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Epiretinal membranes and neural plasticity of the retina
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ERM in retinoschisis: neurofilament (red), vimentin (gr)
Summary & Conclusions
In this thesis we have looked at different aspects of epiretinal membranes (ERM). ERMs are abnormal sheets of cells and extracellular matrix that can develop on the vitreal surface of the retina. The initial cause for these membranes may be unknown, as in idiopathic epiretinal membranes (iERM) or may be secondary to retinal pathology, such as retinal detachment or diabetes. ERMs can cause a spectrum of problems, varying from minimal metamorphopsia to visual disturbance or blindness due to severe traction on the retina and tractional retinal detachment. In Chapter 1 there is a brief introduction to ERMs and the aims of this thesis.

Removal of symptomatic ERMs or of the ILM in macular hole surgery is a challenging task in retinal surgery, even for experienced surgeons. Using a dye to stain the ERM or ILM facilitates peeling of tissues by improving visualisation and contrast between the tissues. In principle the use of dyes should mean safer surgery. However, certain dyes have been found to be potentially toxic to the retina and retinal pigment epithelium. Trypan blue is considered a relatively safe dye, but requires a fluid-air exchange for adequate staining.

In Chapter 2 we describe a technique of modifying trypan blue for vitreoretinal surgery so as to be able to omit the AFX. AFX in itself increases risk for surgical complications including retinal tears. To eliminate the AFX, we proposed a heavy form of TB, which can be applied into a fluid filled eye without dispersing, by mixing trypan blue with 10% glucose.

We performed two clinical studies for ERMs and macular holes and found that heavy trypan blue (HTB) can be delivered efficiently to the retinal surface without the need for an AFX. Staining was sufficient to allow a safe and efficient peeling of ERMs and the ILM. By eliminating the need for a fluid air exchange repeat applications were easily performed. This leads to a more complete removal of the membrane without having a negative effect on vision.

In the study of the ERMs in Chapter 2 the use of HTB was associated with vision improvement and decrease in foveal thickness, and the absence of adverse events in our series. All patients in our study had an improvement of retinal thickness on ocular coherence tomography and no patients had a decrease in vision.

In Chapter 3 HTB was used in macular hole surgery for improved visualisation of the ILM, to facilitate peeling. In this study we had a similar result to the study in Chapter 2, finding that heavy trypan blue can be delivered efficiently to the retinal surface without the need for an AFX. Staining was sufficient to help visualise and peel the ILM. Repeat applications were easily performed when necessary. The macular hole closure rate was similar to that of other series, with a comparable visual improvement.

Due to the facilitated removal of ERMs, it became easier to collect ERMs for further
examination and research. We examined ERMs for proliferation of different cell types and for neurite proliferation.

In Chapter 4 we look at cell proliferation in different types of ERMs using Ki-67, an antibody that stains all proliferating cells. To identify which cells were proliferating antibodies were used against glial cells, RPE and immune cells, the most common cell types in ERMs. Using these antibodies, we show that there is active cell division in all 4 types of ERMs studied here, regardless of estimated duration. The data do, however, indicate that the amount of cell division varies. We observed that the more rapidly growing membranes, as in PVR, had a higher total number of cells and a significantly higher number of proliferating cells. This indicates that proliferation is most likely a major contributor in the expansion of these membranes. The older membranes had a much lower level of Ki-67 labelling indicating that these may be less “reactive”. This may suggest that in later stages of ERM formation, contraction of these cells, rather than proliferation, may be the cause of the wrinkling and subsequent re-detachment of the retina.

Using a Hoechst stain we show that the membranes contain thousands of nuclei, indicating they are much more cellular than previously thought. There appears to be a significant cellular inflammatory component in all epiretinal membranes, as shown by the ricin labelling, which may play a role in the development and continued expansion of the membrane.

