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In vitro test bench for investigating cell adhesion in hydrocephalus shunts

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Abstract: In the treatment of hydrocephalus, complications caused by shunt occlusion are common and one of the main reasons for shunt failure. With the goal of optimizing the hydrocephalus valves, an *in vitro* testing method is designed and implemented to mimic occlusion. The main unit is a flow chamber, simulating the cell growth inside the shunt. A cell suspension is circulated through a chamber using a syringe pump system. Cell flow can be modified and tested against different materials, surface properties and shapes. After flow simulation, the cell attachment and viability can be assessed using fluorescence microscopy. The designed test bench facilitates the development process of hydrocephalus valves, concerning choice of materials, surface finishes and inner (flow) geometry. The syringe pump allows for a steady or changing flow circulation, creating the dynamic conditions for investigation of growth of tissue in the valve. The presented flow chamber can be used to investigate the prevention of occlusion in hydrocephalus valves.

Keywords: hydrocephalus, shunt occlusion, flow chamber, *in vitro* testing, cell culture, biomedical engineering

1 Introduction

In healthy humans, cerebrospinal fluid (CSF) circulates between the brain ventricles and is absorbed via the venous blood system. Hydrocephalus is a neurological disease in which CSF accumulates due to an imbalance between its production and absorption or due to circulation disfunctions. To avoid the increase of intracranial pressure, excess of CSF is typically shunted from the brain ventricles to another body location. Despite significant improvements in technology, shunting remains associated with complications, leading to a relevant rate of shunt failure. The probability of shunt failure depends on the patient group treated, with paediatric populations being affected the most. One reason for failure of this treatment is the occlusion of the shunt system, which usually occurs either inside the valve or in the ventricular catheter. To date, occlusion in the valves has neither been successfully prevented nor effectively stopped, by any known valve design. Previous occlusion research methods focused mainly on examination of explanted shunts with regard to deposits and brain tissue cell appearance. In order to simulate occlusion *in vitro*, usually only the mechanical aspect was investigated, by using used egg proteins or alginate in the simulation model [1, 2]. Although it has been shown that the clots consist of tissue components such as cells and proteins of the extracellular matrix [3], the cellular aspect was usually not in focus of the test systems. The attempts made with cells were related to the occlusion of (ventricular) catheters, but not the valves. Therefore, the main focus of this study is to establish an *in vitro* test method, which allows to simulate cellular growth inside valves for the treatment of hydrocephalus.

2 Materials and methods

Hydrocephalus valves explanted due to occlusion have been examined at Christoph Miethke GmbH & Co. KG as part of the complaint's handling. Based on the observed clogging, the simulation model was developed. To select the correct cell type for the test bench, pathological examination of occluded valves was performed at Charité Universitätsmedizin Berlin – Pädiatrische Neurochirurgie. Further, engineering methods were used to evaluate and emulate the average flow and shear stress parameters inside a standard shunt. The input of R&D engineers, who are working on the design of future valves for the treatment of hydrocephalus, was considered. Based on available data collected by long-term implantable intracranial pressure sensors as well as literature information, the clinical application of shunts was reproduced considering all parameters relevant for the simulation of clogging, due to cell growth.

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3 Results

3.1 Test bench design

A cell-based *in vitro* bench testing method was developed to mimic shunt occlusion. A closed pump system with a flow chamber, presented in Figure 1, was established to simulate cell growth inside a hydrocephalus shunt. A control unit allows the user to set the desired flow parameters. The pump unit consists of a linear motor and a socket, into which two syringes can be clamped. The tubing system consist of silicone tubes, miniNAV (check) valves in front of and behind the syringes, and Y-connectors for connecting the tubing and inserting a bypass, in case flushing is needed. The syringes are simultaneously filled and emptied. A filter attached to the medium reservoir ensures pressure equalisation and the exchange of CO_2 with the buffered medium, while maintaining sterile conditions in the system. Driven by the pump system, the cell suspension is circulated through the flow chamber, subjecting the implant material to the contact with the cells (see Figure 2A). The inlay was 3D-printed on a Keyence Agilista 3200 printer, using AR-M2 clear, biocompatible resin [4] from Keyence. After printing, inlays were CNC post-processed and autoclaved. The housing of the flow chamber was produced at Christoph Miethke GmbH & Co. KG in Potsdam, Germany. The base material is stainless steel (1.4305).

