Preclinical validation of antifibrotic implantables for use in bleb-forming glaucoma surgery

Summary

Glaucoma is the leading cause of irreversible blindness worldwide. The etiology and pathogenesis of this disease, in which the optic nerve slowly degenerates, are complex and not fully elucidated yet. The hallmark of the disease is progressive visual field loss. There is no causative treatment. Current treatments mainly focus on the reduction of (high) intraocular pressure (IOP), the main risk factor for developing glaucoma. To reduce visual field loss, patients are treated with IOP-lowering medications (usually eye drops), laser procedures, or glaucoma surgery. Unfortunately, despite treatment, glaucoma can still progress in patients. Twenty-five percent of patients will ultimately suffer from unilateral blindness and 10% from bilateral blindness at the end of their lives. For these patients, bleb-forming glaucoma filtration surgery (trabeculectomy or tube shunt surgery) has the highest IOP-reducing potential.

All bleb-forming procedures aim to create an alternative pathway for aqueous humor to exit the eye and reduce IOP. This is achieved by creating a hole/fistula between the anterior chamber and the subconjunctival/sub-Tenon's space (trabeculectomy), or by placing a drainage tube at this site. Aqueous humor will flow under the overlying conjunctiva and the accumulation of fluid there will create a small pouch, called a filtering bleb. However, surgery is often postponed due to fear of severe postoperative complications such as hypotony, bleb leakage, and endophthalmitis. Furthermore, despite the use of antifibrotic drugs in conjunction with filtering surgery (mostly mitomycin C (MMC)), approximately 10% of surgeries fail each year due to the formation of fibrosis and subsequent scarring of the filtering bleb, underlining the unmet need for refinement of current treatments or the development of new treatment modalities.

During the SEAMS project we aimed to develop novel biomedical devices and methods to implement within bleb-forming glaucoma surgery. The work presented within this dissertation can be divided into three sub-goals; validation of an animal model for glaucoma surgery research, development of a novel glaucoma drainage device, and development of a sustained drug delivery system loaded with MMC.

Before novel biomedical devices or drugs can be introduced into the clinic, a preclinical study needs to be performed to assess their safety and efficacy. **Chapter 2** offers a systematic review of currently used animal models and antifibrotic treatments for use in glaucoma filtration surgery research. Throughout the years, several types of animals have been used as models for this research, including mice, rats, rabbits, hamsters, dogs, and owl monkeys. Based on the systematic review,

small rodents (such as mice and rats) are ideally suited to test novel drug targets and to perform molecular studies on, due to readily available molecular tools such as transgenic/knock out animals and molecular analysis kits. Due to its large eye (similar to humans), strong fibrotic response, and ease of handling, the rabbit is ideally suited for the development of biomedical devices. Several types of drugs were evaluated in a pre-clinical setting. Although most drugs showed a higher safety profile compared to the gold standard treatment with MMC, a lower antifibrotic effect was often found. Therefore, further optimization regarding drug dosage, administration route, application frequency, and potentially the development of combination therapies or a drug delivery system (DDS) is needed before the current gold standard treatment with MMC can be replaced.

Standardization of animal research is essential to increase the reliability and reproducibility of experiments across different research groups worldwide. In **chapter 3**, three different tonometers, regularly used in animal research, were compared to find the most reliable device for use in rabbits. Amongst the three different tonometers, the iCare TonoVet had the lowest learning curve and showed the smallest inter- and intraobserver variation in rabbits. An approximately 25% mmHg lower IOP value was observed when rabbits were measured under sedation.

SIBS, or in full “poly(styrene-block-isobutylene-block-styrene)” is an innovative material that is currently used in coronary stents and in the PRESERFLO® MicroShunt, a glaucoma microshunt. Although it is made out of a biocompatible, non-erodible, thermoplastic polymer, bleb fibrosis still occurs in the clinic. To validate our rabbit animal model and find potential new targets for antifibrotic treatment modalities after implantation with a SIBS microshunt, rabbits were implanted with the PRESERFLO® MicroShunt and followed for 40 postoperative days to investigate bleb fibrosis (**chapter 4**). Macroscopically, all blebs failed within 2 weeks. Histologically, a wide influx of cells was visible, including macrophages, polymorphonuclear cells, foreign body giant cells, fibroblasts, and myofibroblasts. Novel methods that reduce the fibrotic response should consider the high variety of cells that attribute to the development of bleb fibrosis. Inhibition of the inflammatory response and the downregulation of fibroblasts can offer novel therapeutic avenues to downregulate both the fibrotic response and foreign body response towards a SIBS based implant.

Upon implantation of a biomedical device, a unique interface between the material and surrounding cells develops. One way to reduce fibrosis is by limiting or skewing the reaction of cells into a favorable (antifibrotic) reaction. Surface topographies have gained popularity within the biomedical community. Surface topographies have

shown to skew the cellular response *in vitro* and *in vivo*. However, their antifibrotic potential in bleb-forming glaucoma surgery hasn't been studied up until now. **Chapter 5** studies the effectiveness of surface topographies upon addition onto a modified microshunt. In this pilot study, a PRESERFLO® MicroShunt was modified with an endplate (with or without surface topographies), placed at the distal end of the tube. This study showed that bleb survival was extended by the addition of an endplate to the tube. An endplate with a smooth surface, induced less inflammation and encapsulation compared to the surface topographies, and extended bleb survival compared to the PRESERFLO® MicroShunt. Although one of the 3 topographies showed some promising results regarding bleb survival, results were inconclusive. Further *in vitro* research (currently ongoing) is necessary to select and validate proper surface topographies, optimized with SIBS, to be implemented onto a microshunt.

To prevent the formation of fibrosis in the filtering bleb, MMC is currently used as the gold standard antifibrotic treatment. The past, current and future use of MMC is discussed in **chapter 6**. MMC can be applied intraoperatively with sponges or injected directly into the bleb postoperatively. Although MMC is an effective drug, it is cytotoxic to surrounding tissue, which can lead to severe vision threatening complications. There is an ongoing search for novel antifibrotic drugs that can limit the fibrotic response. In **chapter 2**, a multitude of drugs that are under investigation have been reviewed. While most were safer compared to the current standard, they often lacked efficacy. Therefore, further research is needed to optimize these novel drugs. A drug delivery system (DDS) can release a low dosage of a drug over a certain period of time. In theory, after implementation of a DDS, a continuous, controlled, low dosage can be achieved. Another advantage of a DDS would be a limitation of cytotoxic side effects, while maintaining an effective dosage (see **chapter 6**). In **chapter 7**, two DDS designs were evaluated *in vitro* and *in vivo*. As carriers, either polycaprolactone (PCL) or polylactic-co-glycolic acid (PLGA) were used. *In vitro* release kinetics showed a comparable release for both the PCL-based DDS and the PLGA DDS. However, a secondary burst of MMC was noted around day 42 for the PLGA DDS, which was possibly caused by hydrolysis of the PLGA polymer. The antifibrotic efficacy *in vivo* was compared to the gold standard treatment of 0.4 mg/ml MMC, intraoperatively applied for 3 minutes. Both DDS designs were as effective as the gold standard treatment, but the PLGA DDS showed more favorable results when compared to the PCL-based DDS. Ninety-nine % of all MMC was released at day 90 *in vitro*, and the DDS showed a fast degradation rate with a shorter foreign body response. However, thin-walled blebs were noted in the early postoperative period, Therefore, further optimization in dosage, release and

dispersion of MMC into the bleb will be required to minimize side effects before the DDS can be used in patients.