Analysis of fibroblasts of the suprachoroidal space for development of a new glaucoma micro stent

D. Buss¹, M. Löbler², W. Schmidt³, O. Stachs¹, A. Wree³, R. Guthoff⁴, and K.P. Schmitz²

¹ University of Rostock, Ophthalmological Department, Rostock, Germany
² University of Rostock, Institute for Biomedical Engineering, Rostock, Germany
³ University of Rostock, Department of Anatomy, Rostock, Germany

Abstract—The medical attendance of glaucoma diseases with implanted drainage systems is frequently accompanied by scarring and complete closure of the new attended outflow pathways. The implantation of a new micro stent into the suprachoroidal space promises less scarring problems. To examine the involved fibroblasts with regard to their response to the stent material, coating and incorporated drugs is aim of this study. As a first step the isolation and differentiation of the fibroblasts of different human ocular tissues (tenon capsule, cornea and with most relevance choroid and sclera) was performed. Morphological description based on fluorescence imaging provides first characteristics, but more detailed analysis is required to provide the possibility to distinguish between the different fibroblasts and refine the drug eluting system accompanied with the stent to its specific environment.

Keywords—intracocular pressure, glaucoma drainage device, fibroblast

Introduction

The intracocular pressure (IOP) depends on production and outflow of the aqueous humor out of the anterior chamber. A raised IOP whether the result of an increased production rate or of a decreased outflow rate can result in glaucoma.

The aqueous humor is built at the ciliary body. Its outflow from the anterior chamber is known to pass two different ways: a conventional route through the trabecular meshwork and an unconventional route through the suprachoroidal space [1]. The latter was described in detail by A. Bill [2, 3] in the 1960th and recently has gained new attention in providing a new way for the regulation of the IOP in glaucoma patients due to the implantation of a stent.

The shunt of aqueous humor out of the anterior chamber with implants was first performed with horse hair or silk thread at the beginning of the 19th century [4]. Aqueous humor was transliminally shunted to various spaces, such as the vortex veins, the nasolacrimal duct and the subconjunctival space. The drainage of fluid out of the anterior chamber with seton like devices was also performed with thread or wire made from noble metals or simple translimbal tube devices. Unfortunately these implants were unsuccessful, leading to hypotony due to a lack of flow control and also inflammations.

In 1969 Molteno established a new sort of implant. He used a combination of a tube and a plate to guide the aqueous humor out of the eye and under the conjunctiva to reduce the intraocular pressure. Other tube and plate devices which drained subconjunctivally followed. The Krupin, Baerveldt or Ahmed implant are amongst them.

The overall success rates, regarding IOP control, seem similar between the different devices but in view of the long term success rates early hypotony and scarring are still major issues [4, 5]. The scarring diminishes the outflow capability continuously until the pressure in the eye rises again.

Recently a new aqueous shunt was described which uses the uveoscleral pathway of the eye, the Gold Micro Shunt (SOLX Inc.). This shunt uses the drop in pressure from the anterior chamber to the suprachoroidal space as described by Emri et al. [6]. A first study showed good results one year after the implantation but shunt dislocation, hypotony and hyphema remain a problem [7]. A detailed analysis of the fluid mechanical properties of commercially available glaucoma drainage devices can be found in [8].

The suprachoroidal space is a virtual space between the sclera and choroid. Little is known about this space. This space does not manifest clinically under physiologic conditions. It is measured to be about 30 to 40 µm wide [6, 9, 10] and includes a few cells such as fibroblasts, melanocytes and macrophages embedded into loose bundles of collagen [2, 3, 9, 10].

Assuming that the suprachoroidal space carries less fibroblasts than the subconjunctival space due to a blood-ocular barrier we suggest that the implantation of a stent will have more success than compared to the subconjunctival.
space due to less scarring. The surface treatment and the addition of a drug eluting mechanism to the micro stent will also increase long term success of our glaucoma implant.

To test these assumptions and our approach regarding the biocompatibility of the stent material and the included drugs we cultivated four fibroblast cultures from the tissues margining the suprachoroidal space (sclera, choroid) and two referencing cultures from other ocular tissues (cornea, tenon capsule).

![Fig. 1 Position of the micro stent: distal end in the anterior chamber and proximal end in the suprachoroidal space](image)

The aim of this study is the analysis of the reaction of the cells towards the implant material and possible active agents. Therefore the isolation and cultivation of fibroblasts from the various tissues was necessary.

**Material and Methods**

Four different cell cultures from the following human ocular tissues were established: sclera, choroid, tenon capsule and cornea.

The fibroblasts were isolated by separating the tissue layers and follow-up cultivation of app. 1 mm² pieces in MEM with 10 % FCS resp. 20 % FCS for scleral cultivation. After five to seven days first outgrowing fibroblasts could be identified. The cells were then cultivated until the fourth passage according to standard protocols. Afterwards the cells were frozen in DMSO and stored in liquid nitrogen.

The fluorescent labeling was performed on fibroblast cells of the fourth passage with a monoclonal Anti-Human Fibroblast Surface antibody (F 4771, Sigma, dilution: 1:100) and the corresponding second antibody (anti mouse, F 0257, Sigma, dilution 1:128). The confocal microscope FluoView FV1000 (Olympus) was used.

The examination of cyto-toxicity of stent material and possible active agents towards the cultivated cells will be made by extract tests and direct contact tests.

The use of human tissue in this study is approved by the regional ethical committee.

![Fig. 2 Light microscopical pictures of the cultivated fibroblasts.](image)

- a: sclera, b: choroid, c: tenon capsule, d: cornea

![Fig. 3 Fluorescent microscopically stained pictures of the cultivated fibroblasts, 40x.](image)

- a: sclera, b: choroid, c: tenon capsule, d: cornea