Collagens and retinal Müller cells in healthy and diseased vitreoretinal interface
Bu, Shao

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Chapter 2

Review: Idiopathic Epiretinal Membrane

Shao-Chong Bu, Roel Kuijer, Xiao-Rong Li, Johanna M. M. Hooymans, Leonoor I. Los

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Chapter 2

Abstract

**Background:** Idiopathic epiretinal membrane (iERM) is a fibrocellular membrane that proliferates on the inner surface of the retina at the macular area. Membrane contraction is an important sight threatening event and is due to fibrotic remodelling.

**Methods:** Analysis of current literature regarding the epidemiology, clinical features, and pathogenesis of iERM and fibrotic tissue contraction.

**Results:** Epidemiologic studies report a relationship between iERM prevalence and e.g. increasing age and posterior vitreous detachment (PVD). Clinically, iERM progresses through different stages characterized by an increased thickness and wrinkling of the membrane. Pathophysiologically, iERM formation is a fibrotic process in which myofibroblast formation and the deposition of newly formed collagens play key roles. Anomalous PVD may be a key event initiating the formation of iERM. The age-related accumulation of advanced glycation end products may contribute to anomalous PVD formation and may also influence the mechanical properties of the iERM.

**Conclusion:** Remodelling of the extracellular matrix at the vitreoretinal interface by ageing and fibrotic changes, plays a significant role in the pathogenesis of iERM. A better understanding of molecular mechanisms underlying this process may eventually lead to the development of effective and non-surgical approaches to treat and prevent vitreoretinal fibrotic diseases.
1. General introduction

Idiopathic epiretinal membrane (iERM) is the most common type of fibrocellular proliferation found at the vitreo-retinal interface, which is significantly associated with aging. While patients with iERM can be completely asymptomatic when the membrane is thin and translucent, its progression to a semi-translucent, thick and contractile membrane may result in macular distortion thus inducing metamorphopsia and loss of central visual function. This clinical entity has also been termed “macular pucker”, “preretinal macular fibrosis”, “epiretinal fibrosis” or “gliosis”, “surface wrinkling retinopathy”, or “cellophane maculopathy”.

At present, treatment options are limited and consist of watchful waiting or vitrectomy surgery. The time point when irreversible damage to the macula will have occurred is presently unknown. Therefore, it is not known whether surgery for iERMs should be aimed for at an early stage with minimal symptoms or whether it can safely be delayed until metamorphopsia and loss of visual acuity are present.

Idiopathic ERM formation is a fibrotic process on the surface of the retina. Fibrosis is a common process throughout the body, and a lot of research in the general area of fibrosis has been and is currently being done. Ultimately, results from such studies may contribute to find new treatment options for fibrotic processes in general, which may also be applicable to iERM formation. Therefore, this overview focuses on the pathogenesis of iERM in relation to fibrosis in general and aims to point out directions for future research in this area.

With regard to its pathogenesis, epidemiologic studies found a clear relationship between iERM formation, aging and posterior vitreous detachment. Also, they found some association with genetic and lifestyle factors. Histological studies of surgically obtained iERM specimens identified cellular and extracellular matrix components. A key element in iERM formation and its progression towards a contractile membrane is the transdifferentiation of cellular components towards
myofibroblasts. The latter are supposed to be responsible for excessive collagen production and deposition, as well as iERM contraction. Myofibroblast formation is probably dependent on soluble factors in the microenvironment of the membrane, in particular transforming growth factor beta (TGFβ), as well as on properties of the substrate to which the cells are attached. The stiffness of the extracellular membrane may be a key factor in myofibroblast formation. Membrane stiffness is the result of the presence and proportion of its various collagenous and non-collagenous components. Also, modifications of membrane macromolecules, e.g. by the accumulation of advanced glycation end (AGEs) products, may increase the stiffness of the membrane and may thus contribute to the fibrotic process. Interestingly, AGE accumulation is an age-related phenomenon, which may also be involved in the pathogenesis of anomalous PVD, a factor that probably contributes to the early stages of iERM formation. Thus, AGEs accumulation may be an important link connecting epidemiologic findings to the pathogenesis of iERM.

2. Epidemiology

Over the last two decades, several population-based epidemiology studies were conducted to estimate the prevalence and risk factors of iERM (Table 1). In these studies, non-mydriatic retinophotography was generally used to evaluate the presence and extent of epiretinal membrane. The Han Dan Eye Study used both retinophotography and optical coherence tomography (OCT) for the detection of epiretinal membrane. Aging is a consistently found risk factor and therefore appears to be important in the pathogenesis of iERM. Increased prevalences of iERM were noted in the population over 60 years of age and peak prevalences were observed between ages 70 and 79 years (11.6%~35.7%). Additionally, since in 70% of patients the iERM was found to be associated with posterior vitreous detachment (PVD) at the time of the diagnosis, PVD has been considered as an
important pathogenic factor in iERM formation. This seems to be underlined by the increased prevalence of iERM after cataract surgery, since the latter is known to accelerate PVD formation.\textsuperscript{1-3} Epidemiologic studies reported a great discrepancy in the prevalence of iERM from 1.02\% to 28.9\% among different ethnic groups.\textsuperscript{1-13} It appears that the overall prevalence of iERM in studies conducted in the US and Australia is higher than that in China, Japan and Singapore. This may indicate the influence of genetic and/or lifestyle factors. However, the prevalences of preretinal macular fibrosis (PMR), the more severe form of iERM that usually needs surgical intervention, are similar in these studies. Another interesting finding is that while the Americans with Chinese origin was identified as a risk factor of PMR in the Multi-ethnic population study\textsuperscript{9}, the prevalences of PMR reported in the Chinese studies are rather low (3.8\% versus 0.39\textasciitilde1.8\%).\textsuperscript{5, 12, 13} Whether this discrepancy is due to selection bias or a difference in life style and level of education is unclear. The increased prevalence of iERM in case of diabetes, hypercholesterolemia and vascular narrowing or occlusion, might point in the direction of an association with metabolic factors. The possible association with lifestyle and level of education may also point in that direction. A possible association with refractive errors has been described, but this is inconsistent, since some studies found a higher prevalence of iERM in hypermetropic and others in myopic eyes.\textsuperscript{5, 10}
## Table 1 Risk factors of idiopathic ERM

