Abstracts

Future of Microfluidics: SMILS 2026

June 11–12, Uppsala

SMILS '26

Swedish Microfluidics Network
June 11-12, 2026, Uppsala University

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Welcome to Future of Microfluidics: SMILS 2026!

It is a great pleasure to welcome you to SMILS 2026, hosted here at the Ångström Laboratory, Uppsala University's vibrant research hub for engineering, materials science, physics, and microfabrication.

The SMILS meeting series was started in 2018 as a national gathering point for the Swedish microfluidics community. From the beginning, a central aim has been to give young researchers and students a friendly forum to present their work in front of a peer audience, share ideas and exchange experiences. The meeting also provides opportunities to network, discuss postdoctoral positions, and explore future career paths. Sweden is a small country and that makes it easy to get to know each other!

The SMILS 2026 programme highlights the many ways in which microfluidics is enabling new science and technology, from lab-on-a-chip systems, droplet and particle microfluidics, organ- and tissue-on-chip platforms, diagnostics, bioanalytical tools, and advanced fabrication methods to applications in life science, healthcare, sustainability, and industry. This year, we are honoured to welcome four outstanding keynote speakers representing a rich breadth of expertise across the microfluidics landscape. In addition, we are pleased to see a session focused on MPS/organs-on-chip chaired by members of the newly established Nordic network NORDVASC3D funded by NordForsk that had their kick-off yesterday!

The meeting series is currently co-organised by Uppsala University, Chalmers University of Technology, Stockholm University, KTH Royal Institute of Technology, and Lund University, rotating between our cities, reflecting the collaborative spirit of the community. However, Sweden’s microfluidics community extends well beyond these institutions, and anyone interested in hosting or contributing to a future SMILS meeting is warmly encouraged to reach out.

We would like to thank all speakers, poster presenters, session chairs, sponsors, exhibitors, and participants for contributing to the meeting. We are also grateful to the local organizing team in Uppsala and to the Scientific Programme Committee for their commitment in shaping an engaging and inclusive conference.

We wish you a stimulating and enjoyable conference in Uppsala!

Maria Tenje
Conference Chair

Patrick Sandoz
Conference Co-Chair

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Thursday, June 11, 2026

09:00 Registration open
9:30 - 10:30 COMSOL Multiphysics® workshop (registration required)
10:30 – 11:00 Registration and Fika
11:00 – 11:15 Welcome and Opening Remarks
11:15 – 12:30 **Probing the cellular environment** Chair: Patrick Sandoz
Keynote Lecture: **2.3D intermediate physiological systems: high-resolution functional cell monitoring**
Lourdes Basabe Desmonts, University of the Basque Country (UPV/EHU)
Imaging LSEC fenestrations in gradient-driven microenvironment
Chinmay Khanolkar, University of Gothenburg
3D microrheology for microstructural analysis of hydrolytic degradation of Collagen-based hydrogels,
Janne T. Koivisto, Tampere University
12:30 – 13:30 LUNCH
13: 30 – 15:00 **Lab-on-a-chip** Chair: Sagar Agnihotri
Vessel-on-a-chip platform for intravenous nanomedicine
Axel Norberg, Chalmer University of Technology
Modulating red blood cell viscosity and deformability for blood on chip applications
Tatiana Turcitu, University of Ottawa
Droplet-based workflow reveals metal exposure reshapes bacterial growth under antibiotic stress
Simona Bartkova, Tallinn University of Technology
Diagnostics of the neglected tropical disease schistosomiasis in resource limited settings with microfluidic platforms
Nicolina Priem, Stockholm University
Enhanced nanochannel tracking for multiplexed analysis of extracellular vesicles
Viktoria de Carvalho, Chalmers University of Technology
Exploring microfluidic neuro-models to study nanoparticle-based therapeutics
Ana P. Spencer, KTH Royal Institute of Technology
15:00 – 16:30 COFFEE BREAK & POSTER SESSION
16:30 – 18:00 **MPS/Organs-on-chip** Chair: Neeraj Katiyar
Keynote Lecture: **Advancing translational cancer research with bioengineering and organoids technologies**
Gaspard Pardon, AGORA Cancer Research Centre, EPFL Lausanne
Vascularized placenta–endometrium integration-on-a-chip
Ludivine Delon, University of Oslo
FN-silk basement membrane mimic on Hyaluronic acid hydrogels support cell alignment under sheer stress
Linnea Gustafsson, Spiber Technologies AB
Innovative strategies for engineering vascularized in vitro models with pluripotent stem cell-derived vascular cells
Maximiliano Arce, Uppsala University
19:30 – Reception and Dinner at Norrlands Nation, Västra Ågatan 14 (registration required)

Friday, June 12, 2026

08:30 Registration open
08:50 – 09:00 Announcements
09:00 – 10:30 **Physics of microfluidics** Chair: Simona Bartkova
Keynote Lecture: **Blood flow in microcirculation: how red blood cells shape the stream**
Marianne Fenech, University of Ottawa
Acoustofluidic thin-film device for high throughput
Andreas Lenshof, Lund University
Surfactant dynamics at liquid-liquid interfaces under shear: experimental study of the kinematic condition
Théo Lenavetier, KTH Royal Institute of Technology
Thermoacoustic streaming
Junjie Cheng, Lund University
10:30 – 11:00 COFFEE & POSTERS
11:00 – 12:30 **Micro- and Nanosystems** Chair: Maria Tenje
A Lab-on-a-Silicon-Chip Platform for Rapid Antibiotic Susceptibility Testing
Zheqiang Xu, Uppsala University
Microsystem with electrical read-out for bacteria
Sofia Johansson, Uppsala University
Acoustofluidic sample delivery and media switching for SX
Patricia Lopez Ramirez, KTH Royal Institute of Technology
Keynote Lecture: **A microfluidically controlled, artificial protein motor**
Heiner Linke, Lund University
Prize ceremony and Closing Remarks
12:30 – 12:45 Announcement of SMILS 2027
End of SMILS 2026
13:45 - 15:00 Lab Visits (registration required)

Committees

Scientific Committee
Maria Tenje, Conference Chair, Uppsala University
Patrick Sandoz, Conference Co-Chair, Uppsala University
Per Augustsson, Lund University
Håkan Jönsson, KTH
Fredrik Westerlund, Chalmers University of Technology

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Karin Magnusson, Project Coordinator
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Samuel Wulfert
Xinyang Dai
Sara Ferreira Cardoso
Florence Maryanna Olan Agcaoili
Tatiana Turcitu

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Exhibitors

Advanced Microfluidics
Small Volumes Matter

BERGMAN LABORA

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Keynote Speakers

Prof. Lourdes Basabe Desmonts

Independent researcher interested in the field of micro bioengineering and lab-on-a-chip technologies. Ikerbasque Research Professor since 2012, Director of the Microfluidics & BIOMICS Cluster UPV/EHU since 2019, Principal Investigator of the BIOMICS-microfluidics Research team since 2014, Young Member of Jakiunde, the Academy of Science and Arts of the Basque Country since 2016.

Dr. Gaspard Pardon

Graduated with a MSc in Microengineering from EPFL in 2008, with a PhD in Electrical Engineering from KTH Royal Institute of Technology in Stockholm, Sweden. His graduate research focussed on the engineering of novel micro- and nanofluidic devices for rapid Point-of-Care diagnostics of infectious diseases, including the detection of airborne pathogens. Since March 2021, Gaspard joined EPFL to become Head of the Bioengineering and Technology platform at the AGORA Translational Cancer Research Center.

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Prof. Marianne Fenech

Professor in the Department of Mechanical Engineering at the University of Ottawa. She leads the "Biofluid and Biorheology" lab, where her research focuses on blood flow behavior in microcirculation. She is currently a Visiting Researcher in the Department of Materials Science and Engineering; Biomedical Engineering in Dr. Maria Tenje’s group at Uppsala University.

Prof. Heiner Linke

Professor in Solid State Physics at Lund University. His research group uses experimental and numerical methods to study transport phenomena far from thermal equilibrium. They research nanoelectronic systems, artificial protein motors, nanotechnological applications of biomolecular motors such as in network-based biocomputation, and explore fluid motion driven by ratchet phenomena.

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Session 1: Probing the cellular environment

Chair: Patrick Sandoz
2026-06-11
11:15 – 12:30

1.1	Keynote Lecture 2.3D intermediate physiological systems: high-resolution functional cell monitoring *Lourdes Basabe Desmonts, University of the Basque Country (UPV/EHU)*
1.2	Imaging LSEC fenestrations in gradient-driven microenvironment *Chinmay Khanolkar, University of Gothenburg*
1.3	3D microrheology for microstructural analysis of hydrolytic degradation of Collagen-based hydrogels *Janne T. Koivisto, Tampere University*

Swedish Microfluidics Network
June 11-12, 2026, Uppsala University

Imaging LSEC Fenestrations in Gradient-Driven Microenvironment

Chinmay Khanolkar[1*], Caroline Beck Adiels[2]

[1] Department of Physics, University of Gothenburg, Gothenburg, Sweden
*E-mail: chinmay.hemant@gu.se

Introduction

Liver sinusoidal endothelial cells (LSECs) regulate exchange between blood and hepatocytes via fenestrations (50–300 nm pores), enabling bidirectional transport of solutes and drugs [1]. These structures are critical for hepatic filtration but are significantly reduced during aging and liver disease (defenestration), impairing substrate exchange. A previously developed liver zonation-on-chip (ZoC) device by Adiels and colleagues enables controlled oxygen and glucose gradients under flow, recreating physiologically relevant sinusoidal zones and thereby inducing hepatocyte (HepG2) specialization, i.e., metabolic zonation [2]. Here we extend this platform to primary mouse LSECs, providing a physiologically relevant in vitro cell culture model for these monolayered cells. Also, we aim to visualize LSEC fenestrations using high-resolution imaging techniques, such as structured illumination microscopy (SIM), which has not yet been demonstrated in this context.

Theory and Experimental procedure

The ZoC device is a dual-layer PDMS system comprising a lower gas channel network and an upper cell culture chamber, bonded to a glass coverslip. Opposing gas flows—oxygen-rich incubator air and nitrogen—establish controlled gradients, generating zonation within the device. For a comprehensive overview of the underlying microfluidic design, fabrication techniques, and zonation simulations, the reader is referred to Reza et al [2]. ZoC chips were sterilized using 70% ethanol (20 min), coated with fibronectin (≥40 min), and washed with PBS three times. Primary mouse LSECs were seeded at a density of 5×10^6 cells/mL into the culture chamber and allowed to adhere for 2 h under static conditions before replacing the RPMI medium with EGM. The device was then connected to media and gas flow systems and perfused at 2.5 µL/min for 24 h. Then flow was stopped, cells were fixed using paraformaldehyde, incubated with BioTracker555 for 15 mins, the top layer was detached to provide optical access and finally the cells were imaged using SIM.

Results and Discussion

LSECs cultured in the ZoC device successfully adhered and maintained their morphology under perfused conditions. Preliminary imaging using structured illumination microscopy (SIM) revealed the presence of fenestration-like structures throughout the cultured LSECs.

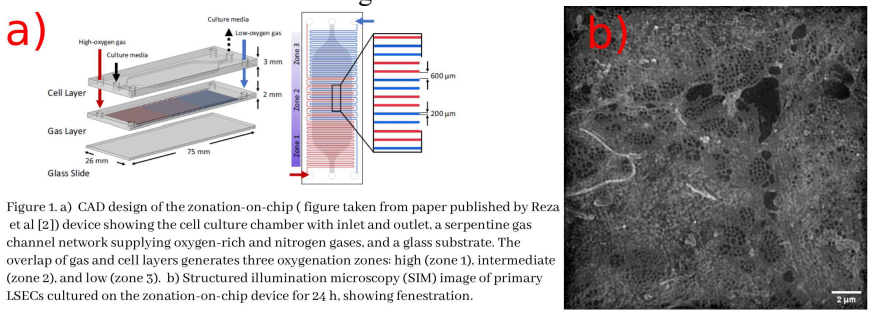

Figure 1. a) CAD design of the zonation-on-chip (figure taken from paper published by Reza et al [2]) device showing the cell culture chamber with inlet and outlet, a serpentine gas channel network supplying oxygen-rich and nitrogen gases, and a glass substrate. The overlap of gas and cell layers generates three oxygenation zones: high (zone 1), intermediate (zone 2), and low (zone 3). b) Structured illumination microscopy (SIM) image of primary LSECs cultured on the zonation-on-chip device for 24 h, showing fenestration.

Conclusion

This study represents an early-stage proof of concept demonstrating the feasibility of culturing primary mouse LSECs in the ZoC platform. We successfully established LSEC culture and visualized fenestrations using SIM. Future work will focus on optimizing the platform design, investigating the effects of pharmacological agents on fenestration size and porosity. In addition, imaging capabilities will be extended to include techniques such as scanning electron microscopy (SEM) and atomic force microscopy (AFM).

References

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[2] Reza Mahdavi, Sameereh Hashemi-Najafabadi, Mohammad Adel Ghiass, Silmu Valaskivi, Hannu Valimaki, Joose Kreutzer, Charlotte Hamngren Blomqvist, Stefano Romeo, Pasi Kallio, and Caroline Beck Adiels. Design, fabrication, and characterization of a user-friendly microfluidic device for studying liver zonation-on-chip (ZoC). Biomedical Microdevices, 27(1):8, 2025.

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3D Microrheology for Microstructural Analysis of Hydrolytic Degradation of Collagen-Based Hydrogels

Tuulia Taipale[1], Kaisa Liimatainen[1], Minna Kellomäki[1] and Janne T. Koivisto[1*]

[1] Faculty of Medicine & Health Technology, Tampere University, Tampere, Finland
*E-mail: janne.t.koivisto@tuni.fi

Introduction

Passive multiple particle tracking (MPT) enables the study of changes in microstructures of hydrogel biomaterials over time. In MPT, Brownian motion of tracer particles embedded in hydrogel is tracked, which reveals separate rigid, viscoelastic, and fluid-like areas inside the hydrogel sample [1]. In our 3D version of MPT microrheology, the sample is imaged with known Z-intervals, and the data is reconstructed into a 3D visualization based on quantified viscoelastic micromechanics with the help of generalized Stokes-Einstein relation [1].

Experimental procedure

We used Nikon Eclipse Ti2 wide field fluorescence microscope to image hydrogels filled with Ø 200 nm fluorescent tracers (Bangs Laboratories). In our proof-of-concept research, we used Geltrex™ and collagen type I from rat-tail, both in two different concentrations. For Geltrex™ concentrations were 10 mg/ml and 7 mg/ml, and for collagen I 1.5 mg/ml and 1 mg/ml. For data analysis and we used our recently developed MuRheo software.

MuRheo does particle detection and tracking based on optimized nearest neighbour algorithm [2]. When 3D data is used, different Z-levels are processed in parallel for faster computations. To study changes in 3D microstructures of hydrogels during hydrolytic degradation, we imaged focus stacks from the same locations in the samples for two weeks. The statistical overview created in MuRheo shows changes in pore volumes of hydrogels, defined by adjustable mean square displacement (MSD) threshold.

Results and Discussion

Our data shows that with Geltrex™ the microstructure is more heterogenous when concentration is higher, while collagen type I hydrogels are more similar between each other's. Furthermore, the difference between heterogeneities of samples was observed only during the first week of incubation. We have found that with collagen type I apparent modulus of polymer network decreases 20% while pore volume increases 60% due to hydrolytic degradation in one week. Figure 1 shows the analysis of highly heterogenous hydrogel microstructure.

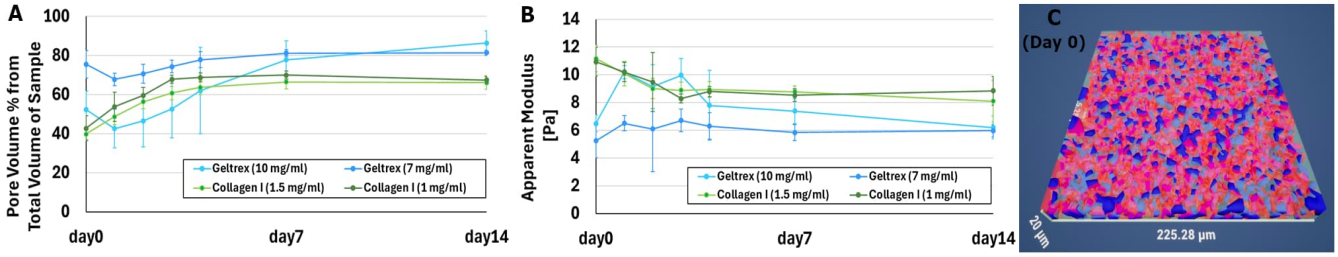

Figure 1: (A) Change in pore volume over time based on highly mobile tracer particles. (B) Average apparent modulus measuring hydrogel network stiffness change over time. (C) Example microstructural visualization of Geltrex™ hydrogel produced in MuRheo with red showing pores and blue molecular network.

Conclusion

Our MPT-based 3D microrheology is shown to work in studying the effect of hydrolytic degradation on hydrogel microstructure, enable by our in-house developed analysis software MuRheo.

Acknowledgments

We thank the Tampere Imaging Facility for support with microscopy, Tampere CellTech Laboratories for access to infrastructure and the Centre of Excellence in Body-on-Chip Research for funding this study.

References

[1] Zia, R. N. *Annu. Rev. Fluid Mech.*, **50** pp. 371-405 (2018)
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Session 2: Lab-on-a-chip

Chair: Sagar Agnihotri
2026-06-11
13:30 – 15:00

2.1	Vessel-on-a-chip platform for intravenous nanomedicine *Axel Norberg, Chalmer University of Technology*
2.2	Modulating red blood cell viscosity and deformability for blood on chip applications *Tatiana Turcitu, University of Ottawa*
2.3	Droplet-based workflow reveals metal exposure reshapes bacterial growth under antibiotic stress *Simona Bartkova, Tallinn University of Technology*
2.4	Diagnostics of the neglected tropical disease schistosomiasis in resource limited settings with microfluidic platforms *Nicolina Priem, Stockholm University*
2.5	Enhanced nanochannel tracking for multiplexed analysis of extracellular vesicles *Viktoria de Carvalho, Chalmers University of Technology*
2.6	Exploring microfluidic neuro-models to study nanoparticle-based therapeutics *Ana P. Spencer, KTH Royal Institute of Technology*

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Vessel-on-a-Chip platform for intravenous nanomedicine

Axel Norberg[1, *], Fan Jia[1] and Alexandra Stubelius[1].
[1] Molecular Bioscience, Chalmers University of Technology, Gothenburg, Sweden
*E-mail: noaxel@chalmers.se

Introduction

Nanoparticles (NPs) are attractive vectors for delivering therapeutic agents. However, intravenous (i.v) injection of NPs struggle to achieve delivery to underlying tissues [1]. Modelling NP and blood vessel interactions is essential for the development of next generation nanotherapeutics [2]. In this project we utilize an endothelialized microfluidic Vessel-on-a-Chip (VoC) platform to study NP interactions and penetration through the vessel wall.

Experimental procedure

We use commercially available microfluidic chips and in-house 3D-printed bioinspired chips as platforms. To mimic the blood vessel wall human umbilical vein endothelial cells (HUVECs) are seeded in the chips to form a monolayer. A peristaltic pump was used to apply flow with shear stresses in the physiological range (1-10 dyn/cm^2) for 24h. Fluorescent NPs (110nm. PDI, loaded with fluorescent Rhodamine 6G) were then introduced into the chips and the chips were subsequently imaged through confocal microscopy to study NP behavior and interaction with the endothelial layer.

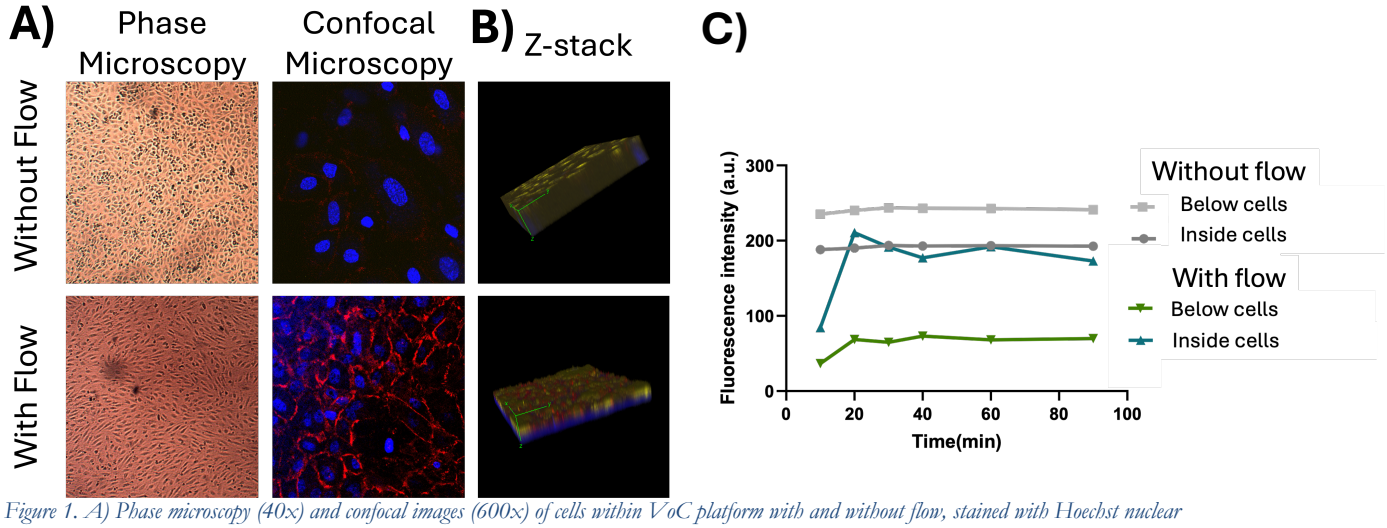

Figure 1. A) Phase microscopy (40x) and confocal images (600x) of cells within VoC platform with and without flow, stained with Hoechst nuclear stain (blue) and VE-cadherin (red). B) Z-stack of fluorescent NP (yellow) distribution in VoC after 20 minutes of exposure. C) Quantification of fluorescent signal of NPs in the VoC platform, from 10-90 minutes.