As has been postulated previously, there seems to be a parallel between wound healing and epiretinal membrane formation, where there is an early proliferative phase with a higher cell division rate in more reactive membranes and a slower cell division rate in the older, less reactive membranes, after which there might be a later contractile phase with the deposition and contraction of extracellular matrix.

After having looked at the proliferation rates of cells in ERMs, we investigated the plasticity of retinal neurons and the growth of neurites from these cells into different types of ERMs. Neurites have been found in sub-and epiretinal membranes in the feline model of RD. In the study described in Chapter 5, we have found that human peri-retinal membranes removed at the time of vitreoretinal surgery also contain neurites and these processes, as in the feline model, appear to prefer a glial substrate over other available surfaces on which they might grow. These neurites could be identified in both PVR and diabetic fibrovascular epiretinal samples. While both horizontal and ganglion cell neurites are routinely observed in the sub- and epiretinal membranes from the feline retina, those in the human membranes almost certainly originate only from ganglion cells since the neurofilament antibody is specific to this cell type in human retina. Neurite growth into epiretinal membranes appears
to be common since all of the samples contained neurofilament labeled processes. It is less common, however, in the subretinal membrane. The growth of neurites through glial scars in the retina seems to be in contrast to other regions in the CNS where gliosis usually impedes the growth of neurites. Our data seems to indicate that retinal glial cells form a permissive substrate for neurite growth, as neurites are always associated with Müller cell processes. They also grow out of the neural retina only at sites where Müller cells grow beyond the outer limiting membrane to form a glial scar.

The growth of these neurites was considered to be induced by the severe retinal trauma of retinal detachment or by proliferative (diabetic) retinopathy, where there are many inflammatory and potential (neural) growth factors present. In the study in Chapter 6 we show that idiopathic epiretinal membranes, removed from eyes with no previous ocular insult, also show the growth of neurites into ERMs indicating that trauma is not necessarily needed to induce their growth.

The neurites found in these idiopathic ERMs are similar in structure to those found in the feline detached retinas and in human PVR and PDR retinas and they behave similarly in that they only grow on a glial substrate. This is in contrast to neurite growth elsewhere in the central nervous system where glial cells are inhibitory to neurite growth. This may be due to differences in the specific cell types in different regions of the central nervous system and the neural growth factors produced by these cell populations. Astrocytes as well as oligodendrocytes are thought to inhibit axon growth elsewhere in the CNS, but the glia in the retina seem to contain factors that are conducive to neurite growth, even without obvious retinal disease or trauma. It is becoming clear from this and other studies that adult retinal neurons retain the ability to remodel given the appropriate cues.

In Chapter 5 and 6 we have shown the presence of ganglion cell neurites in different types of ERMs. These ganglion cell neurites were only stained with neurofilament antibody. To assess whether other neurons sprout neurites we used antibodies against different types of neuronal markers in Chapter 7.

In Chapter 7 we demonstrate the potential for growth, and presence of, rod photoreceptor neurites and three sub-types of retinal ganglion cell neurites in epiretinal membranes. These 3 subtypes of ganglion cells stain with calretinin, melanopsin and neurofilament. The growth appears unrelated to the particular disease condition since neurites were observed in all conditions examined. This illustrates that whether the disease is severely disruptive, such as retinal detachment with epiretinal proliferation, or severe diabetic retinopathy with tractional fibrovascular membranes, or minimally disruptive such as idiopathic epiretinal membranes, neural outgrowth can occur. Most neurites showed synaptophysin nearby, however,
functional synapses were not found. The synaptophysin does seem to indicate the presence of rudimentary synaptic machinery. Future studies are aimed at identifying common factors that may be involved in initiating and guiding neurite growth in the retina.

The causes of and reasons for neurite growth in the retina are as yet unknown. In Chapter 8 we review the literature and attempt to explain possible mechanisms, such as Müller cell glia and microglial activation, that might be involved in creating a permissive environment for neurite outgrowth.

The essential, as yet unanswered question, is whether these neurites can be guided in their growth and be functional after retinal or CNS damage.