<table>
<thead>
<tr>
<th>Study</th>
<th>Year of report</th>
<th>Number of participants</th>
<th>Prevalence of iERM</th>
<th>Prevalence of PMR</th>
<th>Risk factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>The Beaver Dam Eye Study</td>
<td>1994</td>
<td>4926</td>
<td>11.8%</td>
<td>NA</td>
<td>Age, cataract surgery, arteriovenous nicking 2</td>
</tr>
<tr>
<td>The Blue Mountain Eye Study</td>
<td>1997</td>
<td>3654</td>
<td>7%</td>
<td>2.2%</td>
<td>Age, cataract surgery and retinal vein occlusion 1</td>
</tr>
<tr>
<td>The Los Angeles Latino Eye Study</td>
<td>2004</td>
<td>5982</td>
<td>18.5%</td>
<td>2.2%</td>
<td>Age, proliferative retinopathy, retinal lesions, macular holes, and cataract surgery 3</td>
</tr>
<tr>
<td>The Singapore Malay Eye Study</td>
<td>2008</td>
<td>3265</td>
<td>7.9%</td>
<td>3.8%</td>
<td>Ethnicity, age, female gender, hyperopic refraction and narrower retinal arteriolar diameter 10</td>
</tr>
<tr>
<td>The Beijing Eye Study</td>
<td>2008</td>
<td>4378</td>
<td>2.2%</td>
<td>1.8%</td>
<td>Age, higher education, visual field loss 13</td>
</tr>
<tr>
<td>The Funagata Study</td>
<td>2009</td>
<td>1758</td>
<td>5.44%</td>
<td>1.49%</td>
<td>Age and diabetes 11</td>
</tr>
<tr>
<td>The Han Dan Eye Study</td>
<td>2009</td>
<td>6565</td>
<td>3.4%</td>
<td>0.7%</td>
<td>Age and myopia 5</td>
</tr>
<tr>
<td>The Multi-Ethnic Study of Atherosclerosis</td>
<td>2011</td>
<td>5960</td>
<td>26.1%</td>
<td>3.8%</td>
<td>Chinese origin, age, presence of diabetes, and hypercholesterolemia 9</td>
</tr>
<tr>
<td>The Singapore Indian Eye Study</td>
<td>2012</td>
<td>3400</td>
<td>10.2%</td>
<td>3.5%</td>
<td>Age, myopia, and narrower retinal arteriolar diameter 7</td>
</tr>
<tr>
<td>The Beixinjing Block Study</td>
<td>2012</td>
<td>3727</td>
<td>1.02%</td>
<td>0.39%</td>
<td>Diabetes and high level of education 12</td>
</tr>
<tr>
<td>The Melbourne Collaborative Cohort Study</td>
<td>2013</td>
<td>21241</td>
<td>8.9%</td>
<td>3.9%</td>
<td>Age and ethnicity (southern European origin) 4</td>
</tr>
</tbody>
</table>

iERM: idiopathic epiretinal membrane; PMR: preretinal macular fibrosis.
3. Clinical features

Idiopathic ERM is a thin *glistening* membrane over the macula with or without retinal wrinkling. Clinically, a grading system proposed by Gass has been widely used to describe the different stages of the disease.14

Grade 0 (cellophane maculopathy): A transparent membrane without distortion of the underlying retina is observed. Ophthalmoscopically or biomicroscopically, a cellophane light reflex over the retinal surface is seen. The patient is asymptomatic and the diagnosis is usually an incidental finding during routine ophthalmic examination.

Grade 1 (Crinkled cellophane maculopathy): As the disease progresses, shrinkage or contraction of the iERM results in irregular wrinkling of the inner layers of the retina. Fine superficial radiating folds extending outward from the margins of the contracted iERM are often the most easily distinguished biomicroscopic feature. In some cases, the contraction may be sufficient to cause tortuosity of the fine macular capillaries. The reduction in visual acuity from these iERMs is primarily the result of the distortion of the inner retinal layers and not a function of the thickness or opaqueness of the membrane. When the fovea is affected, patients often complain of distorted or blurred central vision of the affected eye. Other reported symptoms include loss of binocularity, central photopsia and macropsia.

Grade 2 (Preretinal macular fibrosis): The iERM is characterized by a thicker and more opaque membrane obscuring the underlying retinal vasculature and a marked full thickness retinal distortion. Increasing vascular tortuosity and size of involved vessels tend to signify more advanced disease. Retinal oedema, small retinal haemorrhages, cotton-wool spots and exudates may all be seen in conjunction with these more opaque membranes. The development of severe macular distortion can induce retinal oedema and breakdown of the underlying
blood-retina barrier that can be shown by fluorescence angiography. Grade 2 iERM is also referred to as macular pucker. Approximately 80% of patients with Grade 2 iERM will have symptoms of blurred vision and/or metamorphopsia.\textsuperscript{15} In general, iERM is a chronic disease and its onset and progression are usually slow. According to the Blue Mountain Eye study, the 5-year cumulative progression rate from grade 0 to grade 2 iERM was reported as 9.3%, and the overall progression, regression and stable rates were 28.6%, 25.7%, and 38.8%, respectively.\textsuperscript{6}

The diagnosis of iERM is primarily based on clinical findings. Moreover, it is a diagnosis of exclusion, which needs a comprehensive ophthalmic examination to rule out the possibility of primary ocular diseases, such as peripheral retinal breaks, retinal vascular occlusive diseases, inflammatory disorders of the retina and choroid, and vitreo-macular traction syndrome.\textsuperscript{16, 17} Besides the subjective ocular fundus examination, many objective examination techniques have been applied to assess the extent of morphological and functional changes of iERM.\textsuperscript{18-20} Optical coherence tomography (OCT) has been extensively used to obtain cross-sectional images of the retina with a 10-micron axial resolution initially. OCT data can be qualitatively and quantitatively analysed to show the morphologic features of iERM and associated macular changes such as macular oedema. Thickening of the macular retina is correlated with decreased visual function and the resolution of macular oedema after surgical removal of the iERM is associated with improvement of visual function.\textsuperscript{21, 22} The recent development of spectral-domain OCT (SD-OCT) offers a higher axial resolution (5~7\textmu m) which allows more precise visualization of the intra-retinal morphologic features such as the integrity of the photoreceptor inner and outer segment (IS/OS) junction, photoreceptor outer segment length (PROS), central foveal thickness, and outer foveal thickness. Clinical researches have been carried out to assess these morphological features and their association with preoperative visual function and postoperative visual recovery. The results
indicated that preoperative disruption of the IS/OS junction, the length of PROS and the thickness of the inner retinal layer were correlated with visual function and thus might be useful in predicting surgical outcome.\textsuperscript{23-25} Fluorescein angiography (FA) has also been used to assess patients with iERM, especially in cases where ocular media opacity precludes a proper fundus examination. FA in patients with iERM often demonstrates intra-retinal leakage or pooling of dye indicating cystoid macular oedema, and retinal vessel distortion indicating membrane contraction.\textsuperscript{26} Recently, the presence of retinal vessel printings on fundus auto-fluorescence imaging which indicates a displacement of distorted retinal vessels, has been reported to be correlated to the degree of metamorphopsia and retinal tissue damage\textsuperscript{27} The newly developed imaging techniques and analytic algorithms may provide novel quantitative measures to detect early retinal damage and to follow-up the postoperative recovery non-invasively. Additionally, macular function examinations such as multifocal electro-retinography (mfERG) and preferential hyperacuity perimetry have also been tested in iERM patients to explore their diagnostic and monitoring value and their correlation with retinal architecture changes and recovery.\textsuperscript{18, 28} However, at present, the limited numbers of patients, short follow-up periods (3 to 6 months) and discrepancy in the results prevent a definite conclusion with regard to their clinical value.

