müller cell layer of the foveola from the outer nuclear layer (ONL) which was associated with a large pseudocyst and a horizontal gap in the central ONL. The traction caused an elongation and subsequent disruption of the stalk of the Müller cell cone in the foveola. A small full-thickness macular hole developed when a portion of the inner Müller cell layer of the foveola was pulled out. After phacoemulsification and shortly before the subsequent spontaneous closure of the hole, there were rapid increases in the number and size of the cystic cavities in the foveal walls resulting in a narrowing of the hole. The hole closed by bridging the gap in the inner part of the central ONL; a new inner Müller cell layer of the foveola was formed, and the gap of the external limiting membrane (ELM) was closed. The cystic cavities in the foveal walls rapidly disappeared within 2 weeks after the closure of the hole. One to 2.5 months after hole closure, the thickness of the central ONL increased which decreased the distance between the central ELM and retinal pigment epithelium. In three of the four other patients, the hole also closed by bridging the gap in the inner part of the ONL.

Conclusion: It is suggested that the spontaneous closure of small macular holes and the subsequent reconstruction of the normal foveal structure are mediated by active mechanisms of Müller cells which resemble those involved in ontogenetic foveal development.

Key words: fovea – macular hole – Müller cell cone – Müller glia – tractional force

Introduction

The fovea is composed of the central foveola surrounded by the foveal walls (Bringmann et al. 2018). The conspicuous shape of the fovea, with the absence of inner retinal layers in the foveola, needs particular structural stabilization. Generally, the retina is mechanically stabilized by glial cells, especially Müller cells; neurons do not provide structural support (Müller 1856; MacDonald et al. 2015). The columnar rows of cell somata in the retinal tissue are arranged and mechanically stabilized by the processes of Müller cells. The stabilization of the tissue structure is supported by the strands of microtubules and intermediate filaments in Müller cells (Reichenbach 1989) as well as by adherent junctions present between astrocytes and Müller cells and among Müller cells (Bringmann et al. 2018). However, in the fovea only type of macroglia is Müller glia; astrocytes are located in the parafovea (Distler & Dreher 1996; Bringmann et al. 2018). Microglia are primarily distributed in the plexiform layers of the foveal walls, whereas the foveola is largely microglia-free (Singaravelu et al. 2017). There are two different populations of Müller cells in the fovea: specialized Müller cells that form the Müller cell cone in the foveola (Yamada 1969; Gass 1999; Syrbe et al. 2018) and Müller cells of the foveal walls and parafovea which have a characteristic ‘z-shape’ because their outer processes run obliquely or horizontally through the Henle fibre layer (HFL) which compensates the spatial shift between the inner and outer layers of the foveal tissue (Reichenbach & Bringmann 2010; Bringmann et al. 2018).

Intermediate filaments like glial fibrillary acidic protein (GFAP) provide resistance to mechanical stress (Lu et al. 2011). Increased expression of
GFAP in Müller cells is a very sensitive retinal stress indicator, e.g., of mechanical stress (Bringmann et al. 2009). Müller cells in the fovea express GFAP (Bringmann et al. 2018). In the foveal walls and parafovea, outer Müller cell processes, which draw through the HFL and outer nuclear layer (ONL), as well as side processes of Müller cells which run horizontally through the inner fibrous part of the outer plexiform layer (OPL) contain GFAP (Bringmann et al. 2018). In addition, cells that form the Müller cell cone in the foveola strongly express GFAP (Bringmann et al. 2018). The expression of GFAP may indicate the presence of mechanical stress in these layers (Bringmann et al. 2018). The distribution of GFAP supports the assumption that tractional forces provided by Müller cell processes in the OPL on the Henle fibres and the elaborated plait of the inner processes of foveolar Müller cells, which spreads along and below the basal lamina of the inner limiting membrane (Syrbe et al. 2018), are the basis for the structural stability of the fovea (Bringmann et al. 2018). The innermost layer of the foveola is composed of the somata and inner processes of the specialized Müller cells which form the Müller cell cone; the vertical stalk of the Müller cell cone in the centre of the foveola is formed by the outer processes of the cells (Bringmann et al. 2018; Syrbe et al. 2018).

The plait of the thin inner processes of the foveolar Müller cells may provide resistance against mechanical stretch resulting from anteroposterior or tangential tractional forces; these occur, for example, after partial detachment of the posterior vitreous and in cystoid macular oedema (Bringmann et al. 2018; Syrbe et al. 2018). In cystoid macular oedema and during early stages of macular hole formation, fluid-filled cysts may be present in the foveola resulting in a detachment of the inner Müller cell layer; the detached Müller cell layer often keeps the foveal walls together (Gass 1999; Byron et al. 2014). Disruption of the inner Müller cell layer of the foveola results in the formation of full-thickness macular holes (Gass 1999).