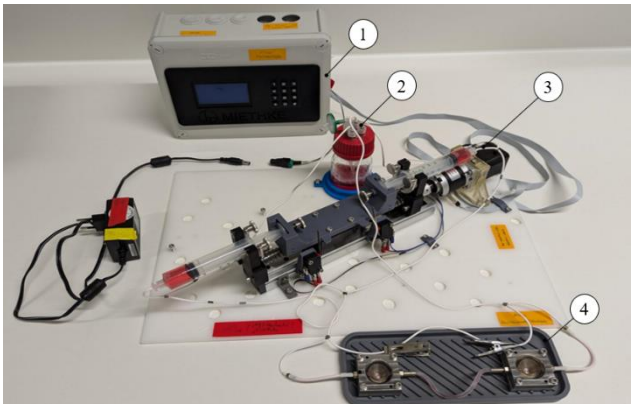

Figure 1: Flow chamber setup consisting of the control unit (1), the medium reservoir (2), the syringe pump (3) and two flow chambers (4).

The assembly of the test bench is done under sterile conditions. For the assembly of the flow chamber, the inlay and two sealings are placed inside the top part of the housing (see Figure 2B). The inlet and outlet are screwed into the sides of the top part of the housing. Sealing rings and a pressure plate are inserted into the bottom part of the housing and the test specimen is placed on top of the pressure plate. Top and bottom parts of the housing are connected by screws. The sealing rings and pressure plate are used to generate a homogeneous force distribution and prevent the system from leaking.

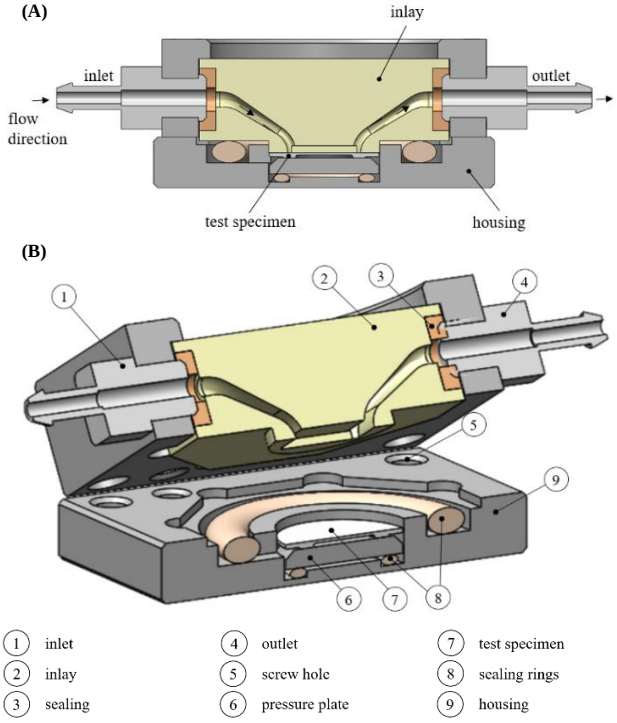

Figure 2: Cross-sectional view of the flow chamber **(A)** fully assembled and **(B)** opened. **(A)** The created channel allows the flow over the test specimen. **(B)** The flow chamber consists of an inlet (1) and outlet (4), a transparent inlay (2), sealings (3, 8), a pressure plate (6), a test specimen (7) and a metal housing (9). Top and bottom part of the chamber are seperately assembled and at the end screwed together (5).