4. Pathology

Idiopathic ERM is a sheet of fibrotic tissue that varies in thickness from a single layer of collagen with interspersed cells to a thicker, multi-layered fibrocellular proliferation that often bridges coarse folds on the retinal surface. Idiopathic ERMs are composed of two major components: cells of retinal and extra-retinal origin and extracellular matrix (ECM) proteins.\textsuperscript{29, 30} Most iERM contain a variety of cell types, including one or more of the following: glial cells (retinal Müller cells, astrocytes
and microglia), hyalocytes, macrophages, retinal pigment epithelial cells, fibroblasts and myofibroblasts.\textsuperscript{31, 32} A precise identification of the origin of cells in iERM by either immunohistochemistry or electron microscopy is hindered by the ability of cells to transdifferentiate during the process of membrane formation. This makes cell marker identification and morphological assessment less specific. Evidence showed that retinal Müller cells, hyalocytes and retinal pigment epithelial (RPE) cells have the ability to differentiate into a myofibroblast-like phenotype that is responsible for excessive collagen production and deposition as well as the contractile activity of iERMs.\textsuperscript{33-35}

From the vitreous (inner) side to the retinal (outer) side, the ERM usually consists of: (1) an inner cellular layer, consisting of one or multiple cell layers, (2) an outer ECM layer containing bundles of extracellular fibrils, which usually are randomly oriented. The outer portion of the ECM layer contains extracellular fibrils, fragments of ILM and - in case of vitreoschisis or partial PVD - residual native vitreous fibrils. The laminar structure is clear in cellophane maculopathy and less prominent in preretinal macular fibrosis. The exact origin of the collagenous components in the ECM of ERM is still an open question. Based on their morphological characteristics under the transmission electron microscopy, Kritzenberger et al suggested that the inner cellular layer and the inner portion of ECM layer were newly formed during the pathogenesis of ERM.\textsuperscript{29}

### 4.1 Cellular components in iERM

By light and electron microscopic analyses, the cellular and extracellular components of iERM have been characterized. In previous studies, various cell types have been identified in iERM, which are thought to be important during its formation. Although the phenotypic transition of the epiretinal cells causes some
uncertainty concerning the origin of the cells, basic morphologic features and specific immunohistochemical cell markers are still informative.

4.1.1. Epiretinal cells of glial origin

Cells of glial origin are either derived from retinal Müller cells and/or astrocytes. It has been suggested that retinal glial cells are one of the major cellular components of iERM.\textsuperscript{36} Retinal glial cells are supposed to be able to migrate through microscopic defects in the ILM that resulted from PVD. Subsequently, cells will then proliferate on the inner surface of the retina to form an ERM.\textsuperscript{37, 38} Ultrastructural features of cells of glial origin include: polarization with an underlying basement membrane, microvilli, and junctional complexes arranged in a monolayer containing masses of intermediate filaments 10nm in diameter. The most common immunohistochemical markers for retinal glial cells include glial fibrillar acidic protein (GFAP), vimentin and cellular retinaldehyde-binding protein (CRALBP).\textsuperscript{39-41} GFAP and vimentin are intermediate cellular filament-forming proteins that are cytoskeleton components. The mature retinal Müller cells predominantly express vimentin whereas retinal astrocytes mainly express GFAP. A limited GFAP expression can be found in the end-feet of mature Müller cells. The expression of GFAP is significantly up-regulated during retinal Müller cell activation and is a hallmark of retinal Müller cell gliosis.\textsuperscript{42} However, a recent study showed co-localization of GFAP and hyalocyte markers.\textsuperscript{32} This finding indicates that GFAP is not strictly expressed in cells of glial origin. CRALBP is expressed in RPE cells and Müller cells, which plays an important role in the visual cycle by functioning as an acceptor of 11-\textit{cis}- retinol from the isomerohydrolase reaction.\textsuperscript{43} Therefore, the expression of CRALBP has been used as a marker for retinal Müller cells.\textsuperscript{42}

4.1.2. Hyalocytes
Hyalocytes are present in the cortical vitreous, with a larger population near the vitreous base and a lower number at the posterior pole. Morphologically, hyalocytes have been described as spindle-shaped, rounded or star shaped cells containing large amounts of secretory granules and a well-developed Golgi apparatus. Morphologic and enhanced green fluorescent protein assisted cell tracking studies indicated that hyalocytes are bone marrow derived cells of monocyte/macrophage lineage.\textsuperscript{44} Morphologically, hyalocytes and macrophages are similar, and both have a paucity of lysosomes. However, hyalocytes are negative to CD68, the classic macrophage marker.\textsuperscript{45} Hyalocyte markers include CD35, CD45, CD64 and CD163, which are markers of the monocyte/macrophage lineage.\textsuperscript{32, 46}

The importance of hyalocytes in the development of iERM has been emphasized since the recognition of the possible role of vitreous cortex remnants on the ILM in the pathogenesis of iERM.\textsuperscript{47} Studies on the biology of hyalocytes revealed that these cells have the ability to proliferate and transdifferentiate into a myofibroblast-like phenotype and may be responsible for the formation of fibrotic tissue and membrane contraction.\textsuperscript{34, 48} Therefore, proliferation and phenotypic transition of hyalocytes on the inner retinal surface after an anomalous PVD represents a plausible theory of the pathogenesis of iERM.

4.1.3. Macrophages

Macrophages have essential functions in wound healing, such as coordinating cell migration and matrix remodelling. These functions are fundamental in the process of iERM formation. The macrophages in the iERM are characterized by the presence of pleomorphic contents, especially melanin and haemosiderin in various stages of degradation within the secondary lysosomes. The other notable ultrastructural features of macrophages are an irregular shape of cell and nucleus, a paucity of lysosomes and the presence of membrane bound granules of varying electron
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density. The most common marker for macrophages is CD68, a member of lysosomal/endosomal-associated membrane glycoproteins that are highly expressed by human monocytes and tissue macrophages. However, since retinal microglia are also considered as tissue macrophages, specific markers, such as Lectin RCA-1, CD11b and Ionized calcium binding adaptor molecule 1 (Iba1) should be used to specifically identify them as microglia.

4.1.4. Fibroblasts

Fibroblasts in iERM are featured by their fusiform shape and the absence of signs of polarity such as apical microvilli or a basement membrane, which is different from fibrous astrocytes of glial origin. They differ from hyalocytes by the presence of longer extensions (with a maximum length of 260 μm) and the absence of PAS-positive granules. The cytoplasm of fibroblasts often contains prominent rough endoplasmic reticulum and Golgi complexes suggesting an active secretory state. In specimens of iERM, fibroblasts were often found in the vicinity of newly formed collagens.

4.1.5. Myofibroblasts

Myofibroblasts are found at sites of wound healing and chronic inflammation, and are believed to play a pivotal role in the healing process and the pathogenesis of fibrosis. By secreting extracellular matrix proteins, and by promoting the contraction of granulation tissue through the expression of the contractile protein, α-smooth muscle actin (α-SMA), these cells are essential for wound repair. The formation of ERMs has been considered as an aberrant wound healing process driven by many growth factors and cytokines, which leads to a contractile scar formed on the inner surface of the retina. The myofibroblast is the key mediator of ECM secretion and contraction during this process. Ultrastructural features of myofibroblasts are their characteristic spindle shape and the presence of large
aggregates of microfilaments about 4 to 5 nm thickness and small fusiform densities. A specific marker for myofibroblasts is α-SMA, an isoform of actin, which has been proven to be important for the contractile activity in several cell types.  