Mechanical forces provided by foveal Müller cells are also involved in the restoration of the fovea after surgical or spontaneous closure of macular holes (Chung & Byeon 2017). However, the cellular mechanisms of macular hole closure are incompletely understood. It was shown that small macular holes with a diameter of less than 400 μm may close spontaneously (Tadayomi et al. 2001; Privat et al. 2007). The rate of spontaneous hole closure is low and ranged from 0 to 6% in various prospective and retrospective studies (Guyer et al. 1992; Yuzawa et al. 1994; Hikichi et al. 1995; Kim et al. 1995; Freeman et al. 1997; Chew et al. 1999; Takahashi & Kishi 1999; Casuso et al. 2001; Privat et al. 2007; Sugiyama et al. 2012).

Here, we describe the full regeneration of the foveal structure after the vitreofoveal traction-induced formation and spontaneous closure of a small full-thickness macular hole. Spectral domain optical coherence tomography (SD-OCT) recordings were obtained over a period of 18 months. In addition, four further cases of spontaneous closure of macular holes were included in the study. We compare recently suggested Müller cell-mediated mechanisms of the ontogenetic development of the fovea (Bringmann et al. 2018) with the putative mechanisms of the closure of small full-thickness macular holes. It is suggested that mechanisms similar to those involved in foveal development may in part explain the mechanisms of spontaneous macular hole closure.

Materials and Methods

The five cases described in this retrospective study were examined between 2014 and 2019 at the Department of Ophthalmology, University of Leipzig, Leipzig, Germany, and the Institute of Ophthalmology (MVZ Augenheilkunde Mitteldeutschland GmbH), Halle, Germany. The study followed the tenets of the Declaration of Helsinki for research involving human subjects. The patients gave their consent for their images and other clinical information to be reported. Cross-sectional images of the macular area were obtained with SD-OCT (Spectralis, Heidelberg Engineering, Heidelberg, Germany). There were no apparent abnormalities in the foveal structure of the fellow eyes of all patients investigated (Figs. 1A and 2Aa, Ca).

All patients were of Caucasian descent. Patient 1 was a 66-year-old man who presented with metamorphopsia and an impairment of vision in the left eye within the last month (best corrected visual acuity [BCVA], 0.5 decimal units, obtained using a Snellen chart). The reduced visual acuity was in part influenced by a nuclear sclerotic cataract. Two years ago, cataract surgery was performed in the fellow eye.

Fig. 1. Development and spontaneous closure of a full-thickness macular hole in the left eye of a 66-year-old man (patient 1). The months after the first examination (0) are indicated left of the images. (A) OCT images of the foveal centre of the healthy right eye. There were no apparent abnormalities in the foveal structure during the examination period. (B) OCT images of the fovea of the left eye. It was found during the first visit that vitreofoveal traction caused a detachment of the inner Müller cell layer of the foveola from the outer nuclear layer (ONL). This was associated with the formation of a large pseudocyst and a horizontal gap in the central ONL. Between 4.6 and 5.5 months, a full-thickness macular hole developed by pulling out a portion of the inner Müller cell layer of the foveola. The hole closed between 7 and 7.5 months by bridging the gap in the central ONL. Arrows, lower part of the disrupted stalk of the Müller cell cone. Arrowheads, operculum. (C) OCT images of the foveal centre (*) and the foveal walls below. The images were recorded 7 (above) and 7.5 months (below) after the first examination. Note the darkness (i.e., hyperreflectivity) of the OPL above the cystic cavities. (D) The outer fovea of the left eye at higher magnification. The following hyperreflective lines are marked: external limiting membrane (ELM), the ellipsoid zone (EZ), the interdigitation zone (IZ), and the retinal pigment epithelium (RPE). The central borders of the EZ line are indicated by white arrowheads, and the central borders of the IZ line are marked by black arrowheads. (E) Time-dependent alterations of the distances between the inner surface of the retina and the RPE, the inner retinal surface and the ELM, and the ELM and the RPE in the foveal centre of the left eye. In addition, the distance between the ELM and RPE in the central foveola of the healthy right eye is shown (asterisks). The distance between the inner retinal surface and the ELM reflects the thickness of the ONL in the foveola. The dotted vertical lines indicate the times of the intravitreal injection of ocriplasmin (left) and cataract surgery (right). The interrupted vertical line indicates the time period of the formation of the full-thickness hole. The continuous vertical line indicates the time of the closure of the hole. (F) Time-dependent alteration of the best corrected visual acuity (BCVA). GCL = ganglion cell layer, HFL = Henle fibre layer, INL = inner nuclear layer, NFL = nerve fibre layer, OPL = outer plexiform layer.
eye. Spectral domain optical coherence tomography (SD-OCT) of the left eye showed vitreofoveal traction and a pseudocyst in the foveola (Fig. 1B). 2.5 months after the first visit, ocriplasmin (Jetrea; 0.1 ml; ThromboGenics NV/Alcon Pharma) was injected into the vitreous of the left eye to relieve vitreofoveal traction. A full-thickness macular hole developed between 4.6 and 5.5 months after the first visit (2.1–3.0 months after injection). Cataract surgery of the left eye with a monofocal lens was performed 6.3 months after the first visit. Because slight zonulolysis was suspected intraoperatively, a capsular tension ring was inserted. Between 7 and 7.5 months after the first examination (0.7–1.2 months after cataract surgery), the macular hole closed spontaneously. Eight months after the first visit, the patient received Nevanac eye drops (1 mg/ml; three times per day). Three weeks later, the Nevanac dose was changed to 3 mg/ml once daily, the pseudophacic eye showed no irritations, and metamorphopsia improved. The recovery of the foveal structure after the closure of the hole was investigated with SD-OCT until 18 months after the first examination. The time-dependent change of the BCVA during the examination period is shown in Figure 1F.