Results and Discussion

We found that HUVECs seeded in the chips formed confluent monolayer both with and without flow. However, ells under flow reach a more mature phenotype, see Fig 1. This is underscored by elongation of the cells and a greater expression of the cell-junction protein VE-cadherin (red), indicating enhanced adhesion and alignment. These tighter junctions also translate to a more complete endothelial layer with improved barrier function. When fluorescent polymeric NPs were added to the no flow condition a clear majority accumulated beneath the cell layer, whereas the endothelial layer formed under flow instead acted as a barrier and limited the NP penetration.

Conclusion

This data highlights that flow is essential for recapitulating physiological phenotypes in HUVECs, which in turn translates into a more representative interaction between the endothelial layer and administered NPs. This VoC platform will act as a model system for investigating future NP formulations.

Acknowledgments

We acknowledge the support of Chalmers Area of Advance Health Sciences for funding this project.

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[2] J. Paoloni, M. Jackson, E. Pandzic, M.S. Alam, E. Meijering, J. Yeow, M.S. Lord, Decoding Endothelial Glycocalyx-Nanoparticle Interactions via a Systematic Polymeric Nanoparticle Library and High-Content Characterization, Small 22(11) (2025).

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Modulating Red Blood Cell Viscosity and Deformability for Blood-on-Chip Applications.

Tatiana Turcitu*, Kyle Champagne, Andy Vinh Le and Marianne Fenech

Department of Mechanical Engineering, University of Ottawa, Ottawa, Canada
*E-mail: tturc092@uottawa.ca

Introduction

Microfluidic blood-on-chip platforms have emerged as powerful tools to study microcirculatory flow, cellular mechanobiology and disease-specific hemorheological signatures under physiologically relevant confinement [1]. In such systems, red blood cell (RBC) deformability, effective blood viscosity and cell morphology critically govern flow resistance, cell margination and oxygen transport, particularly in channels with dimensions comparable to capillaries [1]. To mimic pathological conditions, RBCs are often chemically rigidified using two agents, diamide and glutaraldehyde [2]. Despite their widespread use, the dose-dependent effects of these agents on RBC mechanics remain incompletely understood. In this study, we systematically quantify the dose-dependent effects of these agents on RBC mechanical and rheological properties in confined condition, with direct relevance for the design, interpretation and calibration of microfluidic blood-on-chip.

Theory and Experimental procedure

Blood samples from healthy volunteers were treated with ten concentrations of diamide (0–200 µM) and glutaraldehyde (0–159800 µM) to assess their effects on RBC properties. Key hemorheological parameters were quantified using microfluidic and micro-scale techniques under physiologically relevant confinement. Viscosity was measured in a microcapillary viscometer (channel height 51.6 µm), while RBC deformability was evaluated via microfluidic ektacytometry (channel height 210 µm), a laser-diffraction technique. Morphological changes were investigated by reconstructing the 3D geometry of RBCs using defocusing microscopy, which quantifies cell surface profiles from two bright-field images acquired at different axial positions. Finally, hematocrit (HCT) was measured by centrifuging microhematocrit tubes (inner diameter 1 mm), and the resulting supernatant was analyzed using the Redbance Index, which quantifies its color intensity as an indicator of hemolysis.

Results and Discussion

Diamide produced a biphasic response: the maximum elongation index (EI_{max}) decreased up to 140 µM, then partially recovered at higher concentrations. This coincided with increased sphericity, reduced cell volume and surface area, decreased HCT from hemolysis and a non-monotonous viscosity profile. These effects reflect the combined influence of oxidative stress, vesiculation and altered cell geometry. In contrast, glutaraldehyde induced an abrupt and irreversible loss of deformability at ≥7990 µM, while morphology remained stable. HCT values were consistently lower across concentrations, which we attribute to reduced microtube filling efficiency by rigidified cells. Despite near-zero EI_{max} at high concentrations, viscosity changes were modest due to extreme rigidification.

Conclusion

This study provides a quantitative framework for understanding how chemically induced RBC rigidification alters blood behavior under microfluidic confinement. By systematically characterizing the dose-dependent effects of diamide and glutaraldehyde on deformability, viscosity, and morphology, these results improve the design, calibration and interpretation of microfluidic blood-on-chip systems. The findings also highlight how changes in RBC mechanics can impact flow resistance, cell distribution and measurement accuracy in capillary-scale devices, supporting more reliable modeling of physiological and pathological microcirculation.

Acknowledgments

This work was supported by NSERC (grant number RGPIN-2020-07020).

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[2] A.M. Fosyth et al., *Microvasc. Res.*, **80**(1) 37-43 (2010)
This work has been published as T. Turcitu et al., *Microvasc. Res.*, **165** 104917 (2026)

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Droplet-based workflow reveals metal exposure reshapes bacterial growth under antibiotic stress

David Gonzalez[1,*], Daniel Kacsor[1], Robin Veere[1, 2], Pille Pata[1], Ott Scheler[1], and Simona Bartkova[1]

[1] Department of Chemistry and Biotechnology, Tallinn University of Technology, Tallinn, Estonia
[2] Biology Education Centre, Uppsala University, Uppsala, Sweden
*E-mail: david.gonzalez@taltech.ee

Introduction

Antimicrobial resistance (AMR) is a global emergency, intensified by the co-occurrence of metal contamination and antibiotic exposure. These factors can promote resistance by altering bacterial growth patterns, particularly bacterial self-protective mechanisms such as aggregation and biofilm formation [2]. However, these processes are difficult to study with conventional methods. We use a user-friendly droplet-based microfluidics workflow, where water-in-oil droplets act as independent microenvironments. This enables high-throughput investigation of how sub-inhibitory metal exposure shapes bacterial growth and genomic variation under antibiotic pressure.

Experimental procedure

GFP-labelled *Escherichia coli* were pre-incubated for 9 days in sub-inhibitory concentrations of $ZnCl_2$ or LiCl (Fig. 1a). Antibiotic susceptibility to cefotaxime and kanamycin was assessed before and after metal exposure using a single-cell droplet minimal inhibitory concentration assay (Fig. 1b). Droplets were imaged by confocal microscopy and analysed using CellProfiler™ and CellProfiler Analyst [3] (Fig. 1c). Whole-genome sequencing and comparative genomic analysis further allowed to identify potential genotypic changes (Fig. 1d).

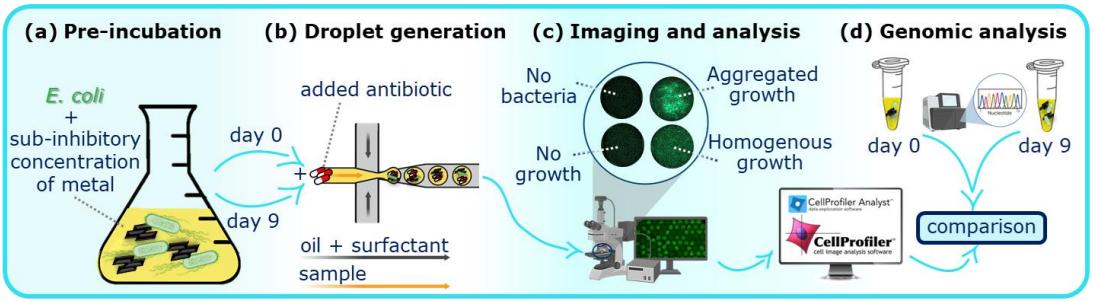

Figure 1. Droplet-based workflow for studying metal induced phenotypic and genotypic dynamics.

Results and Discussion

Our droplet-based workflow enabled high-throughput, quantitative comparison of single-cell growth patterns that are difficult to resolve with conventional methods. Metal-pretreated *E. coli* exhibited reduced aggregation under antibiotic stress, indicating a clear shift in behaviour under selective conditions. Preliminary genome analysis revealed sequence differences in several genes, suggesting underlying adaptation. Their functional role remains under investigation and is expected to shed light on the mechanisms driving the observed phenotypic changes.

Conclusion

This study highlights how combined pollutant exposure can shape AMR in complex ways by altering bacterial growth and potentially selecting for certain variants. Our user-friendly droplet-based microfluidics workflow captures these phenotypic and genotypic dynamics, providing a powerful tool to study this urgent global problem.

Acknowledgments

The project is funded by Estonian Research Council, grants no. PSG1136, TARISTU24-TK14 and ASTRA+ programme co-funded by the European Union, project No. 2021-2027.1.01.25-1163.

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[3] D.R. Stirling, M.J. Swain-Bowden, A.M. Lucas, A.E. Carpenter, B.A. Cimini, A. Goodman, BMC Bioinformatics 22, 433 (2021)

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Diagnostics of the neglected tropical disease schistosomiasis in resource limited settings with microfluidic platforms

Nicolina Priem[1], Pablo Rodriguez-Mateos[2], Harrison Waweru[3], Alexander Iles[1], Jesse Gitaka[3] and Nicole Pamme[1*]
[1]Department of Chemistry, Stockholm University, Stockholm, Sweden
[2]Severo Ochoa Molecular Biology Centre (CSIC-UAM), Madrid, Spain
[3]Mount Kenya University, Thika, Kenya
*E-mail: nicole.pamme@su.se

Introduction

The neglected tropical disease schistosomiasis currently affects >240 million people worldwide with the main burden in resource-limited settings. Currently, widespread diagnostics in endemic areas is hindered by limited access to accurate and affordable tests and the World Health Organization (WHO) has identified a critical need for improved diagnostics [1]. Previous work in the group demonstrated an integrated microfluidic device for detection of *Neisseria gonorrhoeae* [2] and *Sclerotium rolfsii* [3]. Here we present a sample-in-answer-out workflow for point-of-care (POC) diagnostics of *Schistosoma haematobium* (SH) and *Schistosoma mansoni* (SM) in a <1 hour workflow combining nucleic acid extraction, amplification, and detection.

Experimental procedure

The devices were CNC-milled from polymethyl methacrylate with chambers for sample addition and paramagnetic particle-based extraction, wash chambers for purification via immiscible filtration assisted by surface tension for purification, and a final amplification chamber (Fig 1a). Novel primers were designed targeting an identical multi-repeat sequence from Ally, O., *et al.* [4] for two amplification assays – loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA). Post-amplification readout was performed using color change or lateral flow assay (LFA) with gel electrophoresis confirmation or by fluorescence.

Results and Discussion

Initial tube-based amplification assays showed a minimum of 94% primer specificity (true negative rate) against *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, *Treponema pallidum*, *Escherichia coli*, and human genomic DNAs. Results from the integrated on-chip workflows showed isolation and detection down to low fg/mL levels with colorimetric LAMP assays (Fig 1b) and low pg/mL levels for RPA with fluorescence, indicating potential to detect clinically relevant levels.

Figure 1 *(A) Design of the microfluidic device (left) and a picture of a milled platform (right); (B) On-chip integrated workflow workflow with colorimetric LAMP (yellow = positive detection, pink = negative detection).*

Conclusion

This work demonstrates a sensitive and specific integrated on-chip workflow for *Schistosoma* detection that shows potential for POC applications. To further illustrate this, validation with *Schistosoma* eggs spiked in the sample chamber and testing with patient samples to assess the suitability will be assessed next.

Acknowledgments

This research is funded by Stockholm University and Vetenskapsrådet through the project 2024-04229. Adult *Schistosoma* worms were kindly supplied by the Schistosome Snail Resource, UK.

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Swedish Microfluidics Network
June 11-12, 2026, Uppsala University

Enhanced Nanochannel Tracking for Multiplexed Analysis of Extracellular Vesicles

Viktoria de Carvalho[1,*], Elin Persson[2], Alex Lech[2], Aaron Domenzain Del Castillo Cerecer[2], Sriram KK[1], Giovanni Volpe[2], Fredrik Westerlund[1], Elin Esbjörner Winters[1]

[1]Department of Life Sciences, Division of Molecular Bioscience, Chalmers University of Technology, Kemivägen 10, SE-41296 Göteborg, Sweden
[2]Department of Physics, University of Gothenburg, Origovägen 6 b, SE-41296 Göteborg, Sweden
*E-mail: devik@chalmers.se

Introduction

Accurate sizing and characterization of nanoscale particles are essential for many applications. However, conventional techniques such as nanoparticle tracking analysis (NTA) are limited by tracking ambiguities arising from particle diffusion in open volumes. Intersecting trajectories can lead to incorrect track assignment and biased diffusivity estimates, particularly in polydisperse samples. This is especially problematic for extracellular vesicles (EVs), which are inherently heterogeneous in size and molecular composition. Their small size and overlapping subpopulations make reliable characterization challenging with existing methods.

Theory and Experimental procedure

We present the use of a nanochannel-based platform originally designed for DNA analysis [1], that confines particle motion to one dimension under controlled flow conditions. The system features a redesigned chip with funneled nanochannel entrances, enabling efficient particle loading at low applied pressure, improving both throughput and sampling uniformity. Enhanced detection algorithms were applied to improve trajectory extraction and increase tracking fidelity, enabling robust analysis even under low signal-to-noise conditions. In addition, the platform supports four-color fluorescence acquisition, allowing simultaneous detection of multiple labeled particle populations. To demonstrate its applicability to biological samples, EVs derived from HEK293T cells overexpressing different tetraspanins were analyzed. Multiplexed fluorescence detection enabled simultaneous probing of surface markers, facilitating phenotypic characterization of EV subpopulations.

Results and Discussion

The platform was validated using polystyrene beads, demonstrating accurate particle sizing and reliable discrimination in mixed samples. Furthermore, four-color labeled liposomes were analyzed to evaluate co-localization performance. The results show improved specificity and reduced misidentification compared to conventional methods. Additionally, the system produces more well-defined size distributions and enables improved characterization of heterogeneous samples. These findings highlight the advantages of combining nanochannel confinement with advanced detection algorithms for nanoparticle analysis. Preliminary results indicate that this approach can resolve distinct EV subpopulations based on both size and fluorescence signature, providing insights into vesicle heterogeneity. This highlights the potential of the platform for applications in biomedical research and diagnostics, where detailed EV profiling is increasingly important.

Conclusion

In conclusion, nanochannel-based particle tracking, combined with enhanced detection algorithms and multiplexed fluorescence, provides a powerful approach for high-resolution extracellular vesicle characterization. This method overcomes key limitations of traditional techniques and offers improved accuracy, specificity, and throughput for analyzing complex and heterogeneous biological samples.

Acknowledgments

This work is funded by Knut and Alice Wallenberg Foundation.

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SMILS '26
Swedish Microfluidics Network
June 11-12, 2026, Uppsala University

Exploring microfluidic neuro-models to study nanoparticle-based therapeutics

Ana P. Spencer[1,2,3,4]*, Ekaterina O. Kasyanova[3,4], John S. Cognetti[5], Hanna Barriga[1,2,3,4] and Anna Herland[1,2,3,4]

[1] KTH Royal Institute of Technology, Stockholm, Sweden
[2] AIMES - Center for the Advancement of Integrated Medical and Engineering Sciences at Karolinska Institutet and KTH Royal Institute of Technology, Stockholm, Sweden
[3] SciLifeLab - Science for Life Laboratory, Stockholm, Sweden
[4] Karolinska Institutet, Stockholm, Sweden
[5] University of Rochester, Rochester, USA
*E-mail: ana.spencer@scilifelab.se

Introduction

More than 276 million people around the world live with neurological disorders, but treatments are still very limited, largely because the brain requires massive amounts of energy and has a built-in barrier (the blood-brain barrier, BBB) that keeps most drugs out [1, 2]. The neurovascular unit (NVU) regulates brain energy homeostasis, and its dysfunction, seen in Alzheimer's, stroke, or traumatic brain injury, leads to severe outcomes. Current therapies only manage symptoms, while cutting-edge approaches like gene therapy face delivery challenges. Lipid nanoparticles (LNPs) offer a promising solution for delivering mRNA therapeutics across the BBB. However, traditional models (e.g., animal studies, 2D cultures) inadequately mimic human brain complexity.

Theory and Experimental procedure

We have been developing a new human NVU microfluidic (NVU-on-chip) to first assess the LNPs' capacity to cross the BBB, a critical step in central nervous system (CNS) drug delivery, and then evaluate their subsequent therapeutic effects on neuronal targets. The chip has been prepared using induced pluripotent stem cells (iPSCs) from healthy donors, which were successfully differentiated into all essential NVU cell types, brain microvascular endothelial cells (BMECs), pericytes, astrocytes, and neurons, using well-established protocols [3].

Results and Discussion

Cellular characterization confirmed proper differentiation. To ensure physiological relevance, we have been optimizing the dynamic flow using COMSOL software simulations, which allowed us to establish and verify the desired luminal-interstitial connection between the chip's vascular and parenchymal compartments. Moreover, the platform is designed for the integration of cerebral organoids. Unlike many existing models, our two-layer platform is being engineered to incorporate all relevant cellular components in a configuration that facilitates physical interactions, closely mimicking the in vivo physiological milieu. A key design advancement is the provision of sufficient scale within the microchambers. This addresses a common limitation in microfluidic technologies by yielding adequate cell numbers and sample volumes for endpoint analyses, such as genomics and proteomics, thereby enabling an improved evaluation of therapeutic potential. Finally, the platform is designed for seamless compatibility with commercial multi-electrode array (MEA) systems. This alignment will allow future real-time, non-invasive electrophysiological recordings to directly quantify the functional effects of LNPs on neuronal networks or cerebral organoids.

Conclusion

We present a physiologically relevant human microfluidic platform that provides a pipeline from BBB penetration assessment to functional therapeutic evaluation. This can contribute to accelerating the development of next-generation nanotherapeutics for CNS diseases.

Acknowledgments

Funded by the European Union (SciLifeLab PULSE 101177199), Hjärnfonden and Wallenberg Foundations AB.

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Session 3: MPS/Organs-on-chip

Chair: Neeraj Katiyar
2026-06-11
16:30 – 18:00

3.1	Keynote Lecture Advancing translational cancer research with bioengineering and organoids technologies *Gaspard Pardon, AGORA Cancer Research Centre, EPFL Lausanne*
3.2	Vascularized placenta–endometrium integration-on-a-chip *Ludivine Delon, University of Oslo*
3.3	FN-silk basement membrane mimic on Hyaluronic acid hydrogels support cell alignment under sheer stress *Linnea Gustafsson, Spiber Technologies AB*
3.4	Innovative strategies for engineering vascularized in vitro models with pluripotent stem cell-derived vascular cells *Maximiliano Arce, Uppsala University*

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Vascularized Placenta–Endometrium Integration-on-a-Chip

Ludivine Delon[1,2], Thea Wergeland[2], Andrea Dalmao-Fernandez[2], Mathias Busek[2,3], Justyna Stokowiec[2], Alexey Golovin[2], Yuliia Boichuk[2], Stefan Krauss[1,2]

[1]Dep. of Immunology and Transfusion Medicine, Oslo University Hospital, 0424 Oslo, Norway.
[2]Hybrid Technology Hub Centre of Excellence, Institute of Basic Medical Science, University of Oslo, Oslo, Norway.
[3]Department of Microphysiological Systems, Faculty of Medicine of the Eberhard Karls University of Tübingen, Tübingen, Germany
*E-mail: ludivine.delon@medisin.uio.no

Introduction: Pregnancy begins with successful embryo implantation into the maternal endometrium. During this process, trophectoderm-derived trophoblasts invade and remodel the vascularized endometrium to establish functional placental perfusion, which is essential for fetal development and maternal health. Impaired placental development can lead to severe pregnancy complications such as preeclampsia, which remain insufficiently understood and poorly managed. Mechanistic insights are limited by the lack of physiologically relevant human models.[1]

Theory and Experimental procedure: To address this gap, we developed a scalable, pumpless, recirculating organ-on-chip platform that enables dynamic co-culture under controlled flow and oxygen conditions. We first established a Placental Syncytium-on-a-Chip (PSoC), where 0.1 dyn cm-2 fluid shear stress, rather than forskolin, induced not only BeWo cells syncytialisation, but also favorable changes in sFlt-1 and PlGF levels suggesting the development of a physiologically relevant PSoC.[2] We then engineered a Trophoblast Invasion-on-Chip (TIoC) by culturing BeWo cells or primary cytotrophoblasts against defined extracellular matrices in the presence of endothelial cells. Trophoblast migration patterns and phenotypic distribution were modulated by interstitial flow, VEGF gradients, and matrix rheological properties.[3] Building on this, we optimized a robust recirculating Vasculature-on-Chip (rVoC) featuring hierarchical, multilayered vascular networks ranging from capillary-scale vessels to small-artery diameters. We then integrated the vascular network with trophoblast cultures to establish a Trophoblast–Vasculature Remodeling-on-Chip (TVRoC) (**Fig.1**).