Myofibroblasts have heterogeneous origins. By double labelling iERM specimens with α-SMA and other cell markers, several studies have tried to pinpoint the origin of the myofibroblasts. These studies showed that myofibroblasts can form by transdifferentiation of various cell types including hyalocytes, retinal pigment epithelial cells (RPE) and retinal glial cells.  

4.1.6. Retinal pigment epithelial (RPE) cells

The presence of RPE cells in iERM is still a matter of debate. Smiddy et al reported that RPE cells are the predominant cell type in iERM. However, other authors could not confirm this and described that cells of glial origin and/or the hyalocytes were more frequently found in iERM. The RPE cells are more commonly seen in the ERMs secondary to a retinal break and/or prior rhegmatogenous retinal detachment. Kampik et al summarized the typical electron microscopic features of RPE cells in ERM as follows: RPE cells are characterized by their epithelium-like polarity with a well-developed basement membrane and the presence of free-surface microvillus processes. Additionally, RPE cells have junctional complexes, numerous single membrane-limited melanosomes and cytoplasmic microfilaments (5 to 7 nm).  

4.2. Extracellular matrix components of iERM

The ECM of iERM contains native vitreoretinal collagens and/or collagens newly formed by epiretinal cells. By transmission electron microscopy (TEM), variable amounts of extracellular fibrils that form an irregular network are randomly oriented in the ECM layer of iERM. It has been suggested that native vitreous fibres
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have diameters of 8 to 15 nm while newly formed collagen fibrils have diameters of more than 16 nm.\textsuperscript{60} Kampik et al reported that collagenous components of iERM mainly had a smaller diameter of 10 to 15 nm.\textsuperscript{58} Kritzenberger et al reported that fibrils in the ERMs of preretinal macular fibrosis were thicker than those in cellophane maculopathy (18 to 26 nm vs. 6 to 15 nm).\textsuperscript{29} The identities of the collagens in iERM were further clarified by immune-histochemical studies, which reported the presence of type I, II, III, IV and VI collagens.\textsuperscript{29, 61} Furthermore, flat-mount preparations of surgically removed iERM will give an overview of the entire ERM specimen.\textsuperscript{62} Hereby, information on cell density, specific cell populations and the distribution patterns of ECM components can be obtained.\textsuperscript{32, 63, 64}

5. Management

5.1 Surgical management of iERM

Trans pars plana vitrectomy and epiretinal membrane peeling have been used in patients with symptomatic visual disturbances as a standard procedure.\textsuperscript{65} However, in 10 to 21% of cases, the ERMs recurred and around 3% of recurrent cases required a second surgical intervention.\textsuperscript{66, 67} Recurrent ERM is thought to result from incomplete removal. Gandorfer et al showed that ERM removal alone does not completely separate the fibrocellular tissue from the macula. Therefore, additional ILM peeling is advised to achieve a complete removal of epi- and sub- ILM proliferation thus eliminating the scaffold for further proliferation.\textsuperscript{63, 68} Several clinical series reported that ILM peeling seems to give better results than non-ILM peeling. In both groups, equivalent efficacy and safety profiles in terms of final visual outcomes were found, whereas the ERM recurrence rate was lower in the ILM peeling than in the non-ILM peeling group.\textsuperscript{69-71} However, Chang et al. also reported that the reduction in macular thickness was significantly higher in case of single (ERM only) peeling as compared to double (ERM plus ILM) peeling. Another
interesting finding was that patients in the single peeling group who had undergone successful ERM peeling, i.e. without residual ERM at the fovea, had a better postoperative visual function than those in the double peeling group. These findings indicated that ILM peeling is technically challenging and the surgical maneuver might induce additional damage to the underlying retina because ILM is a thin (a few micrometres in thickness) and nearly transparent structure. To improve the visualization of the ILM during the surgery, vital dyes such as trypan blue, indocyanine green (ICG) and brilliant blue have been used. Although some conflicting results have been found in in vivo and in vitro studies, vital dyes, especially ICG, have potential retinal toxic effects, resulting in a deeper retinal cleavage plane and potential retinal cell damage. These retinal changes were suggested to induce visual field defects and a poorer postoperative visual outcome. Furthermore, the application of vital dyes did not improve the surgical outcome in terms of visual function, resolution of macular oedema and decrease in recurrence rate. Therefore, it has been advised to only use vital dyes in case of necessity. Recently, other agents for dye-assisted ILM peeling with less potential adverse effects have been introduced. One possible alternative is the use of triamcinolone acetonide, which can be deposited on the remnants of the cortical vitreous and/or over the ILM. Another alternative is a lutein based-dye, which is theoretically safe to the retina. Maia et al reported on a small series of 12 eyes that underwent ERM and/or ILM peeling using a 0.3% lutein/zeaxanthin and 0.025% brilliant blue based dye. The preliminary results are promising, but further clinical and basic research is needed.

5.2 Pharmaceutical management of iERM and associated ocular disorders
To improve the visual function of iERM patients and to prevent the progression of iERM, several pharmaceutical treatments, including the use of anti-inflammatory and vitreolytic agents have been proposed.

### 5.2.1 Macular oedema

Macular oedema associated with iERM is one of the major anatomic abnormalities that result in a decreased visual acuity both during the pre- and post-operative period. Recent clinical data showed that preoperative cystoid macular oedema (CMO) is correlated to the presence of postoperative persistent intra-retinal cysts, whereas an increased preoperative central retinal thickness is correlated to an increased postoperative central retinal thickness.\(^{87}\) Topical anti-inflammatory agents, including non-steroidal anti-inflammatory drugs (NSAIDs) and Dorzolamide might possibly be beneficial in the resolution of macular oedema after vitrectomy for iERM.\(^{88, 89}\) Schoenberger et al reported that the administration of topical NSAIDs as compared to a placebo, resulted in a more rapid reduction in macular volume.\(^{90}\) Therefore, whether the resolution of retinal oedema could improve the visual function of iERM patients, is an interesting subject for further research. Furthermore, Henderson et al reported that a pre-existing ERM results in a higher risk of postoperative CMO after cataract surgery in non-diabetic patients. They also showed that a combination of topical steroids and NSAIDs shortens the resolution time of CMO.\(^{91}\)

### 5.2.2 Pharmacological vitreolysis for the treatment of iERM

Based on the pathogenesis of iERM (see section 7), a “clean” PVD without any damage to the ILM and without the deposition of residual cortical vitreous collagen onto the ILM, would be beneficial in preventing the formation of iERM. Kampik suggested that pharmacological vitreolysis might be helpful in the treatment of ERM, in case a layer of native vitreous collagen is present between the fibrocellular
proliferation and the ILM. He hypothesized that native vitreous collagens, mainly containing type II collagen, can be degraded by plasmin or other vitreolytic enzymes. However, current clinical studies on the effect of microplasmin have been focusing on vitreomacular traction syndrome and idiopathic macular hole, whereas the clinical value of pharmacological vitreolysis in the prevention of iERM has not yet been addressed. Moreover, Stalmans et al reported that pharmacological vitreolysis was less effective in case an ERM is present.