Patient 2 was a 64-year-old man referred with reduced vision (BCVA, 0.5) and metamorphopsia in the left eye. Spectral domain optical coherence tomography (SD-OCT) showed a detachment of the inner layer of the foveola caused by vitreofoveal traction. Within 3 months after the first visit, a full-thickness macular hole developed which later closed spontaneously. The recovery of the foveal structure was investigated with SD-OCT until 17 months after the first examination. Best corrected visual acuity (BCVA) improved to 0.8.

Patient 3 was a 57-year-old man referred with a sudden decrease in vision in the right eye (BCVA, 0.32) and metamorphopsia. Spectral domain optical coherence tomography (SD-OCT) showed a full-thickness macular hole. One month later, the hole had closed spontaneously, and BCVA was 0.3. Best corrected visual acuity (BCVA) improved to 0.4 (1.4 and 2.4 months) and 0.6 (6 months after the first examination).

Patient 4 was a 65-year-old man referred for a gradual reduction in vision over a period of 4 weeks (BCVA, 0.4) and metamorphopsia in the right eye. Spectral domain optical coherence tomography (SD-OCT) showed a small full-thickness macular hole. Three months later, the hole was closed, metamorphopsia improved, and BCVA was 1.0. Six months after the first visit, the patient did not report any metamorphopsia, and BCVA was 1.0. The right eye of the patient already had a history of multiple surgeries: Within the last 35 years, a cerclage and pars plana vitrectomy either with or without gas tamponade were performed for retinal detachment. Nineteen years ago, cataract surgery with lens implantation had been performed. Two months before the first visit because of the macular hole, a slight vitreous haemorrhage was observed.
which was not associated with a decrease in vision (BCVA, 1.0).

Patient 5 was a 26-year-old man referred for a reduction in vision in the right eye (BCVA, 0.16) after contusion caused by a football. Clinical examination revealed the presence of a Berlin’s oedema and haemorrhage in the fundus and papilla. Spectral domain optical coherence tomography (SD-OCT) showed no hole in the fovea. Within 1.7 months, a small full-thickness macular hole developed which closed spontaneously thereafter. Best corrected visual acuity (BCVA) was 0.16, 0.20, and 0.63 at one, 1.7, and 3 months after the first visit.

Results

Patient 1: Formation of the full-thickness macular hole

The formation and closure of a full-thickness macular hole in the left eye of a 66-year-old man were investigated with SD-OCT during a period of 18 months. Spectral domain optical coherence tomography (SD-OCT) records obtained during the first visit revealed that the inner Müller cell layer was detached from the ONL in the foveola of the left eye due to anteroposterior vitreofoveal traction exerted by the posterior hyaloid adhering to the foveola (Fig. 1B). This produced a stretching and elongation of the stalk of the Müller cell cone in the centre of the foveola and the formation of a large central pseudocyst and a horizontal gap in the ONL of the foveola; the ONL gap became time dependently wider until the hole closed (7 months after the first examination; Fig. 1B). Within the first month, the stalk of the Müller cell cone in the foveola disrupted (Figs. 1B and 3A). Thereafter, the upper part of the disrupted stalk was visible as bulges of the inner Müller cell layer of the foveola (Figs. 1B and 3A). These bulges were retracted between 3.6 and 4.6 months after the first examination (Fig. 1B). The lower part of the disrupted stalk of the Müller cell cone was visible within the gap of the ONL of the foveola until 2.7 months after the first visit (arrows in Fig. 1B). Thereafter, the lower part of the disrupted stalk was resolved (Fig. 1B). Between 4.6 and 5.5 months, a full-thickness hole developed by pulling out a portion of the nasal part of the inner Müller cell layer of the foveola (Figs. 1B and 3A). Subsequently, this portion was visible as operculum at the posterior hyaloid (arrowheads in Figure 1B). Apparently, the intravitreal injection of ocriplasmin performed 2.5 months after the first examination had no effects on the foveal structure with the exceptions of slight fluctuations of the height of the detached inner layer of the foveola and of the distance between the central ELM and RPE (Fig. 1E), likely caused by alterations in the strength of the anteroposterior traction (Fig. 1B).