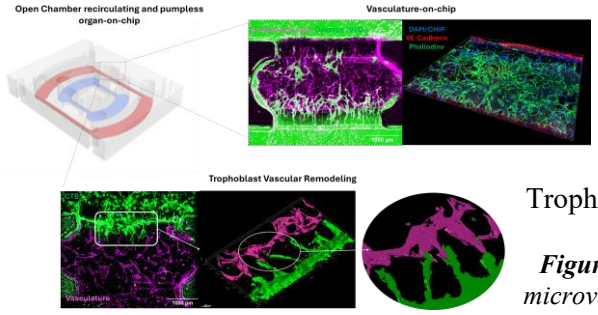

Figure 1. *rOoC device with open chamber used to engineer perfusable microvasculature and study trophoblast remodeling.*

Results and Discussion: This platform enables real-time analysis of interactions between invasive trophoblasts and endothelial networks, showing the replacement of endothelial cells in a vessel by extravillous trophoblasts. Ongoing integration of stromal cells will further supports the investigation of cellular and molecular mechanisms driving maternal–fetal interface remodeling.[4] These findings also provide a crucial framework for integrating stem cell–derived embryo models with vascularized supportive tissues.[5]

Conclusion: Our drug-free, microfluidic platform provides a versatile and human-relevant system to dissect mechanisms of placental integration and vascular remodeling. Ultimately, this approach offers new opportunities to study placental pathophysiology and to identify therapeutic strategies for pregnancy-related disorders.

Acknowledgments: This work was funded by the Research Council of Norway Centre of Excellence scheme contract #262613 and the European Innovation Council grant Supervised Morphogenesis #101071203 and Research Council of Norway verification grant #338249.

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FN-silk Basement Membrane Mimic on Hyaluronic Acid Hydrogels Supports Cell Alignment to Under Sheer Stress

Linnea Gustafsson[1], Savvini Gkouma[2] Philip Lifwergren[3], Daniel Aili[3] and My Hedhammar[2]
[1] Spiber Technologies AB, Stockholm, Sweden
[2] Division of Protein Technology, KTH Royal Institute of Technology, Stockholm, Sweden
[3] Division of Biophysics and Bioengineering, Linköping University, Linköping, Sweden
*E-mail: linnea.gustafsson@spiber.se

Introduction

Hydrogels are in general considered excellent supports for cell culture, however, many lack cell binding sites [1]. In this work we show that the addition of the recombinant protein FN-silk [2] to hyaluronic acid hydrogels results in the formation of a relevant basement membrane mimic [3-4], which promotes endothelial cell adhesion, resulting in the formation of a confluent layer that both responded to and remained intact under sheer stress.

Experimental procedure

FN-silk was added to hyaluronic acid (HA) based hydrogels (synthesized as previously described [1]) crosslinked with PEF-Az_4 and VPM-Az_2. FN-silk membrane formation at the interface of the gel was verified through confocal microscopy, scanning electron microscope (SEM) and nanoindentation. HA hydrogels with FN-silk were cast in a µ-Slide I Luer 3D (Ibidi GmbH, Gräfelfing, Germany) chip and endothelial cells (HUVEC) were seeded on top of the cured hydrogels. The day after seeding, they were exposed to sheer stress of 0, 7.5 or 16 dyne/cm^2 until day 3. Cells were stained with F-actin and DAPI and imaged using fluorescent microscopy.

Results and Discussion

Formation of a FN-silk membrane on top of the HA-hydrogel was verified using SEM and confocal microscopy. Nanoindentation revealed a four time increase in Young's modulus by the addition of FN-silk. The formed FN-silk membrane allowed HUVECs to attach and establish a confluent layer within one day. In contrast, on the bare HA gel surface, the cells did not adhere at all, why these were excluded from flow experiments. However, HUVECs seeded on HA-gels with an FN-silk membrane and exposed to flow, aligned and stretched along the direction of the flow (Figure 1). The HUVECs showed a stress-dependent response and increased in size.

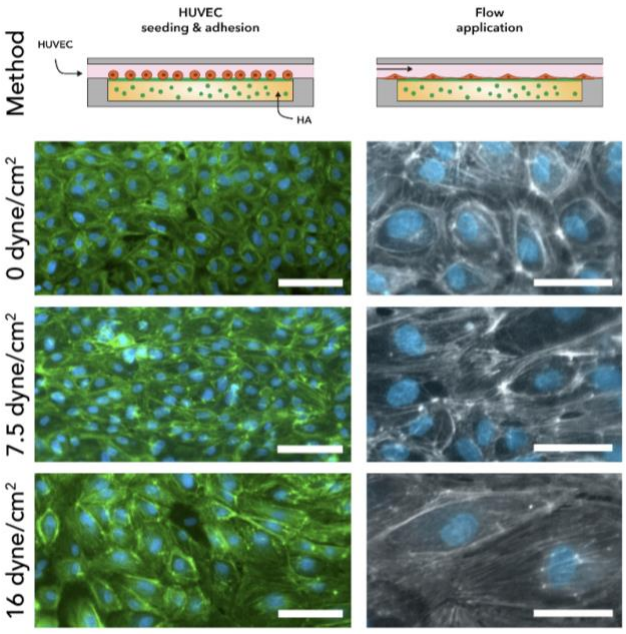

Figure 1. Illustration of the method followed by fluorescent images showing increasing cell size and elongation in the direction of flow.

Conclusion

The FN-silk membrane serves as an *in vitro* basement membrane mimic on HA hydrogels and transforms the inert surface to one that HUVECs cells can adhere to, establish a barrier on, and withstand sheer stresses relevant for large blood vessels.

Acknowledgments

The authors would like to thank Spiber Technologies for providing the FN-silk protein as well as Marina Chatziefraimidou, Tor Wennberg Samuelsson, and Javier Botella-Bobadilla for their contributions to the project.

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Innovative strategies for engineering vascularized in vitro models with pluripotent stem cell-derived vascular cells

Maximiliano Arce[1,*], Iza Erzar[1], Nora Noll[1], and Peetra Magnusson[1]

[1] Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden
*E-mail: max.arce@igp.uu.se

Introduction

Vascularization is critical for advancing in vitro models, enabling efficient nutrient delivery, waste removal, and oxygenation beyond diffusion limits (~200 µm), thereby supporting physiologically relevant tissue function [1]. Incorporation of vascular networks in 3D models enhances long-term cell viability, structural organization, and organ-specific responses, particularly in vascular-associated diseases such as neurodegeneration. Induced pluripotent stem cells (iPSCs) provide a versatile source of human endothelial and perivascular cells, enabling the formation of perfusable vascular networks within organ-on-chip systems and hydrogel-based platforms. Emerging genetic engineering approaches further enable robust and reproducible generation of endothelial cells and pericytes from iPSCs, supporting controlled vascularization of engineered hydrogels.

Theory and Experimental procedure

We genetically engineered iPSCs using the PiggyBac transposase system to facilitate the generation of endothelial cells (iECs) [2, 3] and pericytes. iPSCs-derived endothelial cells and pericytes we co-cultured into biocompatible hydrogels and canonical vascular markers and their potential to generate vascular networks were assessed using confocal microscopy.

Results and Discussion

Endothelial cells and pericytes can be robustly obtained through transcription factor-based lineage specification using genetically engineered iPSCs. iPSC-derived endothelial cells (iECs) express canonical endothelial markers and exhibit a molecular signature associated with an arteriovenous profile. When seeded into biocompatible hydrogels, iECs generate complex, perfusable vascular networks in vitro. Endothelial cells lining these vascular networks form stable, polarized lumens. The addition of pericytes to hydrogels containing iPSC-derived endothelial cells improved the overall organization of the vascular networks, with close spatial association between both cell types, consistent with physiological vessel structure.

Conclusion

We robustly generated induced pluripotent stem cell-derived endothelial cells and pericytes, which successfully vascularized hydrogels in vitro. Altogether, these findings demonstrate the potential of iPSC-derived vascular cell populations for engineering functional vascularized in vitro tissue constructs.

Acknowledgments

We thank our collaborators at the CNSx3 consortium for their contributions into this work.

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Session 4: Physics of microfluidics

Chair: Simona Bartkova
2026-06-12
9:00 – 10:30

4.1	Keynote Lecture Blood flow in microcirculation: how red blood cells shape the stream *Marianne Fenech, University of Ottawa*
4.2	Acoustofluidic thin-film device for high throughput *Andreas Lenshof, Lund University*
4.3	Surfactant dynamics at liquid-liquid interfaces under shear: experimental study of the kinematic condition *Théo Lenavetier, KTH Royal Institute of Technology*
4.4	Thermoacoustic streaming *Junjie Cheng, Lund University*

Acoustofluidic thin-film device for high throughput applications

Andreas Lenshof[1]*, Ramin Matloub[2], Pelle Ohlsson[3], Igor Lubomirsky[4], Henrik Bruus[5], Nini Pryds[5], Vincenzo Esposito[5], Paul Muralt[2,6], and Thomas Laurell[1]

[1]Biomedical Engineering, Lund University, Lund, Sweden; [2]Piemacs SARL, Lausanne, Switzerland
[3]Acousort AB, Lund, Sweden; [4]Weizmann Institute, Rehovot, Israel
[5]Technical University of Denmark, Lyngby, Denmark; [6]EPFL, Lausanne, Switzerland
*E-mail: andreas.lenshof@bme.lth.se

Introduction

Standard bulk acoustic wave (BAW) devices rely on a piezoelectric (PZT) transducer attached to a channel structure commonly made of silicon and/or glass. Although quite reliable, the BAW device suffers from chip-to-chip variations regarding actuation frequency and transducer actuation voltage due to variability in mounting the PZT including the glue layer thickness. Further, electromechanical losses generating heat in the PZT pose an upper limit to the use of high acoustic energy levels needed for high throughput applications.

We present here a novel thin-film actuated acoustofluidic device. The thin-film transducer is deposited directly onto the silicon substrate through sputtering of AlScN, effectively eliminating the glue adhesion layer of the transducer, as done previously on glass chips without microfluidic channels. As predicted theoretically, the thin-film actuator (TFA) device works differently compared to the standard BAW device. The TFA occupies less than 1% (versus 10-60%) of the total device volume, it runs far from its internal resonances (versus near them), and it can only excite shear-strain on the device surface (versus both shear and compression stresses). Consequently, the TFA relies entirely on exciting whole-device resonances, and not by the conventional excitation of channel-width-matched resonances. The shear-strain-induced whole-system resonance is created in the device by the split top electrodes driven at a 180° phase shift and the bottom electrode left floating, see figure 1. The split top electrode was made in three pairs to provide redundancy to possible malfunctioning thin film sections as well as to investigate the reproducibility of the electrode pairs. Results showed that all three transducer pairs had the same resonance frequency of 2.078 MHz, demonstrating the accuracy of the fabrication method.

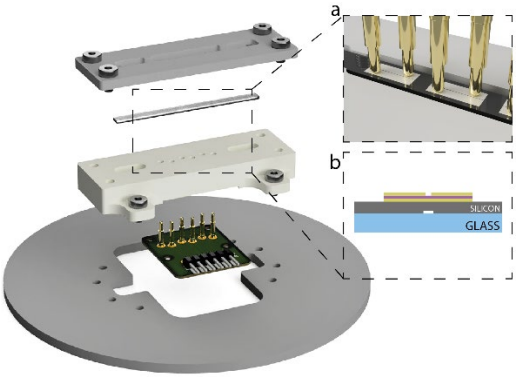

Figure 1: Schematic sketch of the chip holder. a) pogopin connectors to the thin-film actuator electrodes. b) Cross section view of the chip with split top electrode, AlScN layer (purple) and bottom electrode (yellow). The piezoelectric AlScN thin-film transducers were sputtered directly to the silicon substrate eliminating the highly varying glue layer needed when using BAW PZT transducers wielding a much more reproducible device.

Results and Discussion

The different mode of actuation of the thin-film devices produces cleaner impedance spectra with a lot less spurious peaks compared to BAW devices as the mass load and resonance modes of the PZT are eliminated. What is even more intriguing is that the resonance peak, i.e. the transducer driving frequency, is independent of the acoustic properties of the fluid medium allowing for a more robust system compared to the BAW device where the influence of the medium properties is clearly visible in the differential plots. It can be further noted that the thin film actuators are almost completely capacitive with an impedance phase angle of -89.6° at the channel resonance, resulting in a minimal thermal dissipation in the transducer, enabling operation at higher actuation voltage, as compared to the bulk PZT transducers.

The characterization of the TF device was further investigated using 7 µm green fluorescent beads and showed a linear dependency between the flow rate and acoustic energy (transducer voltage squared) while maintaining the beads focused in the center outlet. The device was possible to operate at flow rates as high as 500 µL/min and at 140 Vpp without a noted increase in temperature, compared to standard BAW devices which commonly are not operated above 20 Vpp due to the extreme heat development.

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Surfactant dynamics at liquid-liquid interfaces under shear: experimental study of the kinematic condition

Théo Lenavetier[1*], Zhuxuan Cui[1], Julien Cerutti[1] and Shervin Bagheri[1]

[1] FLOW Division, KTH – Royal Institute of Technology
*E-mail: theole@kth.se

Abstract
Lubricant-infused surfaces (LIS) are engineered materials that incorporate a stable liquid-liquid interface between a trapped lubricant and a working fluid. This configuration is expected to promote remarkable interfacial slip, enabling applications from anti-biofouling to thrombosis prevention. However, unlike controlled lab settings, real environments expose LIS to surface-active chemical contaminants. Even traces amounts of these surfactants can accumulate at the sheared water/oil interface, inducing Marangoni stresses that oppose the flow and reduce interfacial slip.

This raises a key question: what kinematic condition should be imposed at the interface to predict the averaged behavior of the flow, especially in engineering contexts? In the presence of surfactants, accurately determining the stopping point positions and the resulting Marangoni stress poses a significant challenge due to the numerous hidden physico-chemical variables. Consequently, many numerical and analytical models fail to predict the experimental velocity field at the liquid-liquid interface, leading to inaccurate predictions of the flow within the working fluid.

We propose using Doppler Optical Coherence Tomography (D-OCT) to characterize local flows near the liquid-liquid interface of a LIS under controlled surfactant conditions. This technique is relatively new in the soft-matter hydrodynamics community, and enables live in-situ velocimetry with velocities up to v ~10 cm/s and with a spanwise/depthwise resolution of δ ~2µm.

By resolving the 3D velocity field near the interface, we aim to track interfacial motion and compression to infer surfactant flux and kinematic constraints. This allows us to quantify and map Marangoni stresses, revealing surfactant transport pathways and strategies to mitigate contamination issues under realistic operating conditions.

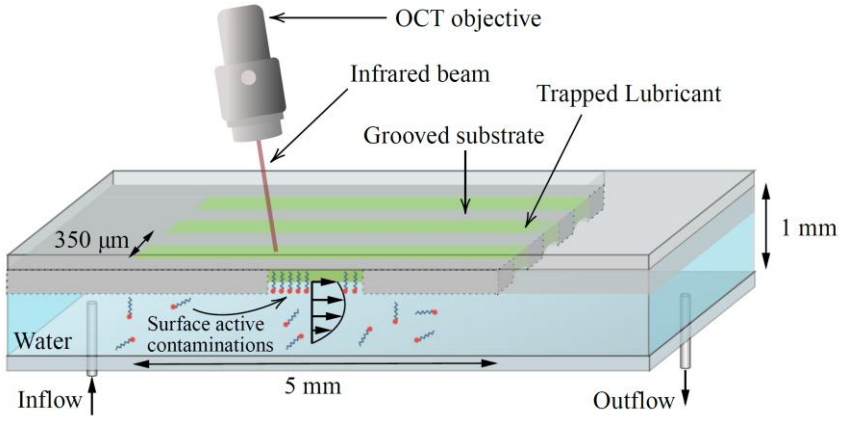

Figure 1 Schematic representation of the experimental millifluidic setup. The surfactants are carried by the working water flow, either because of poorly soluble contamination, or by incorporating them in clean conditions.

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Thermoacoustic streaming

Franziska Martens[1], Junjie Cheng[1], Enrico Corato[1], Ola Jakobsson[1], David VanAssche[1], Wei Qiu[1], and Per Augustsson[1*]

[1] Department of Biomedical Engineering, Lund University, Lund, Sweden
*E-mail: per.augustsson@bme.lth.se

Introduction

Sound carries energy that can scatter and exert forces on suspended microscopic objects such as blood cells and biological nanoparticles. We report our work on forces acting directly in inhomogeneous liquids when a material gradient is present. We tailor such gradients by directing focused laser light into an light-absorbing liquid in an acoustofluidic chamber hosting a standing sound wave. This leads to a fast thermoacoustic streaming flow around the incident laser beam. We will present results from two recently published works [1, 2] and some ongoing work on thermoacoustic streaming reversal.

Theory and Experimental procedure

A focused NIR-laser spot is directed through a glass-silicon-glass chip hosting a microchannel. Light is absorbed by dye molecules in the liquid, causing a local temperature increase. The streaming is visualized by tracking tracer particles in 3D. The λ/2 acoustic pressure field across the channel is generated by a piezo-electric transducer and the resulting 2D-Gaussian temperature gradient can be shifted by moving the laser's point of incidence, Fig.1. The sound scatters when interacting with the inhomogeneous density and compressibility field resulting from the temperature gradient. This results in forces that set the fluid in motion.

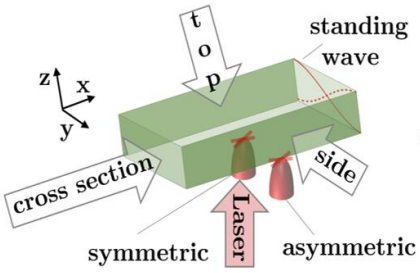

Figure 1: A laser is directed into an acoustofluidic channel hosting a standing half wave

Results and Discussion

We showed in [1] that the thermoacoustic streaming pattern can be tailored by moving the location of the incident laser beam relative to the chamber, Fig 1(a-b). The streaming velocity will be affected by the acoustic amplitude and the intensity of the laser, and it was possible to tailor the streaming flow by altering the amount of absorbing dye in the medium. In [2], we investigated the transient dynamics of the system and found that the build-up time of the thermoacoustic streaming was ~30 ms when activating the laser (for a pre-established sound field), whereas the build-up time is ~3 ms when activating the sound field (for a pre-established temperature gradient). For a no-sound condition, the natural convection of the system is orders of magnitude smaller, and thus the phenomena can be used to define a highly localized flow in a region by constant laser illumination, while the timing can be controlled by toggling the sound. Our ongoing work focus on investigating a reversal of the streaming direction by exchanging the water in the channel by a liquid whose properties changes differently with temperature.

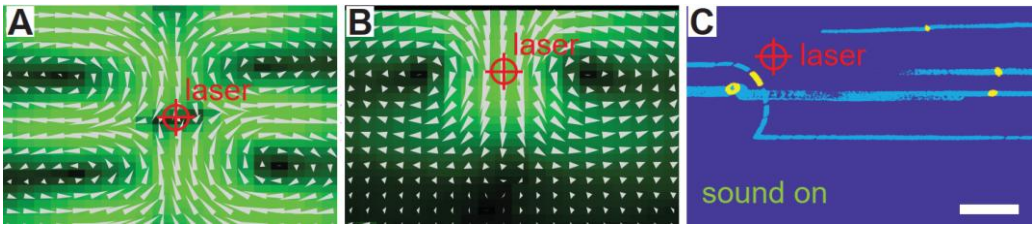

Figure 2: Thermoacoustic streaming for (A) centered and (B) off-centered laser. (C) Image overlay of particles flowing left-to-right through the chamber during a short unset of the sound field showcases a selective re-location of one particle while the others are unaffected due to the highly localized flow.

Conclusions

While currently explorative, we believe thermoacoustic streaming can be used for cell sorting or to control the location and rotation of small objects such as cells or organoids under a microscope inside micro-chambers.

References

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Session 5: Micro- and Nanosystems

Chair: Maria Tenje
2026-06-12
11:00 – 12:30

5.1	A Lab-on-a-Silicon-Chip Platform for Rapid Antibiotic Susceptibility Testing *Zheqiang Xu, Uppsala University*
5.2	Microsystem with electrical read-out for bacteria *Sofia Johansson, Uppsala University*
5.3	Acoustofluidic sample delivery and media switching for SX *Patricia Lopez Ramirez, KTH Royal Institute of Technology*
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A Lab-on-a-Silicon-Chip Platform for Rapid Antibiotic Susceptibility Testing

Zheqiang Xu,[1] Victoria C Nolan,[1] Yingtao Yu,[1] Petra Muir,[1] Sanna Koskiniemi, [*2] and Zhen Zhang*[1]

[1] Ångström Laboratory, Division of Solid-State Electronics, Department of Electrical Engineering, Uppsala University, SE-75121 Uppsala, Sweden.
[2] Department of Cellular and Molecular Biology, Uppsala University, SE-75124 Uppsala, Sweden
*E-mail: sanna.koskiniemi@icm.uu.se; zhen.zhang@angstrom.uu.se

Introduction

Antimicrobial resistance (AMR) requires rapid antibiotic susceptibility testing (AST) to guide treatment. Conventional phenotypic AST relies on bacterial culture and typically requires 24–48 h pre-cultivation plus several hours of testing, delaying clinical decisions. Genotypic methods are faster but cannot reliably detect unknown resistance mechanisms.