The autoclaved and assembled tubing system is directly connected to the nozzles of the flow chamber and interlinked to the syringes and the medium reservoir via Luer Lock connectors. After assembly and addition of the cell suspension, the complete bench test setup is transferred into an incubator. Only the control unit (equipped with an Arduino microcontroller and a key pad), connected with a ribbon cable to the inner part of the incubator, is placed outside the incubator.

The chamber enables easy disassembly of parts, performance of sterilisation and assembly of different test specimens and inlays. The designed test bench has been verified with regard to the compatibility with the sterilization process, the possibility to set the desired parameters and to conduct experiments with various specimens and inlays. It has been concluded, that the developed test bench is suitable for the desired purpose.

3.2 Suitability for occlusion research

3.2.1 Evidence of the test bench functionality

The test bench described in chapter 3.1 was designed to mimic the cell behaviour on different test specimens. After 2 days of simulation, multiple viable cells could be observed on the surface of the test specimen, supporting the functionality of the test system (see Figure 3).

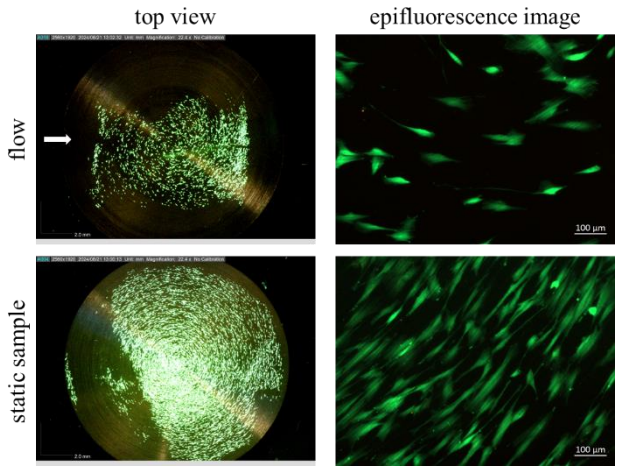

Figure 3: Exemplary images of the live-dead staining of test specimens. Flow chamber sample are shown on the top (flow) and the static sample (control) are shown on the bottom. The top view on the left, with exposure of the live cells (green) and brightfield light for visualisation of the test specimens gives an overview of the complet test specimen. White arrow indicateds flow direction. Epifluorescence images of live and dead (red) cells on the right, reveal the cell morphology.

For the assessment of cell attachment, as well as cell viability after the simulation, a testing protocol has been developed and combined with already established standard procedures (e.g. microscopic observations). All cell experiments have been conducted at Superfunctional Architectures at Biointerfaces (SupraFAB) of Freie Universität Berlin, in assistance of the Core Facility BioSupraMol supported by the DFG. Further description of the protocol is provided in chapter 3.2.2.

3.2.2 Examination protocol

Primary fibroblasts are cultured at 37 °C with 5 % CO_2 in High-Glucose Dulbecco's Modified Eagle Medium (HG DMEM, gibco #31966-021), supplemented with 1% penicillin/ streptomycin (P/S) and 10% fetal bovine serum (FBS). Medium is changed every 2 to 4 days and cells are splitted once a week, using Trypsin-EDTA 0.05% (Life Technologies #15400-054) 1:10 in PBS^{-} (Life Technologies #14190-169).

Cells are detached by aspirating the medium, washing with PBS^{-} one time and incubating the cells with Trypsin-EDTA for around 5 min at 37 °C. When cells are fully detached, HG DMEM with supplemented FBS is added. To remove all Trypsin from the suspension, it is centrifuged at 140 rcf for 4 min. The supernatant is carefully aspirated and the cell pellet is resuspended in fully supplemented, preheated HG DMEM.

Test bench assembly is performed under sterile conditions as described in chapter 3.1. The medium reservoir is filled with supplemented, preheated HG DMEM and the fully assembled tubing and the flow chamber system is being manually filled with the medium. After all air bubbles in the tubing and flow chamber are removed, cell suspension is added to the medium reservoir. Cells and the medium are carefully mixed and distributed in the setup by slowly drawing up and emptying the syringes for two times each. After distributing the cell suspension, the syringes are clamped into the pump construction. Except for the control unit, the entire flow chamber setup, including all tubing and the pump, is placed in the incubator at 37 °C and 5 % CO_2. A flow program is started immediately after and the cells are cultivated under set conditions for the corresponding time.