As long as the macular hole was present, cystic cavities were observed between the OPL and HFL and in the inner nuclear layer (INL) of the foveal walls (Fig. 1B, C). The presence of the cysts was associated with an elevation of the foveal walls around the hole. The number and size of the cystic cavities increased slowly after the formation of the full-thickness macular hole (4.6–6.5 months; Fig. 1B). After cataract surgery (6.3 months after the first examination) and shortly before the closure of the hole (between 6.5 and 7 months), there was a rapid increase in the number and size of the cystic cavities resulting in a high elevation of the foveal walls around the hole; this was associated with an increase in the distance between the centralmost external limiting membrane (ELM) and the retinal pigment epithelium (RPE) (Fig. 1E) as well as a narrowing of the hole (from 105 to 75 µm between 6.5 and 7 months) at the level of the inner part of the ONL (Fig. 1B). During the narrowing of the hole, which was mainly caused by the enlargement of the cysts between the OPL and HFL in the foveal walls, the remnants of the disrupted Müller cell cone moved together (Fig. 3A). The cystic cavities in the foveal walls disappeared very rapidly after the closure of the hole (Fig. 1B, C).

Patient 1: Closure of the macular hole

The macular hole closed spontaneously by bridging the gap at the level of the OPL and the inner part of the ONL (Fig. 1B, C). This was associated with the formation of a newly formed inner Müller cell layer in the foveola and the closure of the central ELM defect (Fig. 1B, C). The Müller cell cone in the foveola regenerated (Fig. 3A). After the closure of the hole, there were oblique vertical hyperreflective lines in the centralmost ONL which contacted the tip of the fovea externa and which likely represent the newly formed stalk of the Müller cell cone (Fig. 1B, D). The thickness of the foveal walls and the width of the foveal pit were unchanged after closure of the hole (data not shown) while the depth of the pit increased slightly (from 175 to 185 µm between 7.5 and 18 months after the first examination).

Figure 1E shows the time-dependent alteration of the distance between the centralmost ELM and RPE. In the healthy right eye, this distance remained unaltered during the examination period (Fig. 1E). This distance was greater in the left eye than in the right eye and increased continuously between 0 and 8.5 months after the first examination; there was a steep transient increase after cataract surgery (between 6.5 and 7 months) which was associated with the rapid increase in the cystic cavities in the foveal walls (Fig. 1E). After the closure of the hole and the disappearance of the cystic cavities in the foveal walls (between 7.5 and 8.5 months), the thickness of the central foveola (distances between the inner retinal surface and ELM and between the ELM and the RPE) remained largely unaltered (Fig. 1E). Between 8.5 and 10 months, there was a rapid increase in the distance between the inner retinal surface and the ELM (from 45 to 65 µm) and a rapid decrease in the distance between the central ELM and the RPE (from 140 to 95 µm) (Fig. 1E). Thereafter, the distance between the ELM and the RPE remained constant and was similar to that observed in the central fovea of the healthy fellow eye (Fig. 1E).

Figure 1F shows the time-dependent alteration of the BCVA during the examination period. Until the formation of the full-thickness hole, BCVA ranged from 0.4 to 0.6. The intravitreal injection of ocriplasmin (2.5 months after the first visit) was associated with mechanical stress on the fovea which caused slight alterations of the distance between the central ELM and RPE (Fig. 1E) accompanied by distinct disturbances of the BCVA (Fig. 1F). After the formation of the full-thickness hole (5.5 months after the first examination), the patient reported an impairment of reading (BCVA, 0.35; Fig. 1F). Cataract surgery (6.3 months after the first visit) was followed by a
slight decrease in the BCVA (Fig. 1F). After closure of the hole, BCVA increased (Fig. 1F). A plateau at 0.8 and 1.0 was achieved when the distance between the central ELM and RPE decreased (9 months after the first visit; Fig. 1F).