In our previous work, we developed a metabolism-based rapid AST approach by monitoring antibiotic-induced extracellular pH changes using nanoscale ion-sensitive field-effect transistors (ISFET). Susceptible bacteria show rapid metabolic inhibition upon antibiotic exposure, producing distinct pH responses compared to resistant strains. This ISFET-based approach reduced the AST time to ~30 min; however, the requirement for high cell density to generate rapid pH changes still necessitates pre-cultivation.

To further reduce the overall sample-to-result time, we eliminate the pre-cultivation step by integrating a cell-collection microfluidic module with ISFET to form a lab-on-a-silicon-chip (LOSC) AST platform, enabling direct testing of urine samples. The microfluidics concentrates bacteria into microscale chambers, increasing local cell density and accelerating metabolism-induced pH changes, thereby enabling rapid detection without pre-cultivation. Using this platform, antibiotic susceptibility can be determined within 20 minutes after sample collection, as demonstrated using a clinical uropathogenic *Escherichia coli* (UPEC) strain.

Experimental procedure

The LOSC integrates an ISFET array with a microfluidic cell-collection module. The microfluidics comprises parallel culture chambers (35×50×20 μm) connected to a main channel through narrow loading channels and smaller exit channels. The loading channels allow bacteria to enter the chambers, while the smaller exit channels prevent their escape, resulting in cell trapping.
Bacterial samples are introduced into the LOSC and guided into the chambers under flow. Test media with or without antibiotics are then perfused, followed by oil sealing to isolate the chambers. Then the ISFETs are used for continuous pH monitoring during the AST.

Conclusion

- Bacterial samples at 10^3–10^6 cells/mL are concentrated to ~10^8 cells/mL into culture chambers in the microfluidics within a 10 min loading step.
- Using 0.9% NaCl with 1% glucose as test medium, the LOSC distinguishes susceptible and resistant strains within 10 min.
- Antibiotic susceptibility is determined within 20 min after sample collection, as demonstrated using a clinical uropathogenic *Escherichia coli* (UPEC) strain.

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Microsystem with electrical read-out for bacteria

Sofia Johansson*, Tobias Hensel, Sagar Narhari Agnihotri, Astrid Olivefors, Maria Tenje

Div. Biomedical Engineering, Dept. Materials Science, Science for Life Laboratory, Uppsala University, Uppsala, Sweden
*E-mail: sofia.m.johansson@angstrom.uu.se

Introduction

The development of fast and reliable diagnostic tools to assist decision-making on administration of antibiotics has been identified as a key challenge in the fight against antibiotic resistance. This project explores electrical sensing microsystems for targeting on-site testing of bacteria with mastitis in cattle from milk samples as a proof-of concept application. Electrical read-out has the potential for more compact and cheap systems compared to microscopy-dependent methods. Possible read-outs include increased impedance at low frequencies due to bacteria growth constricted to narrow microchannels where they grow stacked in a linear fashion. Alternatively, changes of the impedance due changes in the conductivity of the surrounding cell media could be one path of detecting bacteria growth *e.g.* by metabolites secreted by the bacteria.

Platforms for electrical read-out

We explore two different platforms. The first use narrow microfluidic channels for hydrodynamic trapping of bacteria at the end by a small contraction in the channel and this is combined with impedance measurements across the channels. The use of 2-photon 3D printed structures in combination with standardized PDMS devices has been explored for rapid iteration of design options. For example, the 2PP resin failed to cure close to the PDMS, which may be attributed to oxygen inhibition and leading to a change in design with both top and bottom surfaces in glass.

Parallel work investigates droplets placed on high-definition microelectrode arrays (HD-MEA) where electrical measurements are conducted between the array of electrodes on the surface and a common counter electrode. Droplets has previously demonstrated great promise as small bacteria culture containers for detecting antibiotics heteroresistance by optical read-out. As an alternative the secreted metabolites during bacterial growth are known to change the conductivity in the cell media of the droplets and enable electrical read-out.

Acknowledgments

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Acoustofluidic Sample Delivery and Media Switching for SX

Patricia A. López[1,*], Varun Kumar Rajendran[1], Björn Hammarström[1,2], Martin Viklund[1,2], Jonas A. Sellberg[1]

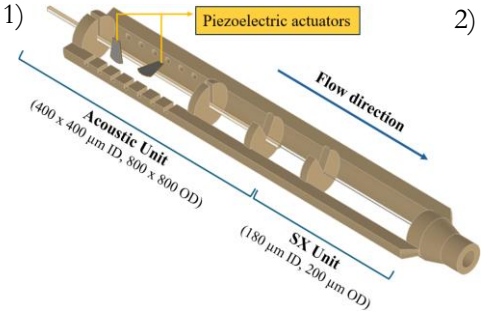
[1] Department of Applied Physics, KTH Royal Institute of Technology, Stockholm, Sweden
[2] SciLifeLab, Stockholm, Sweden
*E-mail: palr2@kth.se

Introduction

Typical conformational studies in Serial Crystallography (SX) combine specialized nozzles with turbulent mixing of protein crystal and ligand solutions, often leading to great sample loss and potential clogging [1]. In this context, the implementation of acoustics succeeds to avoid clogging and reduces crystal consumption by a factor of 6 [2].

Acoustofluidic devices

- The AFX device (Fig. 1) comprehends two different units: the Acoustic Unit, where acoustic resonances triggered inside a square capillary by two perpendicular piezoelectric actuators focus crystals at 5-10 µL/min; and the SX Unit, a circular capillary where the X-ray beam interacts with the sample, producing diffraction data.
- The Media Switching device (Fig. 2) consists of 3 regions: a Pre-Focus channel, where acoustics focus crystals in a narrow line; a Media Switching channel, where acoustics switch crystals from a mother liquid to a new medium across laminar flow; and a Re-Focus channel, where a Kapton X-ray window permits diffraction data collection.



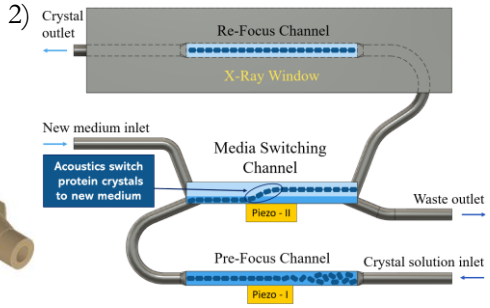
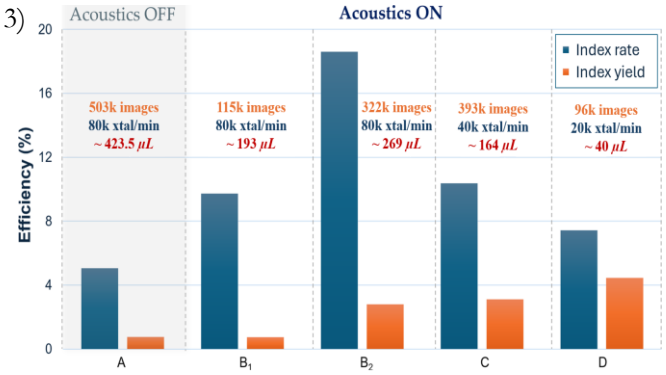


Figure 1. Schematic of the AFX Device inside a 3D printed supporting scaffold (brown).

Figure 2. Schematic of the Media Switching Device, built in glass through In volume Selected Laser-induced Etching (ISLE).

Results and Discussion

The AFX Device succeeds in preventing clogs while consuming up to 6 times less thaumatin crystals (Fig. 3, D) than without acoustics, achieving an index rate up to 4 times higher during data collection (Fig. 3, B_2). On the other hand, the Media Switching Device can switch lysozyme crystals to a new medium without turbulent mixing, allowing for ligand and pH-triggered conformational studies (Fig. 4).



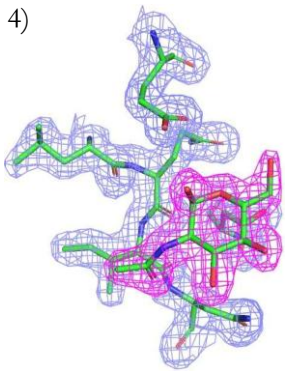


Figure 3. Performance of the AFX Device. All conditions measured at 5 ms exposure time, except B_1 (10 ms).
** Index rate: fraction of diffraction images successfully indexed.*
** Index yield: fraction of indexed crystals out of an estimated total.*

Figure 4. Structure of lysozyme (blue) after switching to N-acetyl glucosamine solution (pink).

Conclusion

The implementation of acoustics in SX delivery systems avoids the appearance of clogs and reduces drastically the sample consumption and the time of collection, significantly increasing the amount of data retrieved per crystal.

Acknowledgments

This work is supported by LEAPS-INNOV (grant no. 101004728) and VR-RÅC (grant no. 2023-66350). We would like to thank our associates at the Technische Universität Hamburg (TUHH, Germany), the Centre for Free-Electron Laser (CFEL) at DESY (Hamburg, Germany), and the MAX IV Laboratory (Lund, Sweden).

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Embedded Biosensors for Localized Cytokine Detection in 3D Microtumor Models

Iro Beligianni[1,*], Gabriel Werr[1], Maria Tenje[1,2] and Patrick A. Sandoz[1,2]

[1]Department of Materials Science and Engineering, Uppsala University, Uppsala, Sweden
[2]Science for Life Laboratory, Uppsala University, Uppsala, Sweden
*E-mail: iro.beligianni@angstrom.uu.se

Introduction

Cancer remains one of the leading causes of death globally, with many types showing resistance to standard treatment approaches. A major obstacle in developing effective therapies is the inability of conventional 2D cultures and animal models to replicate the complexity of the tumor microenvironment. 3D tumor models and organ-on-chip systems offer more physiologically relevant platforms than conventional 2D cultures[1]. However, the limited material availability and constrained volumes inherent to these systems demand new analytical approaches for their full interrogation.
Numerous antibody-based biosensing techniques have been developed to detect secreted cytokines and other soluble factors in cancer models[2]. Most of these platforms are optimized for 2D cell culture systems, while those adapted for 3D cultures predominantly detect signals originating outside the tumor mass. To our knowledge, no antibody-based biosensor has yet been developed that can be integrated directly into microtumors for intratumoral sensing – i.e. capturing precisely where the molecular cues are released. Thus, in this study we aim to develop antibody-based biosensors that can be embedded within 3D microtumor models and on-chip systems to enable localized, intratumoral detection of pro- and anti-tumor signals.

Experimental Procedure

To achieve this, we functionalize microparticles with specific antibodies, incorporate them into tumor spheroids including cancer and immune cells, and capture locally secreted molecules to analyze the dynamics of immune infiltration and effector functions. Specifically, the particles are functionalized via EDC/NHS coupling chemistry, with capture antibodies specific to the pro-inflammatory cytokine interferon-gamma as proof of concept. The beads are then incorporated into microtumors, and a bead-based sandwich ELISA is performed. Conjugation efficiency, signal intensity, and bead integration are systematically optimized across different conditions, and readouts are acquired by flow cytometry and microscopy. The microtumors are cultured in microcavity arrays made of hydrogel, facilitating the spontaneous formation of spheroids and passive diffusion throughout the system.

Conclusion

Together, this ongoing work establishes the foundation for an embedded biosensing approach that overcomes a critical limitation of existing platforms with already promising results including biocompatibility of the particles and detection down to 1 ng/ml interferon-gamma. Future work will focus on optimizing the capture yield and expanding the multiplexing capacity of the platform to simultaneously detect multiple pro- and anti-tumor mediators, as well as validating the system in more complex, heterotypic tumor models incorporating immune and stromal cell components.

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A Paper-Based Colorimetric Sensor for Water Quality Monitoring

Enahoro Asein[1,2], Pablo Giménez-Gómez[1], Nicole Pamme[1,2,*]

[1] Department of Chemistry, Stockholm University, Stockholm, Sweden
[2] Wallenberg Initiative Materials Science for Sustainability, Department of Chemistry, Stockholm University, Stockholm, Sweden
*E-mail: nicole.pamme@su.se

Introduction

Fluoride, although beneficial in limited quantities, can have detrimental effects on dental and skeletal integrity when consumed in excess [3]. Dyes forming complexes with zirconium have been utilised for fluoride detection by spectroscopy, as fluoride forms a stable complex with zirconium resulting in decolourisation or complex formation [4, 5].This work presents an easy-to-use paper-based analytical device (PAD) for quantifying fluoride in water, improving on previous PADs that require pipetting of the sample onto the device [1,2].

Theory and Experimental procedure

We compared three dyes, alizarin red S, eriochrome cyanine R and xylenol orange, using UV-vis spectroscopy. Xylenol orange was selected for its higher sensitivity and used for the development of our PAD. The PADs were fabricated by printing and melting wax through filter paper to create hydrophilic circular areas onto which the dye could be deposited (**Fig 1A**). Each PAD consisted of test zones containing the dye and dye-free sample zones folded on top of the test zone. After lamination, slits were cut into the sample side to allow liquid flow to the test zone after dipping into the sample (**Fig 1B**). Images were captured with a scanner and analysed using ImageJ.

Results and Discussion

The calibration curve (**Fig 1C**) was obtained by plotting relative colour intensity, $I_{relative} = I_{sample} / I_{yellow}$, where I_{sample} and I_{yellow} are the colour intensities from the test zones and yellow colour reference square, against fluoride concentration. We validated the PAD by comparison against a standard method for the analysis of real samples.

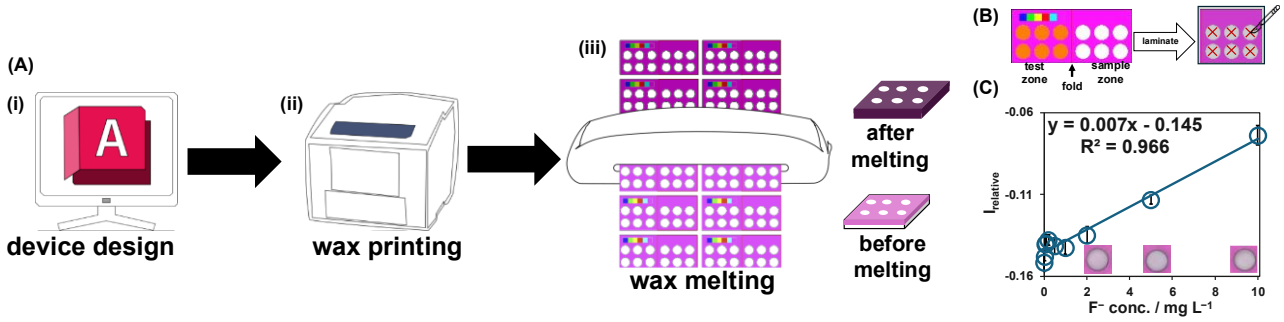

Fig 1. (A) Fabrication of the PAD i) Design in AutoCAD. (ii) Wax printing onto filter paper. (iii) Wax melting with a laminator to create hydrophobic barriers. (B) Design of the dip-style PAD. (C) Calibration curve obtained using the dip-style PAD.

Conclusion

We reached detection and quantification limits of 0.8 mg L^{-1} and 2.4 mg L^{-1} with the PAD, allowing for detection below the 1.5 mg L^{-1} EU limit on fluoride [6] with improved usability compared against other PADs [1,2].

Acknowledgments

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Development of epicardium-integrated cardiac organoids for modelling hypertrophic cardiomyopathy

Martta Häkli[1,*] and Katriina Aalto-Setälä[1,2]

[1] Faculty of Medicine and Health Technology, Tampere University, Tampere, Finland
[2] Heart Center, Tampere University Hospital, Tampere, Finland
*E-mail: martta.hakli@tuni.fi

Introduction

Cardiac organoids are composed of a variety of cell types including e.g. cardiomyocytes (CM) and progenitor cells that form organized 3D structures mimicking cardiac tissue. Traditional 2D models lack the structural complexity and cell-to-cell interactions present in myocardium, which hinders the modelling of diseases [1].

Epicardium is the outermost mesothelial layer of the heart and plays a central role in heart development. It acts as a source of progenitor cells, contributing to cardiac fibroblasts (CF), vascular smooth muscle cell (VSMC) and pericyte populations in heart, and participates in essential signaling guiding CM growth and maturation. Given the contribution of epicardium-derived cells to the major non-myocyte populations of the heart, including epicardial cells (EPI) into cardiac organoids can enable more physiologically relevant disease modelling [2].

Hypertrophic cardiomyopathy (HCM) is the most common genetic cardiovascular disease caused by several known mutations, one of which is the less studied heterozygous Finnish founder mutation c.482C>A, p.(Thr161Lys) in junctophilin-2 (JPH2). As fibrosis is one of the key features in HCM, the cardiac organoid model consisting of multiple cell types including CF with a crucial role in fibrosis [1] can bring significant insight into HCM development and mechanisms.

Materials and Methods

Human induced pluripotent stem cells (hiPSC) from a patient carrying the JPH2 mutation and a healthy control line were differentiated into CM [3] and EPI [4]. CM were enriched using lactate purification. The organoids were generated on day 18 of differentiation. CM and EPI were dissociated into single cells and CM were either used as such or further purified with magnetic-activated cell sorting. Organoids were generated by optimizing a protocol adapted from Fernandes et al. [2]. CM and EPI were mixed 2:1 or 1:1 in medium containing 2 µM retinoic acid to induce epithelial-to-mesenchymal transition in EPI and seeded as 75 000 cells/well or 50 000 cells/well into round bottom 96-well plates coated with poly(2-hydroxyethyl methacrylate) to prevent cell attachment. The plates were centrifuged to support organoid formation. The organoids were cultured for 2-4 weeks, after which they were fixed and stained for fluorescent microscopy to evaluate cellular composition of the organoids.

Results and Discussion

Magnetic-activated cell sorting for CM after lactate purification still significantly improved the CM purity of the differentiation and yielded to organoids with better spontaneous beating characteristics. 75 000 cells/well was observed to yield too large organoids that did not beat well, probably due to limited oxygen and nutrient diffusion into the organoid core. With magnetic-activated cell sorting, CM-to-EPI ratio of 1:1 with 50 000 cells/well was observed to yield to organoids with strongest spontaneous beating characteristics. Based on the fluorescent images, the presence of CM, EPI, CF and VSMC was confirmed.

Conclusion

In this study, generation of epicardium-integrated cardiac organoids from healthy wild type and JPH2 mutation carrying hiPSC lines was optimized. Next, we aim to utilize the model to characterize HCM phenotype and its development in the organoids and elucidate new disease mechanisms related to HCM caused by JPH2 mutation.

Acknowledgments

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From diffusion to cellular signaling: modeling and experimental analysis of VEGF gradients in 3D microfluidic cultures

Neeraj Katiyar[1, 2,*], Nicole Sui Man Luk[1], Tora G. Bratt[1, 2,] and Maria Tenje[1, 2]
[1]Dept. Materials Science and Engineering, Uppsala University, Uppsala, Sweden
[2]Science for Life Laboratory, Uppsala, Sweden
*E-mail: neeraj.katiyar@angstrom.uu.se

Introduction

Chemical gradients of signaling molecules, including growth factors, are central regulators of cellular migration, morphogenesis, and angiogenesis. Yet achieving stable, spatially defined biomolecular gradients in conventional two-dimensional culture systems remains a significant challenge [1]. Microfluidic technologies offer a compelling solution by enabling precise control over fluid dynamics, molecular transport, and the cellular microenvironment [2]. Here, we present a microfluidic platform engineered to study the diffusion, transport, and downstream cellular responses to vascular endothelial growth factor (VEGF) gradients within three-dimensional extracellular matrices. Through the integration of computational modeling, experimental validation, and functional cellular assays, we seek to elucidate the spatiotemporal dynamics of molecular transport and gradient-driven cell signaling in a physiologically relevant context.

Theory and Experimental procedure

Diffusion and transport dynamics were modeled using multiphysics simulations in COMSOL Multiphysics at a perfusion flow rate of 1 µL/min, targeting molecules in the ~45 kDa range corresponding to the molecular weight of VEGF. Experimental validation involved tracking the diffusion of FITC-dextran (45 kDa) through Matrigel (50% v/v) via confocal microscopy and quantitative fluorescence intensity analysis, establishing a correlative benchmark for VEGF transport behavior. Functional studies using RFP-labeled human umbilical vein endothelial cells (HUVECs) embedded within Matrigel in the gradient-generating chip is performed using intracellular Ca^{2+} signaling responses to VEGF gradients monitored by live-cell confocal imaging as read-out.

Results

Preliminary COMSOL simulations demonstrate that at 1 µL/min perfusion, molecular size governs diffusion kinetics and gradient steepness within Matrigel. Molecules in the 45 kDa range exhibit sufficiently slow diffusion to support the formation of stable, well-defined spatial gradients [3]. Experimentally acquired diffusion profiles of 45 kDa FITC-dextran show strong qualitative agreement with simulation predictions, corroborating its use as a reliable transport proxy for VEGF.