The current surgical management of ERMs is not ideal, and improvement of care is needed. Functionally, the visual recovery after vitrectomy combined with ERM/ILM peeling is rarely complete. Anatomically, ERMs can recur and in case of symptomatic recurrence, a second surgical procedure may be indicated. ILM/ERM peeling is a challenging technique which can lead to intra- and post-operative complications including iatrogenic macular damage, infectious endophthalmitis as well as progression of cataract. It has been shown that eyes with lower preoperative visual acuity (VA) improve more frequently and to a greater extent than eyes with better preoperative VA. However, eyes with lower preoperative VA tend to have lower final VA, while the final visual prognosis is better for eyes with better preoperative VA. Clinical research using SD-OCT showed that visual disturbances induced by iERM are associated with intra-retinal changes including the disruption of the photoreceptor integrity. These findings suggest that the damage resulting from iERMs is at least partly irreversible despite surgical management. Therefore, an ideal approach for the management of iERM should be an intervention before permanent retinal tissue damage has occurred. As aforementioned, the visual function of patients with iERM is usually affected when the membrane progresses to grades 1 and 2. This is when the membrane contraction becomes prominent. Fibrocellular tissue contraction is one of the important features of fibrosis. In order to develop valid strategies to prevent the
membrane contraction, the pathophysiology of fibrosis in vitreoretinal interface disease should be clarified.

6. Fibrosis

6.1. General introduction of fibrosis

Fibrosis has been considered as an abnormal wound healing process, characterized by the replacement of normal structural tissue elements by distorted, non-functional fibrotic tissue. Fibrosis is a response to various insults to the tissue, including chronic infections, toxic and metabolic injuries, and idiopathic inflammatory diseases. In this complex process, many fibrogenic factors and cells are involved.\(^{99}\)

In the initial stage of fibrosis, an accumulation of inflammatory cells, such as neutrophils, monocytes, T-lymphocytes and eosinophils is seen. These cells produce a variety of profibrotic cytokines, adhesion molecules and growth factors. The released factors induce activation, migration and proliferation of fibroblastic cells and promote the development of myofibroblasts. The myofibroblast is the crucial cell type in wound healing and fibrotic processes because of its amply clarified function of extracellular matrix protein (mainly collagens) secretion and tissue contraction. In normal wound healing, myofibroblasts usually undergo apoptosis when the wound is healed. In fibrotic diseases, myofibroblasts - driven by various fibrogenic factors - are persistently activated resulting in excessive deposition of collagens and severe tissue contraction. This leads to fibrotic tissue formation and the malfunctioning of organs. The resulting fibrotic tissue contains excessive extracellular matrix proteins in which collagens are the major components. This general scheme of fibrosis applies to many organs including skin, kidney, lung, liver as well as retina after different types of insults as aforementioned (Figure 1).\(^{100}\)
Myofibroblasts have generally been considered to play an important role in producing collagens and establishing tension during wound healing and pathological tissue contractions. They were first observed in granulation tissue of healing wounds.\textsuperscript{101} Subsequently, these smooth-muscle like cells were found in many fibro-contractive diseases, as well as in developing and normal contractile tissues leading to the conclusion that myofibroblasts have a role in producing contractile force.\textsuperscript{102,103} The mature myofibroblast is characterized by the expression of $\alpha$-SMA and the formation of organized stress fibres.\textsuperscript{104} Although the exact
mechanism has not yet been clarified, it has been shown that \( \alpha\)-SMA expressing myofibroblasts have a two-fold stronger contractile activity than \( \alpha\)-SMA-negative fibroblasts.\(^{105}\) All these evidences indicate that myofibroblasts are responsible for generating contractile forces in the fibrotic process. Therefore, developing treatment strategies aiming at preventing the formation of myofibroblasts has been the focus of ongoing research. Understanding the origins, functions and molecular regulations of myofibroblasts in the fibrotic process, may provide ways to develop effective treatment strategies in preventing fibrotic diseases. These may also be of potential benefit to sight threatening vitreo-retinal diseases characterized by fibrotic tissue formation, such as macular epiretinal membrane formation, proliferative diabetic retinopathy and proliferative vitreoretinopathy.

The mechanism underlying myofibroblast genesis is complex. At least three essential elements are needed for the formation of \( \alpha\)-SMA expressing myofibroblasts: (1) accumulation of biologically active TGF\( \beta \), (2) the presence of specialized ECM components such as the ED-A splice variant of fibronectin, and (3) high mechanical tension arising from the extracellular matrix.\(^{106-108}\)

Another important issue in developing effective anti-fibrotic treatment strategies is to clarify the origins of myofibroblasts. It has been shown that myofibroblasts have very heterogeneous origins in different organs.\(^{109}\) Hinz et al suggested that the term myofibroblast describes a functional status rather than a fixed cell type.\(^{110}\) While debate still exists, it has been generally suggested that the precursors of myofibroblasts can be derived from three potential sources: (1) resident mesenchymal cells, such as tissue fibroblasts, can differentiate into myofibroblasts; (2) epithelial cells can become myofibroblasts through epithelial mesenchymal transition (EMT); (3) bone marrow derived cells, consisting of fibrocytes and circulating mesenchymal cells, can be recruited to the site of injured tissue and
differentiate into a myofibroblast phenotype. The myofibroblasts in lung fibrosis seem to be derived from resident fibroblasts (perivascular and peribronchiolar adventitial fibroblasts), circulating fibrocytes and bone marrow derived progenitor cells.\(^{107, 108, 111, 112}\) In liver fibrosis, hepatic stellate cells, hepatocytes and bone marrow derived fibrocytes all contribute.\(^{113-117}\) In fibro-contractive vitreoretinal diseases, retinal Müller cells (of neuro-epithelial origin), hyalocytes (bone marrow derived) and retinal pigment epithelial cells may all differentiate into a myofibroblast-like phenotype and may thus contribute to collagen deposition and membrane contraction.\(^ {34, 35, 118}\)

**6.2. The regulatory role of the extracellular matrix in fibrosis**

Recent advances in extracellular matrix biology indicate that the ECM proteins have diverse cellular effects beyond providing structural support. Growth factors can be activated by binding to specific domains (ligands) of ECM proteins. Activated growth factors can then execute their biological functions, such as regulation of cell proliferation and differentiation. The dynamic remodelling of ECM during fibrosis results in an alteration of the biochemical and biophysical properties of the ECM. These alterations play an influential role in fibrosis.

The newly produced ECM proteins could promote the fibrotic process. In pulmonary fibrosis, an increase in ED-A fibronectin production induces proliferation and myofibroblast trans-differentiation of fibroblasts. In contrast, ED-A deficient mice are less susceptible to fibrosis.\(^{119-121}\) Additionally, there is an increase in the production of ED-A fibronectin in lung fibroblasts in aging mice, which suggests a connection between age, ED-A fibronectin and fibrosis.\(^ {122}\) Type I and III collagens, the most abundant collagens produced during fibrosis, can stimulate the proliferation of cardiac fibroblast in vitro.\(^ {123}\) Type IV collagen can induce an epithelial to mesenchymal transition process in mammary epithelial cells.\(^ {124}\) Finally, 40
type VI collagen, which is upregulated in fibrotic matrix remodelling, promotes myofibroblast transdifferentiation of corneal and cardiac fibroblasts.\textsuperscript{125, 126}