Patient 1: Photoreceptor layer

Figure 1D shows the outer fovea of the left eye at higher magnification. There are four hyperreflective lines in the outer retina: the ELM, the ellipsoid zone, the photoreceptor outer segments, and the mitochondrial zone in the inner photoreceptor segments, the interdigitation zone of the RPE, and the apical part of the RPE containing photoreceptor outer segment fragments, and the mitochondria-containing basal part of the RPE (Cuenca et al. 2018).
The ELM, EZ, and IZ lines showed central defects during macular hole formation (Fig. 1D). The defects of these lines became time dependently larger during the widening of the central ONL defect (Fig. 1D). The defect of the ELM sealed with the closure of the hole (7.5 months after the first examination), and the defects of the EZ and IZ lines became time dependently smaller after the closure of the hole (Fig. 1D). During most stages of macular hole development and closure (until 10 months after the first examination), the central defect of the IZ line was larger than the defect of the EZ line (Fig. 1D). The central EZ and IZ lines, as well as the fovea externa which is visible at the inclined courses of the ELM and EZ lines in the foveola (Schultze 1866), were regenerated 18 months after the first examination (Fig. 1D).

The RPE line showed no disruption during the development and the subsequent closure of the full-thickness macular hole (Fig. 1D). However, as long as the defect in the IZ line was present, the RPE showed a central hyperreflectivity which was surrounded by a hyporeflectivity; the distance of the hyporeflectivity coincided roughly with the distance of the IZ line defect (Fig. 1D). At 11.5 months after the first examination and later, the RPE showed an even reflectivity without any apparent irregularities (Fig. 1D).

**Patient 2**

Figure 2A shows OCT images of the central fovea in the healthy right eye (Fig. 2Aa) and the left eye (Fig. 2Ab) of patient 2. During the first visit, there was a detachment of the inner layer of the foveola in the left eye resulting from vitreofoveal traction (Fig. 2Ab). This was associated with the presence of a central pseudocyst, a horizontal gap in the ONL, and cystic cavities between the OPL and HFL in the foveal walls (Fig. 2Ab). Within 3 months after the first visit, a full-thickness macular hole (smallest diameter, 130 μm) developed by pulling out a portion of the inner Müller cell layer of the foveola; this portion was visible as operculum at the posterior hyaloid (Fig. 2Ab). Between 3 and 5 months after the first visit, the hole closed at the central ONL by regeneration of the inner Müller cell layer of the foveola and sealing of the gap in the central ELM (Fig. 2Ab). Thereafter, the thickness of the central ONL increased which was associated with a decrease in the distance between the central ELM and RPE (Fig. 2Ab). Seventeen months after the first visit, the fovea externa appeared nearly normal (Fig. 2Ab). There remained a small defect in the transition zone between the foveola and the temporal foveal wall with an irregular foveal contour (Fig. 2Ab).

**Patient 3**

Figure 2B shows OCT images of the central fovea in the right eye of patient 3. During the first visit, there was a near full-thickness macular hole (smallest diameter, 388 μm) which was produced by a disruption of the inner Müller cell layer at the temporal side of the foveola; however, this layer remained attached to the nasal foveal walls (Fig. 2B). The large pseudocyst in the foveola produced a widening of the central ONL while there was no gap in the outermost part of the ONL (Fig. 2B). The fovea externa was flattened, and the central photoreceptors were apparently degenerated, as indicated by the defects of the EZ and IZ lines (Fig. 2B). The closure of the hole occurred by a reattachment of the inner Müller cell layer of the foveola to the foveal walls at the level of the OPL and the innermost part of the ONL (1 and 1.4 months after the first visit; Fig. 2B). The hole closed despite the posterior hyaloid remained attached to the foveal centre (1 and 1.4 months; Fig. 2B). The closure of the hole was associated with the formation of a gap in the entire ONL (1 and 1.4 months; Fig. 2B). Thereafter, the central ONL thickened; the thickening was associated with a decrease in the distance between the central ELM and the RPE (2.4 and 6 months; Fig. 2B).

**Patient 4**

Figure 2C shows OCT images of the central fovea in the right eye of patient 4 which were recorded during (Fig. 2Cb) and 3 months after the first visit (Fig. 2Cc). At the first visit, there was a small full-thickness macular hole in the right eye (smallest diameter, 75 μm; Fig. 2Cb). Large cystic cavities in the INL and between the OPL and HFL of the foveal walls produced elevations of the walls around the hole (Fig. 2Cb). Three months later, the hole was closed (Fig. 2Cc). The inner Müller cell layer, the ONL, and the ELM of the foveola had a near-normal appearance while a small defect in the EZ line was still present 6 months after the first visit (Fig. 2Cd).

**Patient 5**

Figure 2D shows the development and spontaneous closure of a traumatic hole in the right eye of patient 5. There was no hole in the fovea during the first visit (Fig. 2D). Within one month, large cystic cavities developed between the OPL and HFL in the foveal walls; this was associated with an elevation of the foveal walls and a detachment of the foveola from the RPE (Fig. 2D). The elevation of the foveal walls caused a centrifugal displacement of the central ONL and a formation of a full-thickness hole (smallest diameter, 105 μm) (Fig. 2D). Between 2 and 3 months after the first visit, the cystic cavities in the foveal walls disappeared; this allowed a drop of the walls associated with a closure of the hole at the level of the OPL and the innermost part of the ONL (Fig. 2D).