Conclusion

This integrated computational - experimental framework yields meaningful insight into the spatiotemporal behavior of biomolecule gradients in 3D extracellular matrices under low-perfusion flow conditions. The platform provides a physiologically relevant model for investigating VEGF-mediated endothelial signaling, and ongoing HUVEC studies will further establish the biological validity of the generated gradients. These findings hold broader implications for angiogenesis research and the development of advanced organ-on-chip systems.

Acknowledgments

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Plant cell communication study on micro-fabricated platform

Dimitra Valadorou[1,2], Dominic Kuang[3], Simon Stael[3], Charles Melnyk[4], Stephane Verger[5,6], and Maria Tenje[1,2,*]

[1]Department of Materials Science and Engineering, Uppsala University, Sweden
[2]Science for Life Laboratory, Uppsala University, Sweden
[3]Department of Molecular Sciences, Uppsala BioCenter, Swedish University of Agricultural Sciences and Linnean Center for Plant Biology, Sweden
[4]Department of Plant Biology, Linnean Center for Plant Biology, Swedish University of Agricultural Sciences, Uppsala, Sweden
[5]Department of Forest Genetics and Plant Physiology, UPSC, SLU, Sweden
[6]Department of Plant Physiology, UPSC, Umeå University, Sweden
*E-mail: maria.tenje@angstrom.uu.se

Introduction

Intercellular communication underpins plant development, differentiation, and responses to environmental stimuli [1]. In plants, signaling occurs through both symplastic and apoplastic pathways. Symplastic communication refers to the direct exchange of molecules between neighboring cells through cytoplasmic connections called plasmodesmata, which physically link the interiors of adjacent cells. In contrast, apoplastic communication occurs outside the cell membranes, where signaling molecules such as reactive oxygen species, peptides, and hormones move through the cell wall and extracellular environment [2]. While in vivo studies have provided valuable insights, the inherent complexity of plant tissues limits the ability to isolate and directly probe the mechanisms governing cell–cell communication. A minimal in vitro system in which isolated plant cells are placed in defined spatial arrangements could overcome these limitations. Such an approach would allow direct investigation of whether symplastic connections, including de novo plasmodesmata, can re-establish when cells are removed from their native tissue context. Protoplasts, plant single cells that have had their cell walls enzymatically removed, provide a useful simplified system for studying these processes, since they preserve physiological activity comparable to whole plants [3]. Combining protoplasts with micro-structured devices offers a promising solution. These platforms provide precise control over cell positioning, physical contact, and microenvironmental cues, while supporting high-resolution live imaging under reproducible conditions.

Experimental procedure

To investigate intercellular communication in a minimal system, we developed a microstructured plant-on-a-chip platform for controlled protoplast manipulation. Protoplasts were isolated from *Arabidopsis thaliana* lines expressing distinct fluorescent reporters. The device consists of 3D-printed micropillars fabricated on a silanized glass coverslip and enclosed within a PDMS well. These micropillars enable precise positioning and confinement of individual protoplasts while allowing controlled physical contact between neighboring cells. The transparent substrate permits high-resolution, time-lapse imaging using inverted microscopy. Protoplast pairs of two different reporter lines (pUBQ10::PM-TdTomato and p35S::GFP, labeling the plasma membrane and cytoplasm respectively) were kept in contact and monitored over a period of three days to assess potential symplastic exchange.

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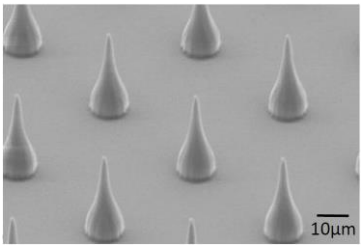

Figure 1: SEM image of the pillar array.

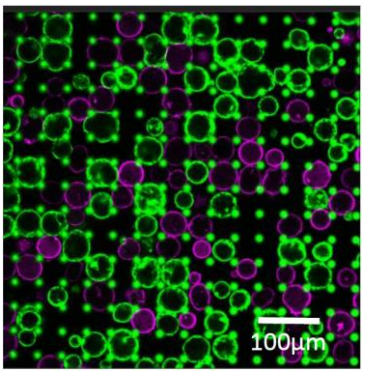

Figure 2: Merged fluorescence microscopy images of mixed pUBQ10::PM-TdTomato and p35S::GFP protoplasts on the chip.

Results and Discussion

The platform successfully enabled stable confinement, positioning, and long-term imaging of individual protoplasts under defined conditions. Controlled cell–cell contact was consistently achieved, demonstrating the system's capability to mimic physical proximity between plant cells. However, no detectable transfer of fluorescent markers between adjacent protoplasts was observed over the experimental timeframe. This suggests that physical contact alone is insufficient to establish symplastic communication in the absence of a cell wall and associated structures such as plasmodesmata. These findings highlight the likely importance of cell wall regeneration, mechanical cues, and tissue-specific context in enabling functional intercellular connectivity.

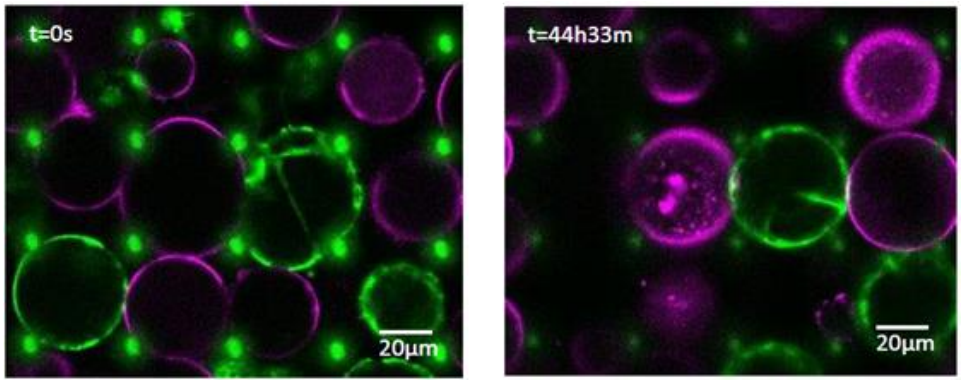

Figure 3: Fluorescence microscopy images of mixed pUBQ10::PM-TdTomato and p35S::GFP protoplasts on the chip at time 0 and after 44h33m.

Conclusion

We present a novel plant-on-a-chip platform that enables controlled investigation of plant cell–cell communication at single-cell resolution. While preliminary experiments indicate that symplastic connectivity does not spontaneously arise between isolated protoplasts in direct contact, the system provides a powerful framework for systematically probing the requirements for intercellular signaling. Future work will focus on incorporating cell wall–regenerated protoplasts to assess the role of structural components in plasmodesmata formation. This approach opens new avenues for reconstructing plant communication networks in vitro and advancing our understanding of the fundamental principles governing plant cell interactions.

Acknowledgments

This study was supported by the Knut and Alice Wallenberg Foundation (grant no. 2022.0029). We thank Milena De Albuquerque Moreira, MyFab Uppsala University for her valuable assistance and insights during the chip fabrication process. We also thank Cecilia Wärdig, Swedish University of Agricultural Sciences for her help with plant growth.

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Swedish Microfluidics Network
June 11-12, 2026, Uppsala University

High-throughput Optical DNA Mapping for bacterial diagnostics

Radhika N. Kunnath[1,2], Anna Johnning[2,3,4], Sriram K. K.[1,2], Albertas Dvirnas[1,5] Karolin Frykholm[1], Marie Wrande[6], Christian Giske[7], Tobias Ambjörnsson[5], Linus Sandegren[6], Erik Kristiansson[2,3,4], and Fredrik Westerlund[1,2*]

[1] Department of Life Sciences, Chalmers University of Technology, Gothenburg, Sweden
[2] Centre for Antibiotic Resistance Research, CARe, University of Gothenburg, Box 440, 405 30 Gothenburg, Sweden
[3] Department of Mathematical Sciences, Chalmers University of Technology and the University of Gothenburg, 412 96 Gothenburg, Sweden
[4] Systems and Data Analysis, Fraunhofer-Chalmers Centre, Chalmers Science Park, 412 88 Gothenburg, Sweden
[5] Centre for environmental and climate science, Lund University, Sölvegatan 12, Lund
[6] Department of Medical Biochemistry and Microbiology, Uppsala University, 751 23 Uppsala, Sweden
[7] Department of Laboratory Medicine, Karolinka Institutet, 171 77 Stockholm
*Email: *E-mail: fredrik.westerlund@chalmers.se

Introduction

The identification of pathogenic bacterial strains is currently performed using labor intensive and time-consuming methods like DNA sequencing. In this report, we propose a faster technique called optical DNA mapping (ODM) for bacterial strain typing. ODM is a single molecule analysis technique in which fluorescently labelled DNA molecules (ranging from 100 kb to 1 Mb in size) are stretched in nanofluidic channels to generate spatial intensity profiles called barcodes that represent the long-range genomic order in the DNA molecules. Previously, we have shown that ODM can be used for bacterial identification and strain typing [1, 2, 3]. In this report, we present a high-throughput ODM system consisting of a multiplexed nanofluidic device along with automated routines for imaging and pumping that can analyze up to ten bacterial samples simultaneously.

Theory and Experimental procedure

In the ODM assay (Figure 1), DNA is stained with YOYO-1 (an intercalating dye that binds to dsDNA) and Netropsin (a non-fluorescent molecule which binds specifically to AT-rich regions) to generate a sequence specific barcode. The stained DNA is then loaded into the wells of the multiplexed nanofluidic device, which works via pressure driven flow. The multiplexed device has 10 sample wells, and each well is associated with a set of 400 parallel nanochannels (100 nm x 150 nm (width x height)). The developed high-throughput automated ODM system consists of a pressure control pump and an epi-fluorescence microscope with a motorized stage and focus drive. The experimental barcodes generated from time series images of the DNA molecules are compared to theory barcodes generated from a database of 47 000 complete genomes to identify the bacteria.

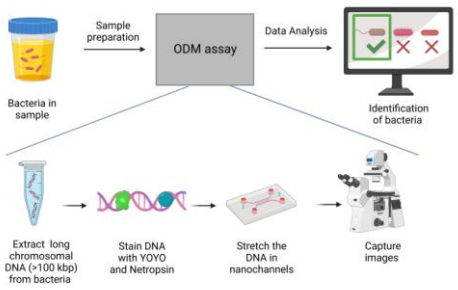

Figure 1 An overview of the ODM assay for bacterial identification

Results and Discussion

The automated multiplexed ODM system significantly improves the speed and the quantity of data collection, and up to 10 samples can be processed at a time using the multiplexed device. The developed system has been used to identify bacteria such as *E. coli and K. pneumoniae* from isolates and spiked clinical samples.

Conclusion

The proposed method has the potential to be deployed for bacterial strain typing in clinical settings.

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SMILS '26
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Development of a Paper-Based Analytical Device Immunosensor for Saxitoxin Monitoring

Sara Santiago[1*], Mònica Campàs[2] and Nicole Pamme[1*]
[1] Department of Chemistry, Stockholm University, Stockholm, Sweden.
[2] IRTA, Marine and Continental Waters, La Ràpita, Spain
*E-mail: sara.santiago@su.se

Introduction

Climate change has increased the frequency of harmful algal blooms in aquatic environments. These events lead to the release of highly toxic compounds produced by cyanobacteria and certain dinoflagellates that threaten ecosystems and public health [1]. Among these toxins, saxitoxin (STX) causes paralytic shellfish poisoning and is strictly monitored in seafood using complex and labour-intense standardised protocols [2]. In this study, we are developing a paper-based analytical device (PAD) based on a competitive immunoassay for rapid on-site colorimetric quantification of STX in water samples. The device is portable (15 cm^2, < 1g), eco-friendly and equipment-free (smartphone app). The single-step assay avoids the handling of reagents and enables a simple readout based on color intensity or distance changes, potentially surpassing conventional approaches.

Experimental procedure

Circular reaction zone PADs and origami-type distance-based PADs (dPADs) were designed with AutoCAD and printed with a Xerox ColorCube wax printer. The capture antibody (cAb) and the colorimetric indicator 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were immobilized within a silk fibroin (SF) membrane on the paper substrate, providing stable and specific recognition of the target analyte. The sample, previously mixed with the STX–horseradish peroxidase conjugate (STX-HRP), was added. Then, STX in the sample competes with STX-HRP for binding sites. Upon addition of H_2O_2, a measurable color change occurs, and the color intensity is inversely proportional to the analyte concentration (Fig. 1a).

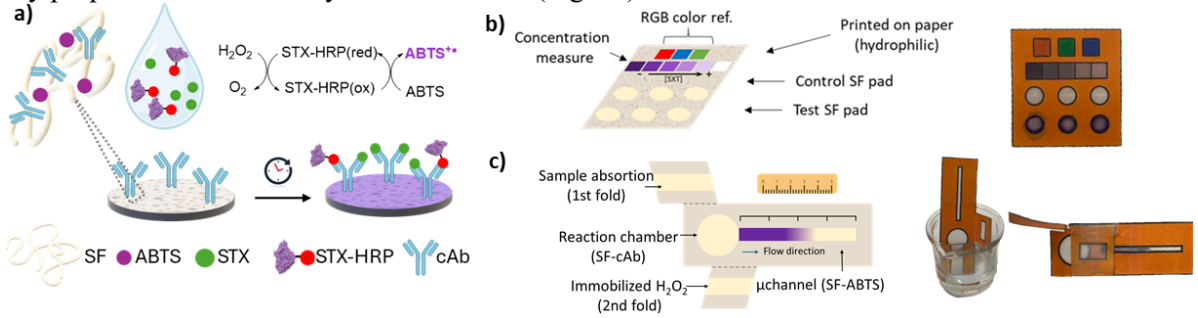

Figure 1. a) Competitive immunoreaction on PADs and dPADs. b) PAD design with circular reaction zones and operation (right: real device). c) dPAD design and operation (right: actual device showing the first fold during the sample soaking and the second fold after H_2O_2-induced color development.

Results and Discussion

The concentrations of bioreagents, colorimetric compound and incubation times, were optimized for the circular PAD design and quantified by ImageJ from smartphone images (Fig. 1b). An origami dPAD with H_2O_2 immobilized in a dedicated chamber was subsequently developed to minimize reagent handling (Fig. 1c). The colorimetric response will be analyzed either by measuring the distance of the color change with ImageJ or visually by the naked eye, demonstrating its potential for user-friendly detection.

Conclusion

A colorimetric PAD for the rapid detection of STX was developed based on a competitive immunoassay. The integration of an origami-type dPAD with immobilized H_2O_2 will minimize reagent handling and will allow a single-step assay with a simple visual readout. The proposed platform represents a portable, low-cost, and user-friendly alternative for rapid on-site monitoring of STX.

Acknowledgments

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June 11-12, 2026, Uppsala University

A Microfluidics Device for Fast Switching of Liquids – For the Controlled Interaction of Single Proteins with Solution

Sina Ritter[1]*, Jason Beech[1], Nils Gustafsson[1], Patrik Nilsson[1], Heiner Linke[1]

[1] NanoLund and Solid State Physics, Lund University, Box 118, 22100 Lund, Sweden
*E-mail: sina.ritter@fysik.lu.se

Introduction

Observing the dynamic response of biomolecular interactions to rapid solution changes remains challenging in single-molecule studies. In particular, controlling the chemical environment on sub-second timescales is essential for probing systems such as chemically clocked artificial molecular motors.[1]
We present a microfluidic device capable of rapidly switching between multiple fluid environments in an arbitrary sequence, within a channel compatible with single-molecule microscopy. The switching is faster than second-scale binding/unbinding events, without drastically increasing flow speed compared to previous devices.[2] Combined with techniques such as single-molecule Förster resonance energy transfer (sm-FRET), this platform enables the investigation of fast biomolecular responses to environmental changes, including the dynamics of a clocked motor protein powered by alternating ligand solutions.

Methods

COMSOL Multiphysics was used to identify issues in the previous designs and to develop an optimised solution. The microfluidic devices were fabricated from polydimethylsiloxane (PDMS) on glass coverslips, enabling real-time microscopy measurements, including total internal reflection fluorescence (TIRF) microscopy. The device features a main observation channel with multiple inlets and outlets (Figure 1 A). The arrangement of inlets ensures that only the desired fluid enters the main channel, while undesired fluids are directed to the corresponding outlets. Fluid selection is controlled by varying the pressure and flow rate at individual inlets, providing precise control over which fluid enters the main channel at any given time.

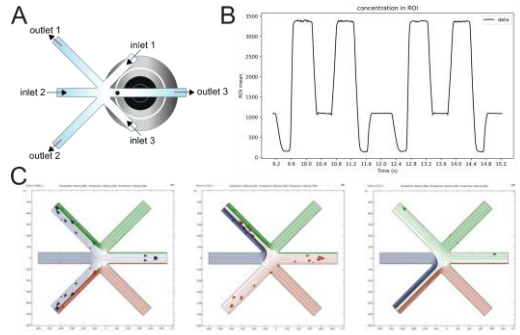

Figure 1: A) Schematic of the device intersection. Single-molecule measurements are performed in the observation channel (right), indicated by the objective (not to scale). B) Results from experiments with fluorescein solutions introduced from three inlets with three different fluorescein concentrations, measured at the start of the observation channel (black circle in A), showing sub-second switching. C) Three simulated steady-state configurations showing dominance of each of the three liquids in the main channel. Each state is achieved by setting the active inlet to a flow rate of 7 µL/min, with the remaining inlets at 1 µL/min.

Results and Discussion

A previous device demonstrated solution switching on a one-second timescale.[2] Preliminary simulation and experiments with the optimised design show fluid exchange within sub-second times with further ability of improvement with better pressure settings and controls supporting faster response times and greater control over the fluidic environment.

Conclusion

We successfully created a microfluidics device which enables sub-seconds switching between three liquids in an arbitrary order. This approach provides precise temporal control of chemical conditions for single-molecule measurements, enabling new studies of fast biomolecular dynamics.

Acknowledgments

This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 951375)

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Swedish Microfluidics Network
June 11-12, 2026, Uppsala University

Development of Heterotypic 3D Lung Tumor Cultures in Stamped Hydrogel-Based Microcavity Arrays

Florence Maryanna O. Agcaoili[1,2*], Gabriel Werr[1], Maria Tenje[1,3], Patrick Sandoz[1,3]

[1] Division of Biomedical Engineering, Department of Materials Science and Engineering, Uppsala University, Uppsala, Sweden
[2] Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden
[3] Science for Life Laboratory, Uppsala University, Uppsala, Sweden
*E-mail: fmo.agcaoili@gmail.com

Introduction

Non-small cell lung cancer (NSCLC) comprises 85% of all lung cancer cases and is characterized by highly heterogeneous tumors with a stiff, desmoplastic stroma due to collagen accumulation during tumorigenesis [1,2]. In addition to genetic and phenotypic variation between patients, immunosuppressive mechanisms that accompany NSCLC comorbidities such as pulmonary fibrosis also increase tumor aggressiveness and resistance to standard treatment approaches [1,3]. Specifically, the collagenous fibrotic stroma prevents infiltration and suppresses activation of immune cells in the tumor [4], emphasizing the role of the tumor microenvironment (TME) in tumor progression. It is then necessary to recapitulate such features of the TME in modeling NSCLC to ensure a more accurate assessment of diagnostic and therapeutic efficacy. Despite the increasing number of NSCLC preclinical models developed over the years, there is still a lack of models that are cost-efficient, easy to generate, and capable of reproducing the role of the desmoplastic stroma as an immunosuppressive physical barrier encapsulating the NSCLC tumor. To address this gap, the present study aims to develop novel, heterotypic 3D tumor cultures recreating the lung tumor microenvironment in stamped hydrogel-based microcavity arrays.

Experimental Procedure and Preliminary Results

The heterotypic 3D NSCLC microtumors consist of A549 cancer and MRC-5 fibroblast cells co-cultured with a collagen scaffold. These were grown in arrays created by stamping microcavities using in-house fabricated stamps onto an agarose base coated with a thin collagen layer containing MRC-5 cells. This design facilitates the incorporation of collagen into the microtumor and builds on the concave microwell design of previously established 3D culture methods, which maximize cell-cell contact to promote aggregation while controlling the size and shape of the formed microtumor. The A549 cells are sequentially seeded after hydrogel polymerization and microcavity formation. The growth, morphology, viability, and organization of the different components within the microtumors are then assessed via bright-field and fluorescence microscopy. Preliminary assessment suggests that the incorporation of collagen results in a more compact and defined 3D microtumor morphology as compared to scaffold-free systems while the microtumors remained viable up to seven days.

Conclusion

Although currently a work in progress, the developed system allows high throughput analysis with parallel replicates of physiologically relevant 3D lung microtumors and enables live cell microscopy at high resolution. Further studies include incorporating additional cell types (e.g., the immune compartment) and evaluating the applicability of this system to further develop complex 3D culture systems and test therapeutic modalities.