The influential role of mechanical tension and stiffness of the ECM in regulating the formation of myofibroblasts has been emphasized by recent experimental findings.\textsuperscript{127-129} Myofibroblast formation may be induced by increased ECM stiffness through the activation of transforming growth factor beta (TGFβ) as has been extensively reported in fibroblasts isolated from heart, lung, liver, and gingiva.\textsuperscript{128, 130-132} TGFβ1 is the key mediator in the induction of the \textit{de novo} expression of α-SMA, an increased expression of ED-A fibronectin, and an increased assembly of stress fibres and focal adhesions in myofibroblast.\textsuperscript{133} TGFβ1 is produced and secreted into the ECM in a latent form. By binding to the proper ligand and/or by being enzymatically cleaved, latent TGFβ1 is activated. Worthington et al suggested that the activation of latent TGFβ can be achieved by its binding to integrins. The biophysical force applied to the integrins by the increased ECM stiffness and tension can result in TGFβ activation, thereby amplifying the fibrotic process in an autocrine manner.\textsuperscript{134}

It is now accepted that both ECM proteins and the mechanical properties of the ECM have an influential role in the formation of myofibroblasts as well as the production of collagens. However, the exact mechanisms by which cells sense these outside-in signals and the cell signalling pathways involved have not yet been clarified and are the focus of ongoing research. Advances in this field will extend our knowledge of the pathophysiology of fibrosis and may help to find novel therapeutic targets to prevent the detrimental effects of this pathological event.

\textbf{7. Pathogenesis of iERM}

The most important risk factors of iERM are aging and the development of PVD. Age-related changes in the ECM of the vitreoretinal interface are thought to induce
PVD. The progression of iERM from cellophane maculopathy to macular pucker can be regarded as a fibrotic process because the pathological findings are an increased ECM protein deposition and the membrane contraction in which myofibroblasts play a crucial role. In order to develop therapeutic strategies to prevent the detrimental effect of iERM on central macular function, two main questions need to be addressed. First, what is the stimulus of iERM formation and second, what is the cause of iERM progression? Recent clinical imaging studies and laboratory researches have begun to explain the pathogenesis of the disease and the way the risk factors, such as aging and PVD are involved.

7.1. Theories on the pathogenesis of iERM

The classic explanation of the initiation of iERM formation was proposed by Foos in his seminary researches. He hypothesized that a detachment of the posterior vitreous could result in minor defects in the ILM allowing the migration of retinal glial cells (Müller cells and astrocytes) to the retinal surface. By demonstrating the glial features of retinal Müller cells and their migration to the inner retinal surface through a defect in the ILM, Foos proposed that the retinal Müller cells are the predominant cells in iERM formation. Subsequently, McLeod et al also reported their findings of cellular proliferation clusters in the vicinity of ILM defects to support this hypothesis. However, subsequent immunohistological studies suggested that defects in the ILM occur only rarely. Therefore, this theory could not be accepted as a general explanation for iERM formation and an alternative theory was formulated.

The alternative theory - which has become widely accepted - proposed that an anomalous PVD has an important role in the formation of iERM. The hyalocytes residing in cortical vitreous remnants that remain on the ILM can be activated by various growth factors. This may result in cellular proliferation and myofibroblast
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differentiation thus leading to iERM formation and contraction. Vagaja et al studied murine hyalocytes in the normal wild type and transgenic Kimba mice (excessive vascular endothelial growth factor produced in RPE cells). They found that the number of hyalocytes increases in response to aging and the local production of VEGF. Sommer et al reported that basic fibroblast growth factor (bFGF) can induce proliferation of hyalocytes and that TGFβ1 promotes ECM production in cultured hyalocytes.

In light of the second theory, we speculate that the mechanical traction induced by PVD could stimulate the retina to produce the growth factors that regulate the hyalocytes. Growing evidence indicates that PVD is a chronic process which starts at the perifoveal region and slowly expands itself to the posterior pole. During this process, the cortical vitreous can exert antero-posterior and tangential traction on the retinal cells. This mechanical traction can induce the expression of bFGF in retinal Müller cells and VEGF in RPE cells. These locally produced growth factors may promote the proliferation and ECM production of hyalocytes in a paracrine fashion. Therefore, factors that result in increased vitreoretinal traction may be involved in the pathogenesis of iERM.

7.2. Advanced glycation end products promote the formation of anomalous PVD

Anomalous PVD is supposed to be the result of extensive vitreous structure collapse and insufficient vitreoretinal dehiscence, which manifests itself as either a partial posterior vitreous detachment or a split of the cortical vitreous (vitreoschisis). This has been accepted as a unifying pathogenic concept of various vitreoretinal diseases. Recently, clinical evidences suggested that increased vitreoretinal traction induced by an anomalous PVD is an important predisposing factor in many vitreomacular diseases, such as idiopathic macular hole, idiopathic epiretinal membrane, vitreomacular traction syndrome, age-related macular degeneration.
and diabetic macular oedema.\textsuperscript{92, 144} By optical coherence tomography/scanning laser ophthalmoscopy examination, Gupta et al found that vitreoschisis was frequently associated with iERM.\textsuperscript{145}

Advanced glycation end products (AGEs) accumulation represents an important molecular mechanism of the formation of anomalous PVD. In diabetic patients, in particular in those with clinically significant diabetic retinopathy, vitreous AGEs accumulation has been found. Also, in diabetic patients, a higher prevalence of incomplete PVD and vitreoschisis has been observed.\textsuperscript{146, 147} In line with this, data from our laboratory indicated that an increased pentosididine accumulation in the vitreous is associated with a lower rate of complete PVD.\textsuperscript{148}

Ageing is commonly associated with an increased modification of proteins, lipids and DNA. Non-enzymatic glycation of aldehyde groups in sugars and dicarbonyls (Maillard reaction) is an important modification, which results in the formation of AGEs. This process was first described in the early 1900’s, when it was noticed that amino acids heated in the presence of reducing sugars resulted in a characteristic yellow-brownish coloration.\textsuperscript{149} During aging (and especially in diabetes mellitus) the rate of AGEs formation increases resulting in a significant accumulation of AGEs. One of the well-known AGEs is glycosylated haemoglobin A1c (HbA1c), which is widely used as an indicator of prolonged exposure to increased blood glucose in the screening and management of diabetes mellitus. In the normal physiological situation, AGEs can only be removed by degradation of the protein on which they are formed. Thus, the accumulation of AGEs will occur in tissues with long-lived macromolecules such as the collagens at the vitreoretinal interface. AGEs and their receptors (RAGEs) represent important pathophysiological mediators in age and diabetes related diseases. They have been recognized as mediators of inflammation in atherosclerosis, Alzheimer’s disease and diabetic complications.\textsuperscript{150, 151}
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AGEs and their receptors in the pathogenesis of age related macular degeneration and diabetic retinopathy has been comprehensively reviewed previously.\textsuperscript{152-154}

AGEs accumulation has been reported in the aging cortical vitreous fibrils, ILM and the Müller cells.\textsuperscript{155-157} This can affect the structure and mechanical properties of the extracellular matrix network, and thus induce liquefaction of vitreous body and promote mechanical vitreous fibrils breakdown. Several studies suggested that AGEs can promote the degenerative changes of vitreous by inducing hyaluronan degradation and its dissociation from the vitreous fibrils.\textsuperscript{155, 158-160}

AGEs accumulation is also associated with an increased tissue stiffness and brittleness in collagen rich tissues.\textsuperscript{161, 162} For instance, the stiffness of human articular cartilage collagens (mainly composed of type II collagen) increased with age and their digestibility decreased proportionally to the extent of AGEs accumulation.\textsuperscript{163, 164} The increased stiffness and brittleness may contribute to the age-related failure of collagen fibrils to resist mechanical stress and represents a potential molecular mechanism whereby ageing is a predisposing factor for collagen network damage. Recent research on the pathogenesis of vitreous liquefaction found evidence of enzymatic collagen degradation within the vitreous but also at the vitreo-retinal interface.\textsuperscript{157, 165} AGE accumulation may alter the mechanical properties of vitreous fibrils as mentioned above, and it may interfere with enzymatic vitreolysis and PVD. Thus, mechanical failure of vitreous fibrils and the formation of an anomalous PVD may result.