**Discussion**

One mode of macular hole formation is mediated by a detachment of the inner Müller cell layer from the ONL in the foveola; this is associated with the formation of a large pseudocyst and a horizontal gap in the central ONL (Fig. 3D) (Chung & Byeon 2017). Localization of the sites of low mechanical stability in the foveola may explain the locations of the tissue disruptions in the early stage of macular hole formation (Bringmann et al. 2018). The detachment of the inner Müller cell layer from the ONL of the foveola was explained by the fact that there are no cellular connections between both layers (Bringmann et al. 2018). The inner layer of the foveola is formed by the specialized foveolar Müller cells while the ONL of the foveola is stabilized by the outer processes of the Müller cells of the foveal walls (Fig. 3B).

The cellular mechanisms of the spontaneous closure of macular holes are
 incompletely understood. Because the fovea is free of astrocytes (Bringmann et al. 2018), the closure is likely mediated by Müller cells. It was suggested that small macular holes allow glial proliferation to bridge the gap (Tadayoni et al. 2001). Glial proliferation may proceed by process outgrowth from Müller cells and/or cell division. However, it is rather unlikely that glial proliferation plays a major role because the volume of the foveal tissue does not alter during the closure of macular holes, suggesting that there is no loss or increase in cellular components (Itoh et al. 2014). Other authors suggested that the closure of macular holes after surgery proceeds without glial cell proliferation (Funata et al. 1992). The release of vitreous traction on the foveola is also unlikely to explain the closure of macular holes, as (in patient 1) this traction was already released 3 months before the closure (while the closure proceeded rapidly during 2 weeks; Fig. 1B). In patient 3, the hole closed despite the posterior hyaloid remained attached at the foveal centre (Fig. 2B). A similar closure of traumatic macular holes despite persistent vitreous attachment at the fovea was described previously (Yamashita et al. 2002).

As indicated by the SD-OCT images, the cardinal event in the closure of small full-thickness macular holes is the sealing of the gap of the central ONL. In patients 1, 3, and 5, the hole closed via a fusion of the remnants of the Müller cell cone somata at the level of the ONL (Fig. 3A, D). In patient 2, the hole closed in the mid of the ONL (Fig. 2A).

The closure is likely mediated by two tissue movements exerted by the Müller cells of the foveal walls: (i) an annular contraction of the horizontal Müller cell side processes in the OPL and (ii) an annular contraction of the Müller cell structures which envelop the photoreceptor cells at the ELM causing the centripetal shift of the central photoreceptors; both movements result in a centripetal shift of the foveal walls and the closure of the hole at the level of the OPL/inner part of the ONL (Fig. 3A, D). In patient 2, the hole closed in the mid of the ONL (Fig. 2A).

Annular contractions are suggested by the finding that the upper and lower foveal walls around the hole are interlinked at the level of the OPL (Figs. 1C and 2Cb); therefore, it is likely that a ring of Müller cell processes surrounds the hole at this level. After the closure of the hole, the normal foveal structure is restored by an increase in the thickness of the central ONL which is produced by a Müller cell-mediated movement of photoreceptor cell somata towards the foveal centre (Fig. 3D). This causes a reduction in the distance between the central ELM and the RPE. It is likely that these movements, which are aimed to remove the central scotoma, are dependent on light stimulation and central information processing.

Because the distance of the Müller cell-mediated centripetal movements may be restricted, only small holes close spontaneously. In addition, we assume that remnants of the inner Müller cell layer of the foveola (which contains the somata and inner processes of the foveolar Müller cells; Fig. 3B) should be present in the foveal walls; these cells regenerate the Müller cell cone after the closure of the hole (Fig. 3A). Larger holes can be closed when the inner Müller cell layer of the foveola remains adhered to the foveal walls as in patient 3 (Fig. 2B). This mechanism of macular hole closure may not function when the diameter of the hole is greater than the diameter of the foveola (in average, about 350 µm; Curcio et al. 1990). Further mechanisms of macular hole closure are possible. It was shown, for example, that a closure of a hole can occur although the inner Müller cell layer of the foveola remains detached from the central ONL; in this case, the ONL is displaced centripetally, the hole closes only at the ELM, and a new Müller cell cone is not formed (Takahashi et al. 2010).