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Investigating the complexity of phage-bacteria interactions through single-cell genomics using semi-permeable capsule technology

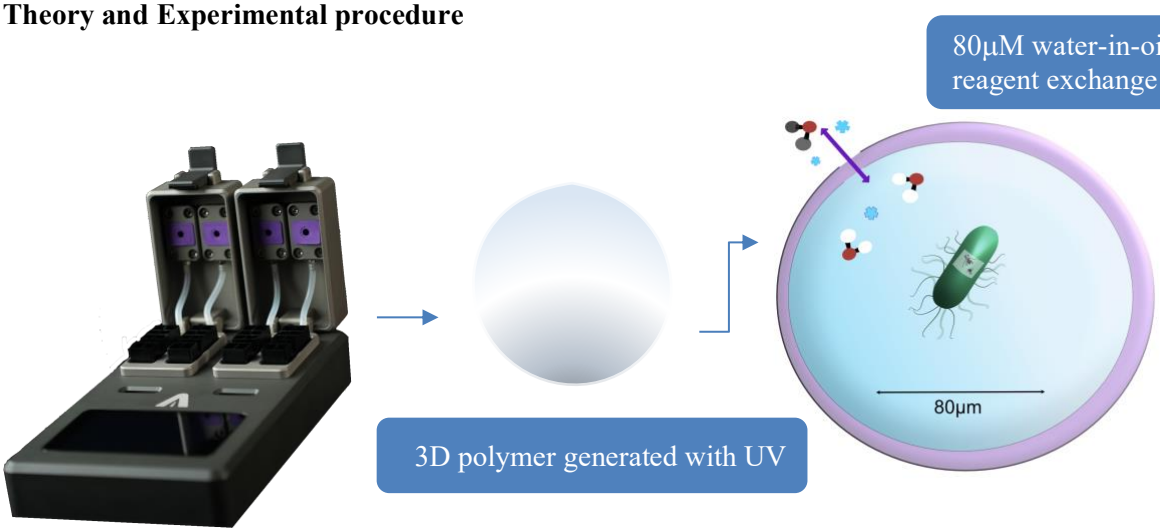
Lauren Davies[1*] and Maliheh Mehrshad[1]

[1] Department of Aquatic Sciences and Assessment, Science for Life Laboratory, Swedish University of Agricultural Sciences, 75007 Uppsala, Sweden
*E-mail: lauren.davies@slu.se

Introduction

Single-cell genomics holds a great potential for disentangling complex interactions between bacteriophages and their bacterial host. Here we utilize Semi-Permeable Capsules (SPCs), a microfluidic technique developed by Atrandi, to investigate phage-bacteria interactions in freshwater ecosystems. After compartmentalizing cells into SPCs and sequencing each individual cell, we will differentiate between infected and non-infected cells and construct an infection network with high temporal resolution to assess the impact of phage-host interactions on ecological and evolutionary dynamics in freshwater.

Theory and Experimental procedure



(1). Semi-permeable capsule technology using the Flux™ device. Figures taken and developed from Atrandi

Unlike water-in-oil droplets, SPCs have a semi-permeable hydrogel shell which allows for reagent exchange both in and out of the capsule. This means that all steps of a single cell microbial workflow: lysis, whole genome amplification and library preparation, can be performed in-capsule without the loss of compartmentalization. We will utilize this compartmentalization and apply it to samples collected over several weeks both in winter and in summer in lake Erken, Sweden.

Conclusions

After the application of a high-throughput single cell workflow and using semi-permeable encapsulation, we will be able to assess phage infection prevalence and link phages with their host and create a network of interactions with elucidation of rare variants through single cell resolution.

Acknowledgments

I would like to acknowledge my bionomics-mmlab group members for help with sampling Maliheh Mehrshad, Vesna Grujcic, Vinicius Silva Kavagutti, Zahra Goodarzi and Fanny Persson. This project is funded by an ERC grant (Grant agreement No. 101117028 — MULTIPHAGE — ERC-2023-STG to Maliheh Mehrshad) Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Research Council Executive Agency. Neither the European Union nor the granting authority can be held responsible for them. I would also like to thank Atrandi for their help in answering queries and questions regarding their technology.

Flux™ instrument image taken from Atrandi webpage: https://atrandi.com/products/flux

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µPATHFINDER: Bridging High-Throughput Microarrays and Single-Molecule Microscopy for Mapping Transcription Factor Binding Landscapes

Tadija Kekic[1,*], Nils Gustafsson[1], Carolina Jerez-Longres[2], Jory Lietard[3], Birte Höcker[2], Paul M. G. Curmi[4], Heiner Linke[1]

[1] NanoLund and Solid-State Physics, Lund University, Lund, Sweden
[2] Department of Biochemistry, University of Bayreuth, Bayreuth, Germany
[3] Institute of Inorganic Chemistry, University of Vienna, Vienna, Austria
[4] School of Physics, University of New South Wales, Sydney, NSW 2052, Australia
*E-mail: tadija.kekic@fysik.lu.se

Introduction

Transcription factors (TFs) regulate gene expression through sequence-specific interactions with DNA. Palindromic DNA motifs are preferred binding sites for many homomeric TFs. These sequences constitute a self-symmetrical and highly constrained subset of sequence space. They are therefore sparsely represented in randomly generated libraries. Production of large, systematically defined palindromic libraries is most readily achieved via *in situ* synthesis. Existing high-throughput approaches rely on ensemble screening. They lack temporal and mechanistic resolution, and detailed kinetic insight of low-throughput single-molecule methods [1–3]. Beyond transcriptional regulation, systematic optimization of DNA–protein interaction is directly relevant to the protein engineering and design of artificial molecular systems, such as DNA-guided protein walkers, where control over binding kinetics is essential for directional motion and functionality [3–5].

Platform Concept, Methodology and Initial Results

Here, we introduce µPATHFINDER, a modular platform that integrates photolithographic *in situ* synthesis of high-density DNA microarrays with microfluidic control and Total Internal Reflection Fluorescence (TIRF) microscopy. We demonstrate the successful synthesis and functional validation of palindromic DNA libraries on a #1.5 coverslip, comprising 800,000 distinct sequences. Fluorescence scanning of patterned arrays reveals spatially resolved binding signals consistent with the underlying sequence design, confirming sequence-specific TF interactions. Initial experiments with transcription factors DtxR, MetJ, and TrpR show reproducible binding patterns across the array. We present initial screening results focusing on 18 nt palindromic sequences in the flanking regions of conserved core binding motifs for these TFs.

Conclusion

µPATHFINDER establishes a scalable framework for systematic investigation of transcription factor binding to palindromic DNA sequence space. Ongoing work focuses on optimization of microfluidics and TIRF microscopy to enable dynamic and single-molecule analysis on the same platform. This platform provides a foundation for advancing our understanding of gene regulation and supports future developments in synthetic biology, molecular diagnostics, and programmable biomolecular systems.

Acknowledgments

This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 951375)

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June 11-12, 2026, Uppsala University

Droplet microfluidics with image texture quantification for detection of rare antibiotic-resistant subpopulations from bloodstream infections

Sagar N. Agnihotri [a,b,c], Nikos Fatsis-Kavalopoulos [c], Emma Vikdahl [c], Jonas Windhager [d], Agustin A. Corbat [e], Dan I. Andersson [c*], and Maria Tenje [a,b*]

[a].Department of Materials Science and Engineering, Uppsala University, Uppsala, Sweden
[b] Science for Life Laboratory, Uppsala University, Uppsala, Sweden
[c] Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden
[d] Department of Information Technology and SciLifeLab BioImage Informatics Unit, Uppsala University, Uppsala, Sweden
[e] Instituto de Física de Buenos Aires, Departamento de Física, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina.
[*]Corresponding author: maria.tenje@angstrom.uu.se

Introduction

Heteroresistance (HR) is an antibiotic resistance phenotype characterized by the presence of rare resistant subpopulations (frequency ≈ 10^{-7} to 10^{-4}) within a main susceptible bacterial population[1]. During antibiotic exposure, these subpopulations can be enriched and cause treatment failure. Standard antibiotic susceptibility testing (AST) often fails to detect HR, and the current gold-standard population analysis profile (PAP) test is labor-intensive and time-consuming.

Theory and Experimental Procedure

To address this diagnostic challenge, we have developed a droplet microfluidics-based platform combined with a texture-based image analysis tool to detect rare resistant subpopulations with frequencies as low as 10^{-6}. The microfluidic chip consists of two main sections: (i) a droplet generation unit featuring a T-junction where an oil phase and an aqueous phase (containing bacteria in Mueller-Hinton broth mixed with antibiotics) are introduced to form discrete droplets (Fig.1), and (ii) an incubation chamber capable of housing approximately 2000–3500 droplets. In our previous work, we developed a droplet shrinkage–based method to detect HR in *E. coli* bloodstream isolates[2]. Although highly suitable for HR detection in *E. coli.* This approach depended on the specific growth medium composition and bacterial metabolism, making it less effective for Gram-positive species such as *S. aureus.*

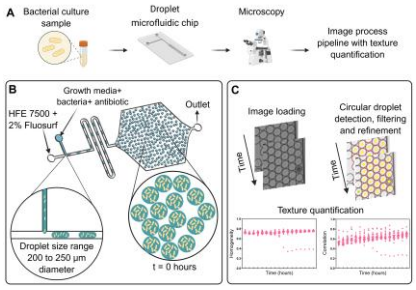

Figure 1: Schematic representation of the HR detection in Gram-negative and Gram-positive bacteria from bloodstream infection. **(A)** The main steps involved in the workflow from overnight bacterial culture to the image process pipeline, involving texture quantification. **(B)** Illustration of the microfluidic geometry for droplet generation and storage involving HFE-7500 with 2% Fluosurf as a continuous phase and growth medium with bacteria and the antibiotic of choice as the dispersed phase. **(C)** Main steps involving the texture quantification from the time-lapse image sequence. Each dot in a plot of homogeneity and correlation represents the value of the texture quantification of every droplet analyzed.

Results and Discussion

Our study demonstrates that integrating droplet-based microfluidics with quantitative image texture analysis provides a robust approach for detecting HR in bacteria isolated from bloodstream infections. Using this method, we successfully identified HR in *A. baumannii*, *K. pneumoniae*, *P. aeruginosa*, and *S. aureus*. The total time required for imaging varied depending on the bacterial species: 24 hours for Gram-negative bacteria and 48 hours for Gram-positive bacteria. Based on the current results, the emergence of antibiotic-resistant subpopulations was detectable as early as 12 hours for *A. baumannii*, 6–12 hours for *K. pneumoniae*, 12 hours for *P. aeruginosa*, and 15–30 hours for *S. aureus*.

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Resealable 3D printed perfusable microwells for organoid differentiation

Samuel Wulfert*[1,2], Patrick Sandoz[1,2], Fredrik J. Swartling[2,3], Maria Tenje[1,2]

[1] Department of Materials Science and Engineering, Uppsala University, Sweden
[2] Science for Life Laboratory, Uppsala University, Sweden
[3] Department of Immunology, Genetics and Pathology, Uppsala University, Sweden

*E-mail: samuel.wulfert@angstrom.uu.se

Introduction

3D cell cultures, such as spheroids and organoids, are becoming frequently used in medical research due to their superiority in modelling *in vivo* responses compared to 2D cultures in Petri dishes. However, current practices with maturing organoids in shaker plates or spinning bioreactors is labour intensive and has limited throughput as they require media exchange every few days [1]. To simplify the process, recent publications have presented perfusable culturing systems for organoids [2]. Current systems mostly lack the flexibility to easily load and unload organoids for in depth analysis during or after the experiment. Here, we present a design that can maintain an organoid in a microwell exposed to a flow of different profiles that can easily be opened and resealed using magnetic snap-fit seals. All the parts are 3D printed which offers manufacturing freedom with free-form design capabilities to generate systems that are compatible with multiplexing flow and generating chemical gradients. While 3D printing is already used for perfusable culture [3],[4] these systems are not constructed for reopening during experiments.

Experimental procedure

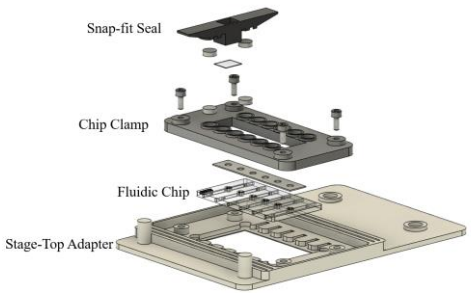

Figure 1 CAD drawing of the system developed at Uppsala University, Sweden. Only one of 6 snap-fit seals shown.

The design consists of three major parts: A fluidic chip that contains channels and microwells, an adapter with the foot-print of a culture well designed to fit standard holders of microscopes, and a chip clamp that the individual seals snap onto. An explosion view of the system is shown in Figure 1.
The parts are 3D printed using FDM (fused deposition molding, BambuLab X1C) and DLP (digital light processing, Asiga Max X27) with ABS (Acrylonitrile butadiene styrene, BambuLab) for the snap-fit seals and stage-top adapter, PPS-GF (Polyphenylene sulfide glasfiber filled, Fiberon) for the chip clamp, and methacrylate based biocompatible resin for the fluidic chip (Pro3dure GR-10). The gasket is cut out of PDMS using a cutter plotter.

Results and Discussion

First results using the device show that it is possible to open the microwells, load a brain organoid, and reseal it reliably without any leaks when the flow is started. Also, a laminar flow with two different fluids flowing besides each other can be established in the chips even when an organoid is present in the microwells.

Conclusion

Here, we present a 3D printed chip for organoid perfusion experiments with resealable wells. We demonstrate that the wells can be opened and closed multiple times. This opens the potential of the design for analysis during on-chip maturation experiments of organoids.

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SMILS '26
Swedish Microfluidics Network
June 11-12, 2026, Uppsala University

Imaging-based analysis of infiltrating immune cells in microtumour spheroids

Xinyang Dai[1], Maria Tenje[1,2], Patrick Sandoz[1,2]

[1] Department of Biomedical Engineering, Uppsala University, Uppsala, Sweden
[2] Science for Life Laboratory, Uppsala University, Uppsala, Sweden
*E-mail: daixinyang693@gmail.com

Introduction

Three-dimensional (3D) tumor models are increasingly used as more realistic alternatives to traditional two-dimensional (2D) cultures [1]. In 2D systems, cells experience non-physiological mechanical and nutrient conditions, making it difficult to recapitulate the tumor microenvironment. In contrast, 3D spheroids allow the formation of spatial gradients, including limited oxygen diffusion, nutrient depletion, and metabolite accumulation [2] all of which directly affect immune cell behavior, especially the ability of infiltrating cells to reach the tumor core and maintain function. γδ T lymphocytes, innate-like immune cells, show strong initial cytotoxicity, but their anti-tumor activity is often not sustained [3]. The underlying mechanisms remain unclear but may be linked to metabolic stress and its associated impact on mitochondrial function.

Theory and Experimental procedure

To explore this, human renal carcinoma A498 cells were used to form 3D spheroids in patterned microcavities, a platform that supports the development of spatial heterogeneity associated with Hypoxia-Inducible Factor 1-alpha (HIF-1α) expression [4]. Mitochondrial membrane potential (ΔΨm) of γδ T cells was assessed using a tetramethyl rhodamine derivative (TMRM) and monitored in real time by confocal microscopy. For volumetric imaging, optical clearing conditions were systematically optimized. Refractive index matching enhanced signal collection, though residual light scattering persisted in deeper regions. To improve reagent penetration throughout the full depth of the sample, a brief dehydration step was introduced prior to re-immersion in clearing solution.

Results and Discussion

A498 microtumor spheroids formed in the platform with uniform size and morphology, as assessed by a quality score. Microtumor spheroids remained viable over several days when assembled directly within the microcavities, as monitored by time-lapse microscopy. The optimized clearing protocol improved optical accessibility to the spheroid core, yielding more consistent fluorescence signals across imaging depths and reducing background scattering compared to standard approaches. Ultimately, this enabled full-depth 3D reconstruction of the microtumors.

Conclusion

This study provides a framework for linking spatial structure, metabolic stress, and immune cell function in 3D tumor spheroids. By improving optical accessibility through optimized clearing strategies, it becomes possible to monitor mitochondrial dynamics with higher reliability. Understanding how mitochondrial function constrains γδ T cell activity may offer new directions for enhancing T cell persistence through metabolic reprogramming.

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Analysis of label-free droplet images using open-source software assisted by supervised machine learning

Dániel Kácsor[1,*], Merili-Saar Abroi[1], Triini Olman[1], Simona Bartkova[1] and Ott Scheler[1]

[1] Department of Chemistry and Biotechnology, Tallinn University of Technology, Tallinn, Estonia
*E-mail: daniel.kacsor@taltech.ee

Introduction

We present image analysis pipelines developed in the open-source software CellProfiler™ capable of detection of microfluidic droplets and objects inside of droplets, such as microplastic particles, on label-free images. The pipelines do not require images with molecular labels, and integrate various supervised machine learning methods for improved detection quality.

Theory and Experimental procedure

We have used DIC images from a confocal microscope taken of microfluidic droplets with microplastic particles and bacteria inside of them. We have used CellProfiler™, CellProfiler Analyst™, ilastik, and Cellpose to detect and classify all objects of interest. We have assessed the accuracy, precision, and analysis time required for each approach. Figure 1 shows a schematic of the experimental setup and the image analysis pipelines.

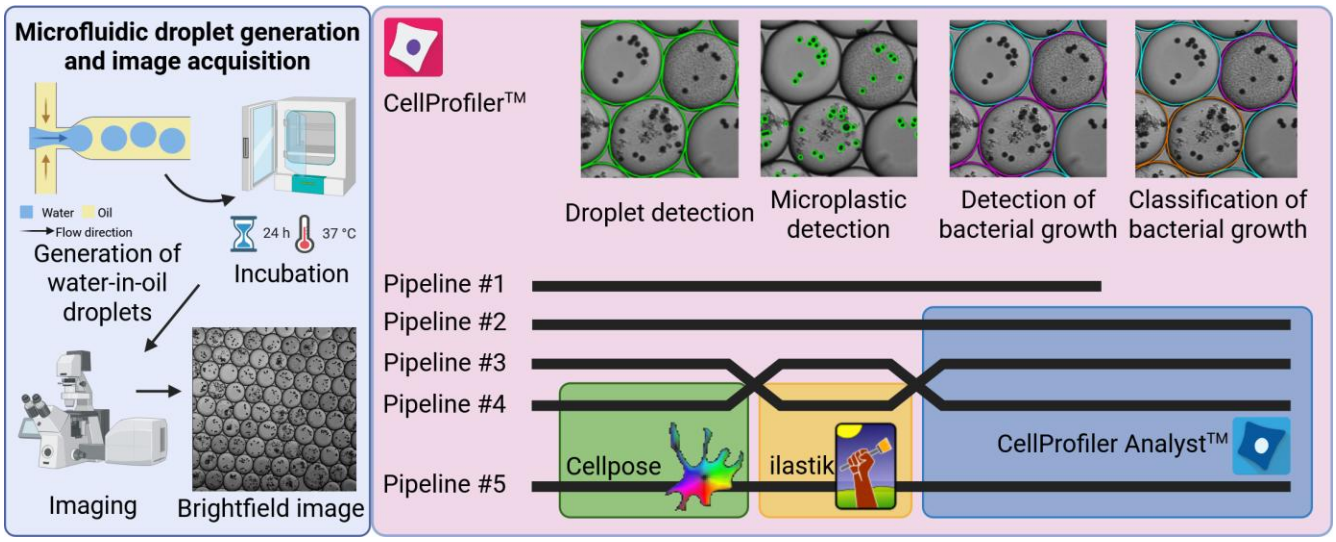

Figure 1: Schematic of experimental setup and image analysis steps of different pipelines.

Results and Discussion

The pipeline with the highest detection accuracy incorporates supervised machine learning methods into every step of the analysis. It can detect droplets at 100% accuracy, microplastic particles at 90% accuracy, the presence of bacterial growth at 100% accuracy, and is able to classify the type of bacterial growth at 95% accuracy.

Conclusion

We have developed image analysis pipelines for use in droplet microfluidics which do not rely on the use of molecular labels and are capable of different types of object detection and classification. We have assessed their performance in terms of quality, as well as computing load and resulting analysis time required for each approach. These pipelines should serve to make analysis of droplet microfluidic image data more accessible to all.

Swedish Microfluidics Network
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Single Extracellular Charge Characterization via Nanofluidic Fluorescence Microscopy

Elin Persson[1,*], Viktoria de Carvalho[1], Sriram KK[1], Elin K Esbjörner[1] and Fredrik Westerlund[1]

[1] Department of Life Sciences, Chalmers University of Technology, Göteborg, Sweden
*E-mail: perelin@chalmers.se

Introduction

Nanofluidic channels are versatile devices applicable to characterization of a plethora of biomolecules. While traditional uses include studies of DNA and DNA-protein interactions, such devices also hold great potential for studies of single nanoparticles, such as extracellular vesicles (EVs). EVs are nanosized membrane bound particles which are released into the extracellular space by all cell types and play a key role in cell-to-cell communication. During the last decades, EVs have gained a great deal of attention due to their involvement in normal cell function, as well as being mediators of several diseases. However, despite years of efforts, EV studies are still challenging due to large sample heterogeneity, complicated purification procedures, and a need for methods that can determine multiple characteristics on the single particle level. We here present a single EV characterization method based on a nanofluidic device coupled to fluorescence microscopy, where we characterize hydrodynamic radius, protein content and the often-overlooked characteristic surface charge, within the same device in a high throughput manner.