Furthermore, AGEs accumulation may also induce the up-regulation of certain growth factors, which were found to be upregulated in iERM and suggested being involved in promoting epiretinal cell proliferation and transdifferentiation. The production of VEGF and bFGF in retinal Müller cells induced by AGEs was reported in a mouse model and cell culture studies.\textsuperscript{166, 167}
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7.3. Collagens that promote iERM formation and contraction

While the origins of myofibroblasts may vary according to the aforementioned theories, the subsequent formation of myofibroblasts and fibrotic tissue are the main cause of visual disturbance in all iERM patients regardless of an association with complete or anomalous PVD. To prevent the detrimental effects of the iERM, one of the most promising approaches is to prevent the formation of myofibroblasts or to promote their apoptosis. By examining factors that induce myofibroblast formation more closely, novel treatment targets may be identified.

7.3.1. Fibrillar collagen: Type I and III collagens

Type I and III collagens belong to the fibrillar collagen family and are the most abundant ECM proteins in the human body. They are expressed in every major organ and tissue. During the fibrotic process, the production of type I and III collagens by fibroblasts and myofibroblasts is enhanced. These collagens form the main structural network of the fibrotic scaffold. At the vitreoretinal interface, the possible fibrillar collagen producing cells are the retinal Müller cells and hyalocytes. The retinal Müller cells have been shown to express the gene of type I collagen and this expression can be upregulated by many fibrogenic growth factors under the influence of substrate stiffness.\textsuperscript{168, 169} Hyalocytes can produce glycoproteins, proteoglycans and collagens as we discussed in the previous section.

Upon the formation of ERM, type I collagen along with other fibrosis related collagens are expressed forming the major structural framework of ERM.\textsuperscript{29, 170} This framework facilitates cellular adhesion and migration and also stimulates cellular proliferation by up-regulation of DNA synthesis through binding to integrin receptors. Several in vitro studies showed that immobilized and soluble forms of type I collagen potentially simulate the adhesion, migration and proliferation of fibroblasts.\textsuperscript{171} Schlie-Wolter et al suggested that the cellular behaviour in response
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to extracellular matrix substrates is cell type specific. For example, type I collagen has a stronger effect on chondrocytes compared to that of fibroblasts and osteoblasts. Type III collagen is known to be expressed in the early stage of fibrosis and has been suggested to modify the existing fibrillar network by covalent cross-linking. Fibrotic collagens have been found to contain higher amounts of hydroxyallysine derived cross-links compared to those of normal tissue. The elevation of hydroxyallysine cross-links in collagen fibres leads to an increase in mechanical strength and reduction of collagen digestibility.

7.3.2. Non-fibrillar collagens: Type IV and VI collagens

Type IV collagen

Type IV collagen belongs to the basement membrane associated collagen group, which is ubiquitously found in the majority of the tissue boundaries near epithelial, endothelial, fat, muscular and nerve cells. There are six different type IV collagen α chains numbered α1(IV) through α6(IV) and encoded by COLα1(IV) through COLα6(IV) genes. This results in only three types of heterotrimers: α1α1α2(IV), α3α4α5(IV) and α5α5α6(IV). The COL4A1 and COL4A2 genes are highly conserved through all species and their protein products are present in almost all basement membranes, whereas COL4A3 to COL4A6 are more spatially and temporally restricted. Mutations in COL4A1 and COL4A2 are associated with congenital cataract, anterior segment dysgenesis including Axenfeld–Rieger anomaly, juvenile-onset glaucoma, optic nerve hypoplasia and retinal degeneration. Mutations in COL4A3, COL4A5 and COL4A6 result in Alport’s syndrome, which can affect the retina, cochlea and kidney. Type IV collagen heterotrimers are assembled intracellularly in the rough endoplasmic reticulum and secreted into the extracellular matrix. After secretion, the heterotrimers form
a mesh-like network and integrate with other basement membrane components, such as laminin, nidogen/entactin and perlecan, to form basement membranes. Type IV collagen is the predominant extracellular protein in the human retinal inner limiting membrane (ILM) and its abundance increases with age. Candiello et al using western blot analysis, estimated that type IV collagen accounts for 57% of the total proteins in human ILM and the proportion of type IV collagen increases with age.\textsuperscript{179} In patients with Alport’s syndrome where the production of type IV collagen is compromised, a thinned ILM/nerve fibre layer was revealed.\textsuperscript{180} The distribution of type IV collagen α chains in the ILM changes during the embryonic and postnatal periods. Recent research on the developmental distribution of type IV collagen isoforms in mouse eyes showed that the ILM contains α1(IV), α2(IV), α5(IV) and α6(IV). The signal intensity of α1(IV)α1(IV)α2(IV) in ILM decreased with age and α5(IV)α5(IV)α6(IV) appeared to be the predominant heterotrimer after birth.\textsuperscript{181} These findings suggest that the α1(IV)α1(IV)α2(IV) heterotrimer is essential during the embryonic period and α5(IV)α5(IV)α6(IV) is important in the mature ILM.

Type IV collagen is critical for neuron survival and angiogenesis and may be a regulator of matrix remodelling. Native type IV collagen is present in the ILM and may act as a substrate for cell growth and migration. Furthermore, the native form of type IV collagen can induce an epithelial to mesenchymal transition, an increase in N-cadherin and vimentin expression, an increase of matrix metalloproteinase-2 (MMP-2) secretion and activation of focal adhesion kinase (FAK) and nuclear factor κ-light chain-enhancer of activated B cells (NFκB) in mammary epithelial cells.\textsuperscript{124} During wound healing in the central nervous system, the expression of type IV collagen was upregulated and found to induce astrocytic expression of thrombospondin-1, a potent transforming growth factor beta activator. Also, type IV collagen expression is upregulated during ERM formation.\textsuperscript{182} The COLa1(IV)
mRNA was found to be significantly increased in epiretinal membranes of patients with iERM or proliferative diabetic retinopathy.\textsuperscript{170}

Type VI collagen

Type VI collagen (Col VI) is an anchoring collagen which forms a distinct microfibrillar network in most connective tissues and basement membranes. It was originally discovered in pepsin extracts of aortic intima in 1983.\textsuperscript{183} Mutations in COL6A1, COL6A2, and COL6A3 genes cause a group of inherited muscular dystrophies, namely Myosclerosis myopathy, Bethlem myopathy (autosomally dominantly inherited) and Ullrich congenital muscular dystrophy (autosomally recessively inherited). In these diseases, the deficiency and malfunction of Col VI leads to muscle weakness caused by a faulty attachment of muscle fibre cells to their adjacent extracellular matrix.

