for inflammation, extracellular matrix, and senescence processes. Genes involved in the identified pathways and processes can be further investigated to identify new treatment options. The identification of known points of action for drugs as RHO GTPase, NF-κB and corticosteroids in this study supports the value of this approach in the identification of new targets for new treatment options.

Glaucoma-on-a-chip: An in vitro model for glaucoma drug discovery based on mimicking the mechanical stress of high eye pressure

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Purpose: The pathology of glaucoma is characterized by optic neuropathy. At the cellular level, there is the loss of retinal ganglion cells (RGCs). Since the loss of these specialized neurons is irreversible, it is urgent to develop treatments that protect these cells. To achieve this, an in vitro glaucoma model would be very useful. The purpose of this study is to develop such a model by culturing RGCs and exposing them to conditions that mimic increased ocular pressure (IOP), the main risk factor of glaucoma.
Methods: Elevated IOP leads to increased hydrostatic pressure in the eye, causing, amongst others, deformation and stretching of cells. To mimick this, we need a device that can apply both hydrostatic pressure and stretch simultaneously to cultured cells. Stretching should be applied dynamically, in order to account for fluctuations in IOP that occur naturally in vivo. To realize this, we modified a recently developed device for stretch and shear stress (Sinha *et al*. Lab Chip 2015; 15 429–439). We use the immortalized neuronal PC12 cell line to establish the model; later RGCs will be used. Output parameters include cell density, survival and morphology.
Results: A medium-throughput cell culture device has been constructed that can apply hydrostatic pressure via the culture medium (0–90 mmHg). In addition, simultaneously, cells can be dynamically stretched anisotropically and isotropically ranging from 0 to 20% (in 5 steps). All experimental conditions are represented in fourfold on the device. First experiments using PC12 cells indicate that cyclic (1 Hz) stretch (10%) applied for 2 days, result in reduced cell density as compared to control (no stretch). Variation in cell density was large, probably related to variation in seeding density. To cope with this variation, we now also record the initial cell density, before applying mechanical strain, and use this for normalization.
Conclusion: We constructed a cell culture device that can apply pressure and stretch to cultured cells. This glaucoma-on-a-chip will help to identify the molecular mechanisms of mechanically-induced RGC death and help to design neuroprotective treatments. In addition, it can serve to characterize sensitivity of patients' cells to mechanical stress.

Isolation and culturing of rodent retinal ganglion cells for glaucoma disease modeling

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Purpose: Glaucoma is a major eye disease characterized by pathology of the optic nerve and irreversible loss of Retinal Ganglion Cells (RGCs). Since there is currently no cure available to restore lost vision, it is paramount to protect the RGCs. In order to study RGC death and develop neuroprotective therapies, we develop an in vitro glaucoma model. Such an in vitro glaucoma model requires RGCs in culture. The aim of the present study is to set up and characterize purification and culture of primary rat RGCs.
Materials and methods: We reviewed literature and selected the two-step immunopanning protocol for purification and culturing of rodent RGCs (Winzeler & Wang, *ColdSpringHarbProtoc, 2013(7):643–52*). Briefly, following this protocol, dissociated retinal cell suspensions were purified using antibody-coated Petri dishes to first deplete unwanted cells and then select for RGCs. Early postnatal rats of the inbred Brown Norway rat strain were used to obtain retinas. Yield and viability were monitored and optimized step-by-step using cell counting, trypan blue staining, and live cell imaging. Purified cells were cultured and fixed for immunocytochemistry.
Results: After initial experiments with early postnatal pups from postnatal day 4–9, titrating enzyme and antibody concentrations, a standard protocol was established, using papain (Worthington Biochemical LS003126) to dissociate resected retinas, a negative panning step to remove non-neuronal cells, a positive panning plate with anti-CD90 antibodies (Thy1.1 antibody; AbD Serotec T11D7e) to select RGCs, and trypsinisation to retrieve bound RGCs from the panning plate. With this protocol, up to 10–20 million cells were dissociated per retina, and these were purified in successive steps to 20 000 RGCs per retina. All experiments yielded viable RGCs, immunopositive for the RGC marker protein Rbpms, growing elaborate neurites in culture.
Discussion and conclusion: Using a two-step immunopanning protocol, we obtained viable rat RGCs. These are now available for analysis (e.g. gene expression), culturing, glaucoma modeling and for transplantation experiments.

Mapping expression of glaucoma risk genes in the mouse eye

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Purpose: More than 100 genes have been associated with glaucoma and/or glaucomatous endophenotypes. Despite this wealth of genetic information, our understanding of the pathophysiology of glaucoma is still limited. One notable factor which complicates the discovery of molecular pathways based on these genetic data is that many different ocular tissues are involved in glaucoma pathogenesis and that detailed information on the cellular expression of glaucoma risk genes is lacking. In this study we investigate whether RNA in-situ hybridization is a suitable general method to localize gene expression at the (sub)cellular level. We will test this technique for four glaucoma risk genes of which the ocular expression is not precisely known.
Methods: *Tnf, Tgfβr3, F5* and *Dusp1* mRNA expression was measured in healthy, 8-week-old pigmented (C57BL/6; $n = 4$) and albino mice (C57BL/6BrdCrHsd-Tyrc; $n = 4$) by RNA in-situ hybridization (RNAscope, Advanced Cell Diagnostics, CA). Expression levels were semi-quantitatively scored.
Results: A clear and detailed ocular expression pattern was obtained for all four candidate glaucoma genes. *Tnf* expression was low and found in the corneal epithelial layer and in the inner and outer nuclear layer of the retina. No expression was found in other tissues. Expression of *F5* was predominantly found in the non-pigmented ciliary body epithelium where a very high expression was observed. *Tgfβr3* was highly expressed in the tissues of the anterior segment. Low expression was found in the retina. *Dusp1* was expressed in all ocular tissues.
Conclusions: The employed RNA in-situ hybridization method provides detailed ocular maps of the expression of glaucoma risk genes, which will contribute to our understanding and treatment of glaucoma.