Experimental procedure

Synthetic liposomes with increasing negative charge were synthesized and used as a model system. The nanofluidic chip was attached to a specialized chip holder enabling both attachment of electrodes and tubing to apply a pressurized nitrogen flow. Particles were loaded onto the chip and their movement at either 15 V/cm or 30V/cm was detected via an in-house machine learning based single particle tracking algorithm. The method development included diluting liposomes in different ionic strengths to investigate electrokinetics of device, reference measurements using electrophoretic light scattering to create a calibration curve and establishing a protocol to determine size and charge of particles simultaneously. Next, EVs were obtained from HEK293T cells containing a genetically encoded fluorescent tag on the tetraspanins CD63, CD9, TSPAN2 or TSPAN3. The cultured media was purified using tangential flow filtration followed by either ion-exchange chromatography and/or ultrafiltration. Purified EVs were loaded onto the nanofluidic device and characterized for their charge, size and biomarker content, including staining with fluorescent carbohydrate binding lectins.

Results and Discussion

Synthetic liposomes of different charge were resolved as distinct populations on-chip, and their electrophoretic mobility could be determined. This mobility was further used to determine zeta potential on the single particle level. Subsequently, we show that decomposition of the total displacement along the channel can be divided into deterministic and stochastic components, to simultaneously determine size and charge on the single particle level, for particles of both homogenous and heterogenous size distributions. Single particle EV characterization revealed large internal charge distributions spanning approximately -30 to -60 mV. Additionally distinct charge-to-size relationships across subpopulations, defined by the four tetraspanins investigated, could be found. Lectin staining further demonstrated that charge-defined EV subpopulations exhibit distinct glycan signatures.

Conclusion

Together, our findings suggest that surface charge is a defining and previously underutilized property of EV heterogeneity. By enabling multi-parametric characterization of charge, size and biomarker content on the single particle level, this method opens the door to deeper understanding of how electrostatic interactions play a part in cell-to-cell communication of complex biological systems.

Acknowledgments

This project has received funding by the Knut and Alice Wallenberg Foundation. Cell-lines used in this project were a kind gift by the lab of Samir El Andaloussi at the Karolinska Institute, Stockholm, Sweden. Fabrication of nanofluidic devices was executed at the Chalmers Nanofabrication Laboratory MC2.

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Curvature-sensitive proteins on nanowire-supported lipid bilayers

Eliz Hafazismailolu[1, *], Julia Valderas Gutiérrez[1], Rubina Davtyan[1], Heiner Linke[1] and Fredrik Höök[2]

[1] NanoLund and Solid State Physics, Lund University, Lund, Sweden
[2] Nano and Biophysics, Chalmers University of Technology, Gothenburg, Sweden
*E-mail: eliz.hafazismailolu@fysik.lu.se

Introduction

A lipid bilayer prefers a flat configuration because this minimizes its bending energy. However, curvature is required for regulating organelle shape, vesicle trafficking, and processes such as membrane fusion, scission, and protein organization. The formation of curvature cannot be driven by thermal energy alone. Instead, the significant energy required to create complex membrane shapes is provided by proteins that interact with lipid bilayers to sense and generate membrane curvature [1][2][3]. This project aims to understand how the local membrane curvature affects the binding and diffusion of these proteins at the single-molecule level. We use nanowire-supported lipid bilayers to provide both convex and concave curvatures with radii on the 100 nm scale.

Theory and Experimental procedure

We use vertically aligned silicon nanowires. In addition to providing curved topology, they also enhance fluorescence of surface bound molecules in three ways. They increase the local electromagnetic field, and improve excitation of nearby fluorophores, enhance emission through additional electromagnetic modes, and guide emitted photons along their axis [4]. For the experiments a microfluidic device is made from PDMS using soft lithography, where a microchannel is formed to place the nanowire substrate. The channel is sealed with a glass slide and consists of one inlet and one outlet, allowing pressure-driven fluid flow. Following this, a negatively charged bilayer is formed on nanowires using vesicle rupture method. Once bilayer mobility is confirmed by fluorescence recovery after photobleaching (FRAP), the curvature sensitive protein EHD2 is introduced at increasing concentrations. EHD2 is known to form oligomeric scaffolds at the neck of caveolae. Using an epifluorescence microscope, several analysis are performed, including FRAP, z-stack imaging, and time-resolved analysis to study the binding and diffusion of this protein.

Results and Discussion

FRAP analysis confirmed the formation of a fluid supported lipid bilayer, with diffusion coefficients of 0.8 - 0.9 $\mu m^2/s$ and mobile fractions up to 0.9. Protein EHD2 showed low mobility (mobile fraction 0.03 – 0.1), decreasing with increasing concentration. Z-stack imaging on negatively charged supported lipid bilayers showed preferential binding of EHD2 at convex regions of the nanowire in the presence of ATP, while in the absence of ATP fluorescence intensity was highest at the center of nanowire. A potential binding preference of EHD2 with ATP is also observed on neutral charged bilayers, but this requires further investigation. Additionally, the control protein, streptavidin, known to be non-curvature sensing, did not show any curvature preference.

Conclusion

The results show that the protein EHD2 shows curvature preference under specific experimental conditions. However, further studies are required to understand its low mobility. In addition, the nanowire platform can be used to study whether other curvature sensitive proteins show specific localization behaviour.

Acknowledgments

This research is funded by the Knut and Alice Wallenberg Foundation (Wallenberg Scholar).

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Patient-specific brain phantom in PDMS

Daniel P.G. Nilsson[1], Madelene Holmgren[2,3], Petter Holmlund[4], Anders Wåhlin[2,4,5], Anders Eklund[2,5], Tobias Dahlberg[1], Krister Wiklund[1], and Magnus Andersson[1,6,*]

[1] Department of Physics, Umeå University, 901 87, Sweden
[2] Department of Diagnostics and Intervention, Biomedical Engineering and Radiation Physics, Umeå University, 901 87, Sweden
[3] Department of Clinical Science, Neurosciences, Umeå University, 901 87, Sweden
[4] Department of Applied Physics and Electronics, Umeå University, 901 87, Sweden
[5] Umeå Center for Functional Brain Imaging (UFBI), Umeå University, 901 87, Sweden
[6] Umeå Center for Microbial Research (UCMR), Umeå University, 901 87, Sweden
[*] magnus.andersson@umu.se

Introduction

Creating 3D phantom models of patient's arteries have many roles in the medical field, from education to clinical research. However, current phantom models are generic, expensive, and time-consuming to fabricate. Thus, there is a need for a cost- and time-efficient pipeline from medical imaging to patient-specific models.

Method

In this work, we propose a method for creating full-scale phantom models from computed tomography angiography images (CTA) [1]. For this, intracranial CTA scans from a previous study on stroke patients are used to generate a 0.3 mm resolution 3D model of the arterial tree called the circle of Willis [2]. This model is 3D printed at a resolution of 47 µm using a $200 SLA printer (*Photon S*, Anycubic) and 4.4 mL water-soluble resin (*IM-HT-WS*, 3Dresyns), for a material cost of $2. The support pillars that holds the arteries while printing are removed before the part is cleaned and cured under UV light for 15 min. The resulting sacrificial mold is then placed in a 10 x 6 x 4 cm Plexiglas container filled with 250 g premixed PDMS silicone (*SYLGARD 184*, Dow Corning) and cured for 2 h at 80°C. Finally, the PDMS block is removed from the outer container and the sacrificial arteries are dissolved using an ultrasonic cleaner and deionized water. The resulting phantom model can be seen in *Fig. 1* and consists of a complex network of Ø1-5 mm wide channels branching out in all directions inside of a flexible, transparent, and biocompatible silicone block.

Results and Discussion

We analyzed the phantom model with magnetic resonance imaging (MRI) and compared it with the patient data. The results show good agreement and smooth surfaces for the arteries, with an average cross-sectional error of -9.8% for the whole model. This method can fabricate a patient-specific phantom model in the lab, with less than 2 hours of total labor time and at a low cost.

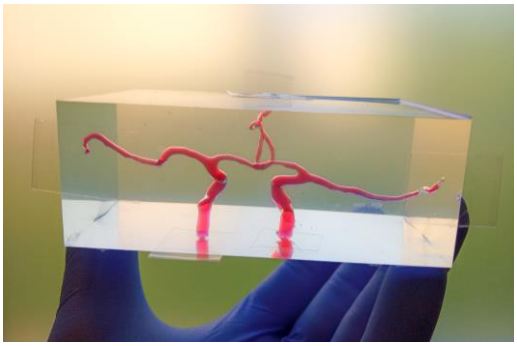

Fig. 1 *Patient-specific phantom model of the anterior cerebral network molded in PDMS. The arterial channels are filled with water and red food dye, with tape covering the ports.*

Conclusion

Using water-soluble resin with an SLA printer has provided an easy way to make sacrificial molds for patient-specific phantom models suitable for education and training in MRI/CT imaging or ultrasound measurements. Additionally, these phantom models can also help with dynamic flow simulations in neuroscience.

Acknowledgments

Thanks to supporting staff and volunteering patients at the University Hospital of Umeå, Sweden. Financially supported by the Swedish Foundation for Strategic Research; Vetenskapsrådet (2019-04016). Swedish Ethical Review Authority (2019-05909) approved the study and consent was obtained from the participant.

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Determining the effect of oscillating flow and flow rate on nutrient transport into 3D cellular constructs

Tora Godow Bratt[1,2,*], Neeraj Katiyar[1,2], Willy V. Bonneuil[3], Shervin Bagheri[3], Maria Tenje[1,2]

[1] Dept. of Materials Science and Engineering, Uppsala University, Uppsala, Sweden
[2] Science for Life Laboratory, Uppsala University, Uppsala, Sweden
[3] Dept. of Engineering Mechanics, KTH Royal Institute of Technology, Stockholm, Sweden
*E-mail: tora.godow_bratt@angstrom.uu.se

Introduction

Three-dimensional (3D) cellular constructs provide clear advantages over two-dimensional (2D) cell cultures, as they more accurately mimic the structural and functional characteristics of the *in vivo* environment, resulting in a widespread use as an alternative to animal models [1]. However, a significant limitation of these 3D constructs is the formation of a necrotic core as a consequence of the restricted nutrient diffusion into the densely packed cellular structure. Previous theoretical work has shown that the application of an external unidirectional flow enhances nutrient transport and reduces cell death on the inflow side of the construct [2]. Building on these findings, this study explores the effect of an oscillating flow on nutrient distribution and necrotic core formation inside a 3D cellular construct, hypothesising that a bidirectional perfusion could improve cell viability symmetrically throughout the entire construct.

Experimental procedure

Experiments were performed using a custom-designed PDMS-based microfluidic device comprising two perpendicular channels with a two-photon printed barrier at their intersection for immobilisation of the 3D cellular construct, Figure 1. The first channel is used for loading and entrapment of the construct, while the other is used for application of the oscillating flow on the construct. The flow is managed using a syringe pump system connected to the microfluidic device programmed with an oscillatory flow pattern. To evaluate the size of the necrotic core over time, 3D constructs are planned to be embedded and frozen for cryosectioning on-chip, followed by staining for dead cells, allowing 3D visualisation within the construct.

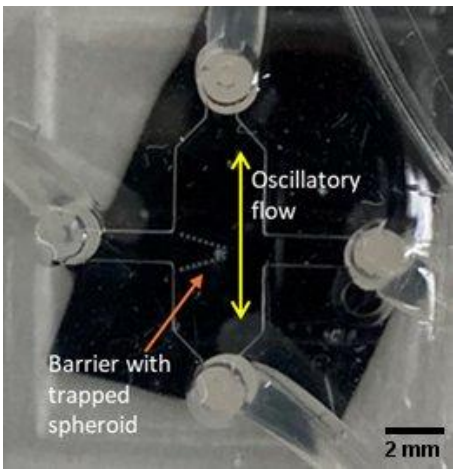

Figure 1. Top view of the microfluidic device with a spheroid trapped by the barrier (orange arrow) and the oscillatory flow direction indicated (yellow arrow).

Results and Discussion

Preliminary results demonstrate the successful loading and entrapment of human lung fibroblast spheroids by the printed barrier, verifying the functional performance of the microfluidic device. The barrier effectively secures the spheroids in place, even at higher flow rates, while still allowing access to nutrients, thereby maintaining viability conditions. An oscillatory flow of 50 µL/min has been successfully applied to a spheroid in the device over a period of 48 hours, confirming the practicality of the platform for studying necrotic core formation. Ongoing experiments aim to assess necrotic core development under various flow profiles and over longer time frames.

Conclusion

This microfluidic platform enables realistic 3D cell culturing under controlled oscillatory flow conditions, making it ideal for studying nutrient transport, necrotic core formation, and tissue viability. Further results will conclude the platform's potential of providing a simple method for decreasing cell death while studying 3D cellular constructs.

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Smart Fluidic Interconnects: Surface Energy-Guided In-Air Bridges for Modular Microphysiological Systems

Souvik Paul[1*], Thomas winkler[1]

[1] Department of Micro and Nano systems, KTH, Stockholm, Sweden
*E-mail: Souvikpa@kth.se

Introduction

Modular microfluidic technology is essential for moving beyond the limitations of monolithic microfluidic devices, enabling rapid reconfiguration and flexibility [1]. However, standard physical connectors introduce significant dead volume and mechanical displacement. Recent efforts to create gasketless interfaces using superhydrophobic surfaces for vertical stacking [2] are effective, while vertical interfaces often require mechanical clamping and hinder structural resolution. Here, we present an in-line, lateral modular integration strategy using surface energy-guided in-air liquid bridges to enable the hot-swapping with zero mechanical and the same time minimizing the dead volumes less than 0.5 µl.

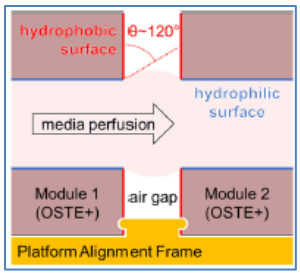

Figure 1 A schematic of the in air interconnect transporting media from one module chip to another

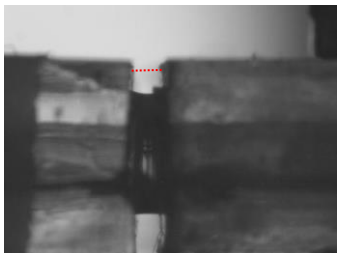

Figure 2 A microscopic image of the in air liquid bridge that shows a gap of 110 µm.

Theory and Experimental procedure

OSTE+ modules were fabricated, and surface patterning was achieved by Hydrophilization *($\theta < 30^0$)* and Hydrophobization *($\theta > 110^0$)* by grafting HEMA and FDMA respectively. An in-house experimental setup was developed to validate liquid transfer across a lateral air gap between two in-line modules. The perfusion stability was characterized using a syringe pump at flow rates ranging from 0.01 to 0.5 µl/min. This in-line setup allows for zero-displacement module exchange while monitoring the leakage as a function of both flow rate and gap distance.

Results and Discussion

We have successfully demonstrated the fluid transfer across a 100µm gap without any mechanical contact between two modules, using a flow rate ranging between .01-.5 µl/min, we achieved a leakage percentage goes below 10%. The in-air bridge architecture reduces the dead volume at a significant rate.

Conclusion

We have developed a robust, virtual fluidic interface for modular MPS that eliminates the need for physical connectors. By leveraging the surface energy contrast between HEMA and FDMA on OSTE+ substrates, we achieved zero-displacement coupling with minimal dead volume.

Acknowledgments

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Swedish Microfluidics Network
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Vascularized Tumor-on-chip
Functional vessel network remodeling by Tumor-on-chip

Andrea Dalmao-Fernandez [1], Ludivine Delon[1], Thea Josefine Wergeland[1], Mathias Busek[1], Yuliia Boichuk[1], Jonas Aakre Wik[1], Alexey Golovin[1], Justyna Stokowiec[1], Stefan Krauss[1]

[1] Hybrid Technology Hub Centre of Excellence, Faculty of Medicine, University of Oslo, Norway
*E-mail: andrdalm@uio.no

Introduction

Tumor vasculature is a key driver of cancer progression and metastasis, functioning not only in nutrient delivery but also as a regulator of tumor cell behavior. Cancer cells actively remodel surrounding blood vessels through pro-angiogenic signaling, leading to abnormal, leaky, and heterogeneous vascular networks. These changes promote epithelial-to-mesenchymal transition (EMT) and enhance invasive potential [1]. To better understand these interactions, advanced in vitro models that recapitulate tumor–vascular crosstalk are needed.

Theory and Experimental procedure

We developed a vascularized tumor-on-chip model using a pumpless recirculating organ-on-a-chip (rOoC) platform [2]. Human umbilical vein endothelial cells (HUVECs) were embedded in a fibrin matrix to form a perfusable vascular network under controlled flow conditions. A 1mm punch was made into the gel and aggressive melanoma spheroids (A375MA2) were introduced into the system to study tumor–vascular interactions. Vascular remodeling, integrity, and perfusability were assessed using fluorescent dextran and microspheres, alongside immunostaining for endothelial markers such as CD31 and VE-cadherin. Imaging and orthogonal views by IMARIS v9 software enabled spatial analysis of vessel–tumor interactions.

Results and Discussion

The model successfully generated a functional vascular network that remained perfusable after co-culture with tumor spheroids. Tumor cells induced significant vascular remodeling, including reorganization of vessel architecture and degradation of the surrounding extracellular matrix. Despite matrix disruption, physical and functional connections between endothelial cells and tumor cells were maintained. Evidence of cancer cell invasion into the vasculature (intravasation) was observed, along with dynamic changes in vessel permeability and structure.

Conclusion

This vascularized tumor-on-chip platform provides a physiologically relevant system to study mechanisms of metastasis, including EMT and intravasation. The model shows potential for evaluating tumor–vascular interactions and therapeutic strategies.

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Vascularization-on-chip
Functional vessel network on-chip for disease modeling and drug testing

Andrea Dalmao-Fernandez [1*], Ludivine Delon[1*], Thea Josefine Wergeland[1], Mathias Busek[1], Yuliia Boichuk[1], Jonas Aakre Wik[1], Alexey Golovin[1], Justyna Stokowiec[1], Stefan Krauss[1]
*Shared authorship

[1] Hybrid Technology Hub Centre of Excellence, Faculty of Medicine, University of Oslo, Norway
*E-mail: andrdalm@uio.no

Introduction

Vascularization is a critical component of advanced microphysiological systems (MPS), particularly in cancer research, where blood vessels regulate nutrient delivery, immune cell trafficking, and drug distribution [1]. Conventional 2D and static 3D culture models fail to capture these dynamic processes, limiting their predictive power. To address this gap, we developed a pumpless recirculating organ-on-a-chip (rOoC) platform [2] that enables physiologically relevant flow conditions and supports the formation of functional vascular networks.

Theory and Experimental procedure

The rOoC system consists of a microfluidic open chamber designed to generate unidirectional flow without external pumps. Human umbilical vein endothelial cells (HUVECs) were embedded in a fibrin matrix and cultured under controlled flow conditions with angiogenic factors to promote vascular network formation. Perfusability and vessel integrity were assessed using fluorescent dextran and microspheres (200nm-1µm). Imaging techniques, including fluorescence microscopy and 3D reconstruction, were used to evaluate vascular morphology and particle flow within the network.

Results and Discussion

After eight days of culture, a perfusable and interconnected vascular network was successfully established within the chip. The vessels exhibited barrier functionality, allowing controlled transport of fluorescent particles and dextran. Flow dynamics supported endothelial organization and lumen formation, while particle tracking confirmed active circulation within the network. These findings demonstrate that the rOoC platform effectively mimics key aspects of vascular transport and microenvironmental regulation.

Conclusion

The pumpless rOoC platform provides a robust and physiologically relevant model for vascularized tissue systems. By enabling functional vasculature and controlled flow, this system offers significant potential for studying tumor biology, immune interactions, and drug delivery. Ultimately, it represents a promising tool for improving preclinical testing and advancing precision medicine.

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Modeling flow and diffusion across the blood-brain barrier in a novel neurovascular unit microfluidic model

Ekaterina O. Kasyanova[1,2]*, Ana P. Spencer[1,2,3,4], John S. Cognetti[5], and Anna Herland[1,2,3,4]

[1] Karolinska Institutet, Stockholm, Sweden
[2] SciLifeLab - Science for Life Laboratory, Stockholm, Sweden
[3] KTH Royal Institute of Technology, Stockholm, Sweden
[4] AIMES - Center for the Advancement of Integrated Medical and Engineering Sciences at Karolinska Institutet and KTH Royal Institute of Technology, Stockholm, Sweden
[5] University of Rochester, Rochester, USA
*E-mail: ekaterina.kasyanova@scilifelab.se

Introduction

Neurological disorders affect 276 million people worldwide, and pharmaceutical development is challenging due to the restrictive nature of the blood-brain barrier (BBB) [1]. Current preclinical animal and in vitro models lack physiologically relevant complexity and do not facilitate full analysis. Our project focuses on developing a model of a human neurovascular unit (NVU) on a two-layer microfluidic chip (NVU-on-chip), which integrates the BBB and relevant brain cell compartments. The key design advancement of the model is the optimised microchambers and intercompartment microchannels, allowing for adequate flow across the entire model, and for necessary cell numbers and volumes for various real-time and endpoint analyses.

Theory and Experimental procedure

The NVU-on-chip has been designed in Onshape (PTC), and laminar flow was modelled in COMSOL Multiphysics (COMSOL AB) (Figure 1). Velocity (m/s), shear rate (1/s) and pressure (Pa) were visualised in a stationary study. Diffusion of cascade blue across a porous membrane between the endothelial and astrocyte compartments was visualised as concentration (mol/m^3) in a time-dependent study as a representation of therapeutic administration.