Col VI consists of three genetically distinct polypeptide α-chains: α1(VI), α2(VI) and α3(VI), which are encoded by COL6A1, COL6A2 and COL6A3, respectively. The α1(VI) and α2(VI) chains contain one N-terminal (N1) and two C-terminal von Willebrand factor type A (vWF-A) modules. The α3(VI) chain is larger than the other two and is characterized by a short triple helix flanked by large N- and C-terminal globular domains containing 12 vWF-A modules. Recent studies identified three novel Col VI chains, namely, α4(VI), α5(VI) and α6(VI). These chains structurally resemble the α3(VI) chain containing one N-terminal domain made of seven vWF-A modules.\textsuperscript{184, 185} Col VI α-chains are synthesized and assembled into heterotrimeric monomers in the cytoplasm. Intracellularly, the heterotrimeric Col VI monomers assemble in a staggered and antiparallel fashion to form dimers, which then align to form tetramers, which are secreted into the extracellular space. Both dimers and tetramers are stabilized by disulphide bonds. The secreted Col VI tetramers align end-to-end in the extracellular space to form beaded microfibrils.\textsuperscript{186}
Col VI was proposed to act primarily as an anchoring fibre connecting collagens to the surrounding matrix.\textsuperscript{187} Recently, evidence for additional functions of Col VI has been found. For instance, Col VI plays an influential role in many pathophysiological processes, such as angiogenesis, tumour resistance to chemotherapy, neuron protection in Alzheimer’s disease, and induction of fibroblast proliferation and myofibroblast differentiation in fibrosis.\textsuperscript{188-190}

Col VI has a critical role in maintaining connective tissue integrity and anchoring the interstitial matrix to the basement membrane.\textsuperscript{191} The characteristic beaded and highly branched microfibrillar network is associated with the basement membrane of most tissues, such as foetal membranes, large vessels, skin, liver, kidney and skeletal muscle.\textsuperscript{192, 193} As an anchoring collagen, Col VI has been found to interact with many ECM components such as type I, II, IV, and XIV collagens, fibronectin, perlecan, biglycan, decorin and hyaluronan.\textsuperscript{191, 194-200}

Col VI has been identified in vitreous, at the ILM and in the basement membranes of retinal blood vessels.\textsuperscript{201, 202} At these sites, it may interact with other described components such as type II and IV collagens, hyaluronan, and fibronectin. This may imply that Col VI is one of the important molecules involved in vitreoretinal and vitreovascular adhesion.\textsuperscript{203} Vitreous collagen fibres, containing mainly type II collagen and hyaluronan may penetrate the ILM and thus mediate vitreoretinal adhesion itself.\textsuperscript{204} In addition, vitreous fibres may penetrate deeper and adhere to basement membranes surrounding the retinal blood vessels.\textsuperscript{202} Thus, Col VI may be involved in vitreoretinal disease processes, such as tearing of the retina and retinal blood vessels during posterior vitreous detachment.

Furthermore, Col VI may also have an influential role in the pathogenesis of iERM formation. A growing body of evidence suggests that Col VI plays an important role in the fibrotic process by regulating myofibroblast behaviour and ECM remodelling.
An over expression of Col VI was found in liver and lung fibrosis. Shamhart et al demonstrated that Col VI can induce myofibroblast differentiation of cardiac fibroblasts. Col VI can induce proliferation of fibroblasts and other mesenchymal cell lines in vitro and these effects are independent of growth factors such as platelet derived growth factor, basic fibroblast growth factor and transforming growth factor beta-2. The way Col VI affects cellular function involves its binding to integrins resulting in cytoskeletal changes. This may cause a structural alteration of the integrins leading to activation of the focal adhesion complex that initiates the cell signalling cascade. Ruhl et al reported that Col VI can induce tyrosine phosphorylation and activate mitogen-activated protein kinase ERK-2 in fibroblasts, which is partially mediated by integrin β1. Bryant et al reported that integrin α3 interacts with Col VI to promote myofibroblast differentiation of cardiac fibroblasts in post cardiac infarction remodelling.

The proposed regulatory role of Col VI in the fibrotic process promoted us to investigate Col VI in relation to the formation of ERM in vitreoretinal diseases. We observed that Col VI is present in idiopathic epiretinal membrane (unpublished observation) but not in the ERM associated with idiopathic macular hole. Clinically, the former is associated with more pronounced tissue contraction than the latter. Furthermore, Col VI can induce α-SMA up regulation in retinal Müller cells in vitro, which suggests that Col VI promotes myofibroblast transdifferentiation of retinal Müller cells (unpublished observation). Since the retinal Müller cell is one of the important cell types involved in ERM formation and contraction, the expression of Col VI in this pathophysiological process may not only contribute to the mechanical property of the ERM as Kritzenberger et al suggested, but may also promote the activity of myofibroblasts in a paracrine fashion.
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The accumulation of Col VI has also been found in several other ocular pathologies. Gottanka et al reported an increase of Col VI around the optic nerve bundle and the basement membrane of the central retinal vessels in primary open angle glaucoma. Astrocytes in the optic nerve may be responsible for Col VI production. Knupp et al found Col VI deposition in the posterior cortical vitreous in patients with age related macular degeneration. The exact role of Col VI in these pathological conditions is unknown. However, given the potential active role of Col VI in the pathophysiological process, an understanding of the underlying mechanisms may provide new targets for clinical therapy.

8. Summary and perspective

Remodelling of the ECM at the vitreoretinal interface, both resulting from ageing and fibrotic changes, plays a significant role in the pathogenesis of iERM. The age-related modification of ECM, such as AGEs accumulation, increases the rigidity and brittleness of the vitreoretinal collagens, thus creating an aged vitreoretinal interface prone to the formation of anomalous PVD and ERM. Collagens newly produced during iERM formation, including type I, III, IV and VI collagens, promote the fibrotic process by: (1) inducing fibroblast proliferation and myofibroblast transdifferentiation and (2) forming a rigid collagen scaffold on which the myofibroblast precursor cells are more susceptible to fibrogenic factors. Recent advances in understanding the biochemical and biomechanical roles of aged ECM in fibrosis permit the construction of a plausible sequence of events that lead to the development of iERM.

However, several questions remain regarding the pathogenesis of iERM. First, how does PVD trigger the migration, proliferation and transdifferentiation of retinal Müller cells and hyalocytes? Although the evidence suggests that antero-posterior traction can induce the activation of retinal Müller cells and the formation of
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cystoid oedema at the foveal retina, the exact molecular mechanisms have not yet been clarified. Second, the origin of the myofibroblasts in the ERM that are responsible for its contractile activity has not yet been fully specified. The current understanding is that retinal Müller cells, hyalocytes and RPE cells are all possible precursor cells of myofibroblasts. Third, molecular mechanisms underlying the profibrotic effect of collagens, and the role of cellular receptors and cell signalling pathways should be clarified. An understanding of these factors may eventually lead to the development of effective and non-surgical approaches to treat and prevent vitreoretinal fibrotic diseases.
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