After the closure of the macular hole in patient 1, we observed obliquely arranged vertical hyperreflective lines in the centralmost ONL which contacted the tip of the fovea externa (Fig. 1D). The origin of these lines is unclear. Possibly, these lines are reflections at the inner borders of the ONL in the foveola covered by (glotic) Müller cells and represent the regenerating stalk of the Müller cell cone in the foveola (Fig. 3A).

After the formation of the full-thickness macular hole in patient 1, the number and size of the cystic cavities in the foveal walls initially increased slowly (Fig. 1B). After cataract surgery and shortly before the closure of the hole, the number and size of the cystic cavities increased rapidly (6.5–7 months after the first examination; Fig. 1B). This caused a centripetal protrusion of the foveal walls around the hole resulting in a narrowing of the hole at the level of the inner part of the ONL and a narrowing of the remnants of the disrupted Müller cell cone (Fig. 3A). We propose that the oedematous protrusion of the foveal walls contributes to the closure of the macular hole. This assumption is supported by the findings that both the narrowing and the closure of the hole occurred at the level of the inner part of the ONL (Fig. 3A). However, as observed in patient 5, the increase and subsequent decrease in the size of the cystic cavities in the foveal walls may be also a cause of the formation of macular holes (Fig. 2D).

The cystic cavities in the INL and between the OPL and HFL of the foveal walls (Figs. 1B, C and 2Ab, Cb) are likely caused by a leakage of the vessels of the vascular ring which delimitates the foveal avascular zone in the foveal slope (Provis et al. 2000). As previously suggested for the development of cystoid macular oedema, a dysfunction of Müller cells may contribute to the development of oedematous cysts, by a dysregulation of the fluid clearance through the cells (Bringmann et al. 2004). This dysregulation is reflected in the rapid swelling of Müller cells when they are exposed to osmotic stress (Reichenbach & Bringmann 2010). Mechanical stress is a known inducer of Müller cell swelling (Matsumoto et al. 2018). Müller cells were shown to respond rapidly to mechanical tissue stretch with transient increases in the cytosolic calcium level and changes in protein expression (Lindqvist et al. 2010; Agte et al. 2017). It is likely that mechanical stress resulting from the vitreofoveal traction onto the foveola induces a dysfunction of Müller cells which contributes to the development of the cystic cavities in the foveal walls during the formation of macular holes. Mechanical stress exerted by the hydrostatic pressure within the cysts, resulting in an enlargement of the cysts, may contribute to the induction of Müller cell dysfunction.
This assumption is supported by the finding that the cysts disappeared very rapidly after the closure of the holes (Figs. 1B, C and 2A, B, D). Cataract surgery is a well-known pathogenic condition which may induce macular oedema characterized by the development of intraretinal fluid-filled cysts in the fovea (Irvine 1976). The postoperative cystoid macular oedema is caused by the action of inflammatory mediators, especially prostaglandins (Miyake & Ibaraki 2002). Prostaglandins were shown to induce Müller cell swelling under osmotic stress conditions (Uckermann et al. 2005). It is possible that, in patient 1, cataract surgery induced the large transient increase in the cyst size shortly before the closure of the hole (Fig. 1B).

During most stages of macular hole widening and closure in patient 1, the central defect of the IZ line appeared larger than the defect of the EZ line (Fig. 1D). The defects in both lines became larger during the widening of the hole and smaller after the closure of the hole (Fig. 1D). The absence of a regular EZ line in the central fovea between 6.5 and 8.5 months after the first examination (Fig. 1D) and the defect of the IZ line may indicate a degeneration or a loss of the regular arrangement of the photoreceptor segments in the foveola. The coincidence between the hyporeflectivity of the RPE and the defect of the IZ line (Fig. 1D) may suggest that the first is caused by a detachment of the photoreceptor segments from the RPE, i.e., a disruption of the outer segment-/RPE junction. Because the RPE line in OCT images arises from light reflection at the mitochondria in the basal part of the RPE (Cuenca et al. 2018), the hyporeflectivity of the RPE may reflect a loss of mitochondria in RPE cells which lost their contact to photoreceptors. Disruption of the contact between photoreceptors and RPE may be associated with a decreased need of energy production required for photopigment recycling and metabolism of phagocytized photoreceptor segment tips. However, other explanations are also possible, e.g., altered light transmission properties through the thickened foveal tissue. In addition, the cause of the central hyperreflectivity of the RPE, which was observed during the formation and after the closure of the full-thickness hole (until 9 months after the first visit; Fig. 1D) is unclear. It is possible that it is caused by the improved light transmission through the hole.

In patient 1, the distance between the centralmost ELM and RPE increased roughly linearly until one month after the closure of the hole; thereafter, the distance decreased rapidly to a value similar to that found in the healthy fellow eye (Fig. 1E). The decrease in the distance coincided with the regeneration of the central EZ line which occurred after 8.5 months after the first examination (Fig. 1D) and is thus a precondition for the regeneration of the central photoreceptor segments.