Results and Discussion

The new microfluidic platform was found to have adequate flow through all compartments and microchannels (Figure 2). Dimensions of the cell compartments, flow velocity and porosity of the membrane are currently being optimised to ensure enough shear stress for adequate functionality of the endothelial chamber and for sufficient diffusion of cascade blue.

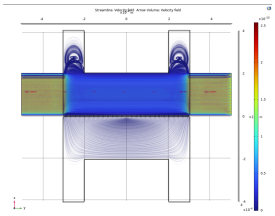

Figure 1. COMSOL modeling of flow velocity in the microfluidic platform, top view.

Conclusion

In summary, we present a flow model of an optimised physiologically relevant NVU-on-chip allowing for in vitro assessment of therapeutic crossing of the BBB. This model further contributes to the development of a platform for real-time analysis of therapeutic effect on the NVU compartments, hence enhancing preclinical testing of neurological disorder therapeutics.

Acknowledgments

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Microplastic influence on bacterial co-cultures in droplets

Triini Olman[1,*], Saari Anete Loog[1], Merili Saar-Abroi[1], Daniel Kacsor[1], Robin Veere[1], Simona Bartkova[1], Ott Scheler[1]

[1] Department of Chemistry and Biotechnology, Tallinn University of Technology, Tallinn, Estonia
*E-mail: triini.olman@taltech.ee

Introduction

Antibiotic resistance is worsening, partly due to microplastics that promote bacterial aggregation and biofilm formation [1], [2]. Traditional methods miss these microscale interactions [3]. Using droplet microfluidics, we isolate bacteria with microplastics to study them at the single-cell level, revealing species- and antibiotic-dependent effects on aggregation and susceptibility.

Experimental procedure

Monodisperse nanoliter-scale droplets were generated with a flow-focusing microfluidic chip, co-encapsulating *E. coli*, *S. aureus*, different antibiotic concentrations, and 10 µm polystyrene microplastic beads. After overnight incubation at 37ºC, droplets were imaged using confocal microscopy and analyzed with image-processing pipelines in CellProfiler™ and CellProfiler Analyst™ to quantify bacterial growth and aggregation.

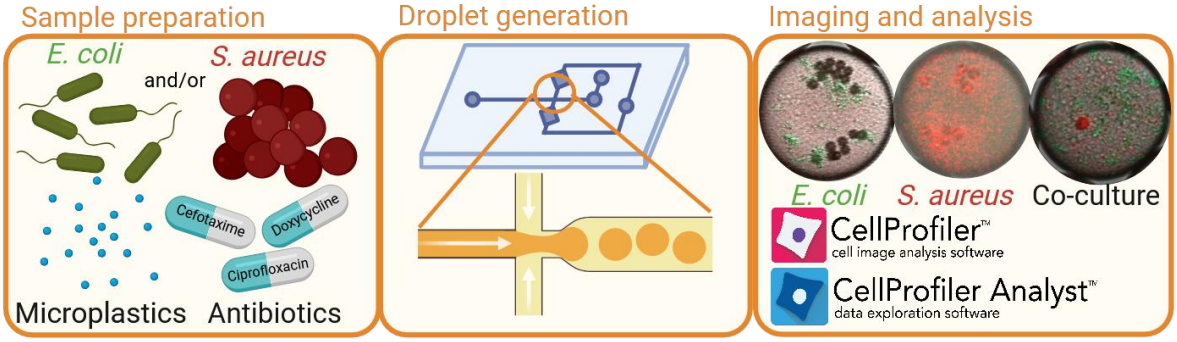

Figure 1. Experimental procedure.

Results and Discussion

Results show increased aggregation in *S. aureus* with microplastics under ciprofloxacin, while *E. coli* shows variable aggregation and antibiotic-dependent susceptibility. Similar effects in co-cultures reveal interspecies interactions and competition, and give insight into how microbial communities respond to the presence of microplastics and antibiotics.

Conclusion

Microplastics alter bacterial aggregation behavior and influence antibiotic susceptibility. Droplet microfluidics provides a platform to isolate and quantify these interactions at the single-cell level, revealing effects that are not observable in bulk systems. This approach improves understanding of how microplastics contribute to microbial behavior and antibiotic response.

Acknowledgments

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Streaming by Design: Sharp-Edge Acoustofluidic Micromixing

Zahra Ghorbani[1,*], Morteza Bayareh[2] and Per Augustsson[1]

[1] Department of Biomedical Engineering, Lund University, Lund, Sweden
[2] Department of Mechanical Engineering, Shahrekord University, Shahrekord, Iran
*E-mail: zahra.ghorbani@bme.lth.se

Introduction

Microfluidic micromixers are essential for achieving rapid and homogeneous contact between reagents in lab-on-a-chip systems, chemical analysis, biomedical diagnostics, and drug-delivery platforms; however, because flows at the microscale are typically laminar, passive mixing based mainly on molecular diffusion is often too slow for practical applications [1]. Active micromixers overcome this limitation by applying external fields to disturb the flow, and acoustic actuation is particularly attractive because acoustic streaming can generate steady vortical motion near oscillating boundaries without invasive components [2]. In sharp-edge-based acoustic micromixers, vibrating sharp structures produce localized streaming vortices that enhance transverse transport and improve mixing at low Reynolds numbers [3].

In the present work, an acoustic sharp-edge micromixer was investigated in a short-format conference study by combining a numerical framework with experimental visualization of acoustically induced flow and mixing. The mixing performance was shown to be strongly governed by operating and geometrical parameters, including displacement amplitude, background flow velocity, driving frequency, channel width, sharp-edge height, tip angle, curvature radius, number of edges, and edge spacing. Improved mixing efficiency was generally obtained at higher displacement amplitudes and driving frequencies, with taller and sharper edges, lower background flow velocities, and smaller channel widths. The experimental observations further confirmed that acoustic excitation induced vortical streaming around the sharp edges and substantially enhanced the mixing process, thereby supporting the numerical prediction that acoustic streaming is the dominant mechanism responsible for the improved mixing efficiency.

Theory and Experimental\Numerical procedure

The micromixer was modeled as a two-dimensional sharp-edge microchannel in which the acoustic response of a compressible Newtonian liquid was described using generalized Lagrangian mean (GLM) theory and perturbation analysis [3]. The zeroth-order solution represented the pressure-driven background flow, the first-order solution described the harmonic acoustic field, and the time-averaged second-order velocity was considered as the acoustic streaming field. Governing equations for a laminar viscous compressible fluid are continuity and momentum equations [4]:

$$\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho \boldsymbol{u}) = 0 \tag{1}$$

$$\rho \frac{\partial \boldsymbol{u}}{\partial t} + \rho(\boldsymbol{u}.\nabla)\boldsymbol{u} = -\nabla p + \mu \nabla^2 \boldsymbol{u} + \left(\mu_b + \frac{1}{3}\mu\right)\nabla\nabla.\boldsymbol{u} \tag{2}$$

where ρ is the density and **u** is the velocity. μ and μ_b are dynamic viscosity and bulk viscosity, respectively, and p is the pressure. A linear relation for p and ρ is assumed to define the liquid motion:

$$p = c_0^2 \rho \tag{3}$$

where c_0 is the sound speed in the liquid at rest. The liquid motion in acoustic field-driven micromixers is characterized by Eqs. 1–3 and appropriate boundary conditions. The equation of convection–diffusion transport is:

$$\frac{\partial C}{\partial t} + \nabla \cdot (C\boldsymbol{u}) = D\nabla^2 C \tag{4}$$

This velocity field was coupled with the convection-diffusion equation, where C and D denote the species concentration and diffusion coefficient, respectively, to predict the mixing profile. A nonzero velocity was imposed at both inlets, no-slip conditions were applied on the channel walls and sharp edges, and pressure/traction conditions were used at the outlet. The numerical simulations are performed using COMSOL Multiphysics 5.6 software, which is based on the finite element method. Table 1 shows the values of the parameters for water used in the numerical simulations.

For experimental validation, a microchannel with the same two-dimensional geometry was tested using two syringe pumps operated at an inlet velocity of 500 μm/s for each stream. Distilled water was introduced through one inlet, whereas a 10:1 mixture of distilled water and ink was supplied through the other. Acoustic actuation was applied at 5.5 kHz and 20 V. Before excitation, the two streams remained nearly separated and a clear interface was observed. After the acoustic field was switched on, vortical streaming was generated between the sharp edges and rapid mixing was achieved within approximately 5 s. When the acoustic field was turned off, the interface was

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re-formed, confirming that the mixing enhancement was acoustically induced. Additional particle tests with radii of 2, 6, and 10 μm showed that the particles followed the streaming paths and did not measurably change the mixing length or mixing time.

Table 1 Applied parameters in the model at T = 25 ◦C

Parameters	**Value**
Driving frequency, f	5.5 kHz
Amplitude of displacement, d_0	1 μm
Background flow velocity, v_{in}	5×10^{-4} ms^{-1}
Density, ρ_0	997 kg m^{-3}
Shear viscosity, μ	0.890 mPa s
Bulk viscosity, μ_b	2.47 mPa s
Speed of sound (in water), C_0	1497 ms^{-1}
Diffusion coefficient, D	4×10^{-10} m^{2}s^{-1}
Compressibility, κ_0	4.48×10^{-10} Pa^{-1}
Angle of tips, α	15 °

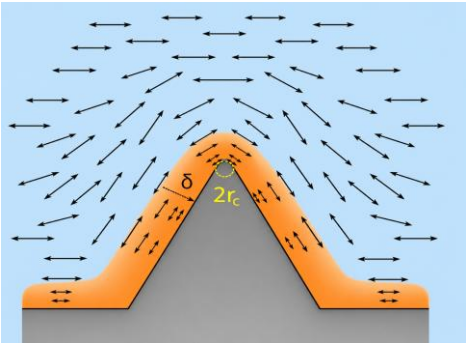

Figure 2 The mechanisms of acoustic streaming generated by a sharp edge.

Results and Discussion

The numerical and experimental results confirm that mixing in the sharp-edge micromixer is governed primarily by acoustic streaming generated near the oscillating edge tips. Without acoustic excitation, the two fluid streams remain largely separated and mixing is limited by molecular diffusion, whereas actuation produces vortical streaming that disturbs the interface and enhances transverse transport. Numerically, the mixing efficiency was found to depend strongly on the balance between acoustic streaming and the background flow. At lower inlet velocity, the longer residence time allowed the acoustic vortices to dominate, giving 99.98% mixing efficiency at $v_{in} = 500\ \mu m/s$, while the efficiency decreased to 47.38% at $v_{in} = 2000\ \mu m/s$. Increasing the displacement amplitude and driving frequency strengthened the second-order streaming velocity and improved concentration uniformity; for example, the outlet mixing efficiency increased from 71.98% to 90.70% when the frequency was raised from 4.75 to 5.75 kHz.

The geometrical parameters further controlled the strength and spatial development of the vortices. Taller sharp edges and narrower channels increased the interaction between the streaming flow and the main flow, with the mixing efficiency increasing from 42.52% to 99.98% as the edge height increased from 150 to 350 μm. Sharper edges were also more effective: increasing the tip angle from 6° to 70° reduced the mixing efficiency from 98.34% to 21.12%, and larger tip radii weakened the second-order velocity. The edge number and spacing affected vortex interaction, as densely packed structures restricted vortex growth, while fewer and properly spaced edges produced stronger disturbance. Experimentally, acoustic actuation at f=5.5 kHz and 20 V rapidly disrupted the dyed/clear-water interface and produced nearly complete mixing within about 5 s; when the acoustic field was switched off, the interface reappeared. Particle tests using radii of 2, 6, and 10 μm showed that particles mainly followed the streaming loops and did not improve the mixing time or length. Overall, the results demonstrate that efficient mixing is achieved when strong, well-developed acoustic vortices are generated close to the fluid interface through appropriate actuation conditions and sharp-edge geometry.

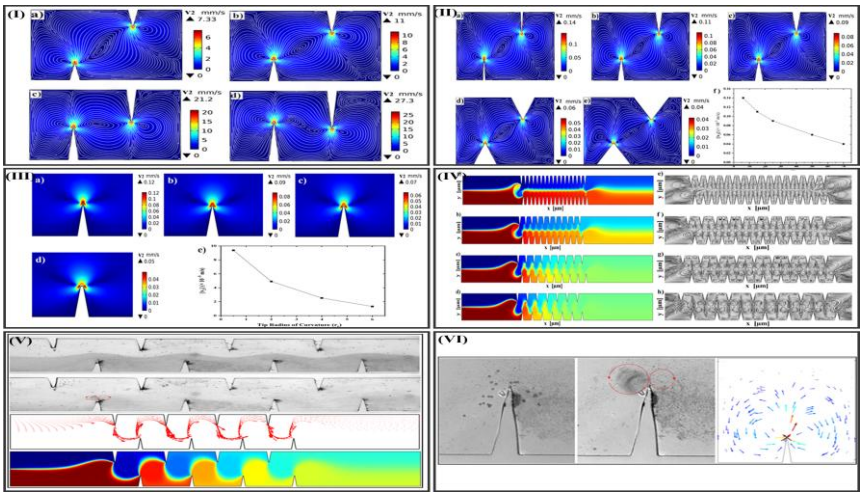

Figure 2 Numerical and experimental characterization of sharp-edge acoustic mixing: (I) effect of sharp-edge height, (II) tip angle, (III) tip radius of curvature, and (IV) number/spacing of sharp edges on acoustic streaming, second-order velocity, and concentration fields. Experimental and numerical particle-tracking results for 6 μm particles are also shown in (V) the full micromixer and (VI) around a single sharp edge, confirming particle motion along the acoustic-streaming vortices.

Conclusion

Acoustic streaming generated by oscillating sharp-edge structures was shown to be the dominant mechanism responsible for rapid mixing in the micromixer. The numerical results demonstrated that mixing efficiency is enhanced by stronger acoustic actuation, lower background flow velocity, taller and sharper edges, smaller channel width, and optimized edge spacing. Experimental observations supported the numerical predictions, showing rapid disruption of the fluid interface under acoustic excitation and re-formation of the interface after the acoustic field was switched off. Overall, the results confirm that properly designed sharp-edge acoustofluidic micromixers provide an effective strategy for fast and homogeneous mixing in lab-on-a-chip applications.

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Investigation of thermoacoustic effects in acoustofluidic trapping

Gianmaria Viciconte[1,*], David Van Assche[1], Ola Jakobsson[1] and Per Augustsson[1,*]

[1] Department of Biomedical Engineering, Lund University, Ole Römers väg 3, 22363 Lund, Sweden
*E-mail: gianmaria.viciconte@bme.lth.se, per.augustsson@bme.lth.se

Introduction

Acoustofluidic trapping is a contactless manipulation technique that involves the use of acoustic waves, usually generated by piezoelectric transducers, inside micro-channels to trap and hold particles in a liquid medium. Despite the use of trapping devices in a wide variety of biomedical and microfabrication applications [1], certain thermoacoustic phenomena observed during acoustic trapping remain poorly understood. For this reason, a rigorous experimental investigation is necessary for an adequate comprehension of acoustic and fluid dynamic phenomenology and to ensure further optimization of trapping devices.

Theory and Experimental Procedure

Several effects are involved in acoustic trapping. Apart from the acoustic radiation forces acting directly on suspended particles, acoustically generated streaming is also present. The acoustic streaming and vortices, generally widely mentioned and studied in literature, lie on a plane perpendicular to the transducer plane [2]. However, experimental observations in microfluidic devices have also reported streaming and vortex structures on a plane parallel to the emitting surface of the piezoelectric transducer. Our study focuses on this streaming, the origin of which is still a matter of debate, with some authors attributing it to thermoacoustic effects and others to acoustic ones [3, 4].
The experimental setup consists of a single-node trapping device placed on a Peltier element for controlling the temperature (*Fig. 1*).

Results and Discussion

The mentioned phenomena are investigated through advanced microscopic imaging techniques: defocusing-based particle tracking, astigmatic imaging, and fluorescence microscopy. The image in Figure 1, acquired with fluorescence microscopy, clearly shows four vortex formations on a plane parallel to the radiating surface of the transducer. Through modelling and experiments, we can show that the four streaming rolls associated with acoustic trapping are related to thermoacoustic streaming, and that we can predictably control this effect by controlling the temperature field in the channel, generated by the piezoceramic transducer.

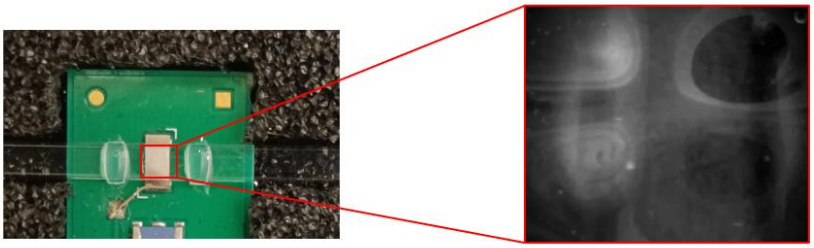

Figure 1. (a) Single-node trapping. (b) Image of the streaming vortices present on a plane parallel to the emitting surface of the piezoelectric transducer.

Conclusion

Our comprehensive investigation of the fluid dynamic structures associated with acoustic trapping clarifies the impact of the thermal and acoustic driving forces on the phenomenon. This new knowledge will be essential for a better understanding and future optimization of trapping devices.

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Standardisation of hybrid acoustochromatography for nanoparticle isolation

Jing Zhang[1], Monika Rosa Fürnsinn[1], Michael Gerlt[2], and Thomas Laurell[1]*

[1] Department of Biomedical Engineering, Faculty of Engineering (LTH), Lund University, Lund, Sweden
[2] Institute for Molecular Systems Engineering and Advanced Materials (IMSEAM), Heidelberg University, Heidelberg, Germany
*Corresponding author: thomas.laurell@bme.lth.se

Introduction

The diagnostic potential of extracellular vesicles (EVs) has been increasingly recognised over the last years due to their composition and cargo, including proteins, nucleic acids and metabolites, that directly reflects the state of their parent cell [1]. However, EVs are highly heterogeneous regarding size, cargo, function, and molecular composition, which makes targeted isolation challenging [2]. Next to their biological complexity, EV analysis from low volume of clinical samples remains difficult due to the trade-off between processing throughput, selectivity, recovery efficiency, and compatibility with downstream proteomics workflows [3]. Conventional EV isolation methods such as ultracentrifugation, size exclusion chromatography, and affinity-based capture often suffer from long processing times, limited scalability, sample loss, or biased EV subpopulation recovery [4]. In contrast, our acoustochromatography method provides gentle and label-free purification of nanoscale vesicles in an experimental time of 5-10 minutes. [5]. Here we present a polymer-based acousto-chromatography platform for nanoparticle isolation, aimed at selectively isolating EVs from complex biological samples for biomarker detection and low-volume proteomic sample preparation. The system integrates acoustic particle manipulation within a packed seed particle column, building upon previously demonstrated acoustochromatography systems [5]. To further improve the efficiency and robustness of the platform, we have focused on process automation through Python-based control and monitoring, ensuring analytical repeatability, standardized operation, and experimental transparency throughout each run.

Theory and Experimental procedure

As part of the standardized process flow of the system, three consecutive experimental runs and corresponding control measurements (bypass configuration) were conducted to assess run-to-run variability. The PBS-containing syringe was operated at a flow rate of 100 µL/min, with the flow regime continuously monitored using a flow sensor (FLUIGENT). The sample syringe filled a 10 µL sample loop with 200 nm fluorescent polystyrene particles, which was injected via an automated six-port valve, directing the sample either through the microfluidic chip or the bypass line. The chamber was packed with 100 µm polystyrene seed beads in an ultrasonic bath to maximize packing density. Acoustic trapping was achieved using a 10 × 10 × 0.4 mm piezoelectric transducer operated at its thickness resonance frequency of 2.05 MHz. The transducer was driven at 2 V via an AnalogDiscovery3 and amplified through a bridged amplifier, resulting in an output power of approximately 900 mW. Particle release was induced at 181 kHz (width resonance) using a 3 V input, corresponding to 1 W output power, with a burst duty cycle of 50%. Nanoparticle recovery was quantified by fluorescence intensity measurements performed in a 0.9 × 0.9 mm glass capillary positioned beneath a microscope.

Results and Discussion

Three control experiments, bypassing the column, yielded a mean peak area of 5203 with a relative standard deviation (RSD) of 4.5% (Figure 3A), demonstrating good reproducibility of the fluidic system. Peak areas obtained in the standard nanoparticle experiments were normalized to the mean peak area of the bypass controls. Based on this analysis, 22.8% of the injected particles passed through the packed bed, while 22.9% were recovered during the release step, corresponding to a recovery reproducibility of 2.8% RSD. The remaining 54.3% of particles were not detected and presumed to be retained within or adsorbed onto the packed bed. This work establishes a standardized platform for acoustochromatographic nanoparticle processing and demonstrates the value of automation and digital monitoring to reduce system variability. These advances improve handling of biological samples and represent an important step toward robust analytical method development. Future developments anticipate to improve EV isolation, fractionation, and low-volume proteomic sample preparation for biomarker discovery and precision diagnostics.

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