For control, test specimens are placed in a 35 mm dish. Cells are seeded on top of the test specimens and cultivated without flow at 37 °C and 5 % CO_2 in parallel to the flow specimens.

The cell viability is assessed by live-dead staining with fluorescein diacetate (FDA, Sigma Aldrich, #F7378) and propidium iodide (PI, Sigma Aldrich, #P4170).

Test specimens are carefully transferred into 35 mm dishes (VWR # 734-2317) and gently washed with prewarmed PBS^{++} (Life Technologies, #14040-174). All samples are then covered with 2.5 mL live-dead staining solution (10 µM FDA and 50 µM PI in medium without FBS) for 3 min at 37°C, followed by another washing step. The stained samples are then imaged in fresh PBS^{++}, using a Dino-Lite High-Speed Real-Time digital microscope, equipped with a SFA-D-BF-RB filter and a separate fluorescent lamp (NIGHTSEA, SFA Fluorescence Adapter for Dino-Lite) for top view images and a Zeiss Observer Z1 from Carl Zeiss Microscopy GmbH (Jena, Germany), equipped with a 10x objective (#420340-9901-000 EC Plan-Neofluar 10x/0.3 M27) for epifluorescence imaging. Dino-Lite top view images are taken first. Then, the test specimens are turned upside down for inverted epifluorescence imaging. Image evaluation is performed with the software DinoCapture 2.0, Zen 2 Version 2.0.0.0 (Carl Zeiss Microscopy GmbH, Germany) and ImageJ.

4 Conclusion

The mechanism that leads to the occlusion of hydrocephalus valves is not fully understood - for example, some observations made in the complaints' evaluation initially seem counterintuitive (e.g. narrow flow channels do not necessarily increase the probability of occlusion). Taking this into account, it is crucial for the development of future generations of hydrocephalus valves to provide engineers with tools that allow the *in vitro* testing of hydrocephalus valves regarding their susceptibility to occlusion.

The possibilities for investigating occlusion processes in a hydrocephalus valve have been limited to mechanical obstruction and the examination of explanted valves. Here we presented a test bench system, that is flexible and that can be used to investigate the occlusion-development factors on a cellular level.

The presented experimental setup can be used to investigate factors which affect the occlusion process, such as materials, flow rates, surface properties and shapes. This is achieved by the modular design of the flow chamber and the possibility to set flow parameters using the control unit.

The cavity for the test specimen allows for the insertion of various test materials, while the 3D-printed inlay can be easily manufactured. Depending on the research question, different geometries can be implemented quickly. The used inlay material is biocompatible and inexpensive, making it ideal for large-scale use in hydrocephalus research.

The pump system exhibits only minor fluctuations in flow and exposes the cells to minimal mechanical stress. This design allows the cell suspension to be seeded and to circulate not just under static conditions. This test system is therefore the first of its kind that can simulate the formation of cell colonization and occlusion in the valve *in vitro*. With the help of the presented bench system, important insights can be gained for the future development of occlusion-resistant valves.

Fibroblasts are a predominant component of the connective tissue and main producers of the extracellular matrix. In order to produce abundant tissue in a short amount of time, primary fibroblasts were selected as a suitable cell type for this setup. Variation of the composition of the cell culture medium allows to boost extracellular matrix and protein secretion.

Flow chamber experiments were conducted/maintained under dynamic conditions for several days to simulate the initial occlusion processes. In the future, an extension of the test period could be considered.

Author Statement

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Conflict of interest: L. Thieme, J. Gumz, A. Daberkow, G. Schwab and M. Kahl are employees of the Christoph Miethke GmbH & Co. KG. Ch. Miethke is the CEO of Christoph Miethke GmbH & Co. KG.

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