Bleb on a chip

Development of a fluidic platform to induce hydrostatic pressure and strain on human tenon fibroblast cells

Cuijpers, Jordy

Award date:
2023

Bleb on a chip

Development of a fluidic platform to induce hydrostatic pressure and strain on human tenon fibroblast cells

Name	Student number	Department
Jordy Cuijpers	1496263	Mechanical Engineering

CONFIDENTIAL Public summary

Supervisors:

Prof. Dr. ir	J.M.J den Toonder	(TU/e)
MSc	I.C. Figueiredo Pereira	(TU/e)
MSc	P.K. Sudarsanam	(TU/e)

"This report was made in accordance with the TU/e Code of Scientific Conduct for the Master thesis"

Eindhoven, April 24, 2023 EC: 45 points

Abstract

Glaucoma is a leading cause of irreversible blindness and is characterized by damage to the optic nerve due to abnormally high intraocular pressure (IOP). Glaucoma cannot be cured, but some methods exist to prevent disease progression such as the implantation of a glaucoma drainage device (GDD). These devices transfer aqueous humor (fluid from the eye) between the sclera and the conjunctival tissue, where a filtering bleb is formed. The bleb is encapsulated by the body and the fluid flows through this capsule and is absorbed by capillaries. For some patients this capsule is replaced by fibrotic tissue due to biological and mechanical triggers. This fibrotic tissue hinders the fluid and negatively affects the efficacy of the GDD. In this Bleb-on-a-chip project, we developed a fluidic device platform that enables researchers to implement artificial capsules of human tenon fibroblast cells (2D and more accurate 3D cell cultures by using hydrogels). The device is able to apply biological and mechanical cues to the artificial capsule while incubating for cell response. Cues include hydrostatic pressure until 40 [mbar], and a capsule strain of 10%. The device should provide the capability to perform cell readouts during or after incubation such as live imaging, optical microscopy, and fluorescence microscopy. We built a leak-tight fluidic device comprised of polymers, magnets, and chemically bonded elastic polymer, which has the ability to pressurize an artificial capsule until 40 [mbar] and strain the capsule 10% while also being compliant with cell culturing. The production of the device was robust enough to create two complete setups that proved to be reusable except for the well which need to be made new for each experiment. Eight wells can be made at once with the provided production process. However, we observed that the membrane in the well can become creased when in contact with IPA, which causes the PDMS to swell. Further research is needed to determine if these creased membranes have an effect on cell behavior. We included an experimental setup to validate the hydrostatic pressure and strain of the device. The device is able to apply a hydrostatic pressure between 0-40 [mbar] within an error of 0.2 [mbar] and apply a radial strain of 10% with an error of 3%. For 2D cell cultures in the device, we executed a coating to test cell adhesion on our membrane which is made of polydimethylsiloxane. The experiment shows that fibronectin coating produces the most favorable outcome in terms of cell adhesion on PDMS. Gelatine coating and no treatment are less effective, with cells growing only on the polystyrene bottom outside of the PDMS surface in the latter case. We also executed a hydrostatic pressure experiment on the device for 2D cell cultures using a fibronectin coating to check the effect of hydrostatic pressure on the fibrotic behavior of cells via either morphology or α-SMA expression with fluorescent dyes (α-SMA) is a good marker for fibrosis). The staining was unsuccessful, likely due to inadequate cell penetration of the dye. Although the cell morphology did not differ between 0 [mbar] and 40 [mbar] applied pressure, the even cell spread and attachment suggest that the experiment was successful. A close-up image demonstrates that high-quality images of cell stress fibers can be obtained using the developed method. The presence of stress fibers may be attributed to the amount of fibronectin used. We executed a 3D culture pilot experiment showing the development of a connective tissue representing an artificial capsule. We included microhooks to potentially mitigate tissue contraction and observed the tissue contraction around the hooks after 17 days. The detachable wells of the Bleb-on-a-chip device allow for the growth of tissues in separate wells and their subsequent installation in the device for experiments. However, further research is necessary to develop methods for the readout of these capsules. Overall, it shows the potential of the system to create more accurate artificial capsule tissue. At last, we provide a cell culture outlook to investigate if there is a causal dependence between the mentioned cues and the stress fiber formation which could lead to fibrotic capsule tissue. The device and methods potentially improve our understanding of fibrotic tissue formation which can enhance the success rate of GDDs in the future.