It is unclear how the central defect of the photoreceptor layer (EZ line) is regenerated after the closure of the hole. There are two possibilities: (i) a centripetal displacement of the photoreceptor cells around the EZ line defect, and/or (ii) an outgrowth of newly formed photoreceptor segments from the photoreceptor cell somata in the central ONL. The rapid increase in the distance between the inner retinal surface and the ELM, which occurred between 8.5 and 10 months after the first examination in patient 1 (Fig. 1E), suggests that during this period a centripetal displacement of photoreceptor cells occurred. The relatively stable thickness of the central foveola after 10 months after the first examination (Fig. 1E) suggests that during this period the photoreceptor layer was regenerated by an outgrowth of newly formed photoreceptor segments from the photoreceptor cell somata.

We propose that Müller cell-mediated tissue movements which create the fovea during the ontogenetic development (Fig. 3D) (Bringmann et al. 2018) may play also a role in the closure of macular holes. It was suggested that the ontogenetic development of the foveal pit proceeds during three main stages: (i) formation of the foveal pit by an anteroposterior contraction of the centralmost Müller cells; (ii) widening of the foveal pit by a horizontal contraction of the astrocytic network in the nerve fibre/ganglion cell layers; this produces a tilt of the inner tissue of the foveal walls and initiates the formation of the foveola; and (iii) erection of the inner tissue of the foveal walls by a horizontal contraction of Müller cell side processes in the inner fibrous part of the OPL in the foveal walls (Fig. 3D) (Bringmann et al. 2018). The two-first steps proceed prenatally while the last step proceeds postnatally and is likely dependent on light stimulation and central information processing (Bringmann et al. 2018). It is likely that the annular contraction of the Müller cell side processes in the OPL, which is implicated in the closure of the macular hole (Fig. 3D), is mediated by Müller cell side processes which produce the ejection of the inner foveal tissue during development.

The centripetal relocation of the (peri)foveal photoreceptors at the ELM during the ontogenetic development creates the high packing of the central photoreceptors (Fig. 3D) (Bringmann et al. 2018). The latter is likely mediated by a radial contraction of the receptor segments and a horizontal contraction of Müller cell structures which surround the photoreceptor cells at the ELM (Bringmann et al. 2018). Photoreceptor and Müller cells are tightly glued together in the HFL and ONL, and at the ELM (Omri et al. 2010; Matet et al. 2015). At the ELM, Müller cells contain contractile rings of filamentous actin which enclose the photoreceptors; these rings are associated with the junctions between Müller and photoreceptor cells and form a structural meshwork in which photoreceptors are embedded (Del et al. 1987). The developmental increase in the packing of the central photoreceptors is associated with a thinning and elongation of the central photoreceptor segments. It is likely that the rapid increase in the thickness of the central ONL, which occurred between 8.5 and 10 months after the first examination in patient 1 (Fig. 1E) and which proceeded by a centripetal displacement of photoreceptor cells, is mediated by a mechanism similar to that which mediates the central photoreceptor packing during development (Fig. 3D).

The development of the fovea externa, i.e., the cone-like arrangement of the elongated cone segments in the foveola which is visible at the inclined courses of the ELM and EZ lines in OCT images, was suggested to be supported by two Müller cell-mediated mechanisms: (i) a centrifugal pulling of Henle fibres due to tractive forces provided by the Müller cells of the foveal walls on the Henle fibres which result from the centrifugal displacement of Müller cell side processes in the OPL, and (ii) an anteroposterior contraction.
of the outer processes of the cells of the Müller cell cone (Fig. 3D) (Bringmann et al. 2018). The first mechanism also produces the central widening of the ONL in the foveola (Detwiler 1943). It was also suggested that the Müller cell-mediated morphological alterations of the foveal shape, resulting in flattening and deepening of the foveal pit, may contribute to the adaptation of the position of the central photoreceptors to changes in the angle of the incoming light and thus may play a role in accommodation and fixation in normal subjects (Fig. 3C) (Fortin, 1925, cited in Kolmer & Lauber 1936; Bringmann et al. 2018). It is conceivable that the first mechanism contributes to the time-dependent widening of the gap in the central ONL during macular hole formation (Fig. 3D).

It was shown that small macular holes with a diameter of less than 400 μm may close spontaneously (Tadayoni et al. 2001; Privat et al. 2007); however, the rate of spontaneous hole closure is low. One characteristic of the five patients examined in this study was that there were no apparent abnormalities in the foveal structure of the fellow eyes (Figs. 1A and 2Aa, Ca). A treatment of small holes may be not required if the vision remains stable and the hole does not enlarge, and if the fovea of the fellow eye appears normal.

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