5th NOVEL FLUIDIC TECHNOLOGIES WORKSHOP WITH AN EMPHASIS ON TISSUE ENGINEERING

WORKSHOP

4-5 May 2023

IZMIR/TURKEY

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Workshop Abstract Book

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Dear Colleagues,

It is with great pleasure that the Organizing Committee extends a cordial invitation to you to attend the upcoming workshop, **"5th Novel Fluidic Technologies Workshop with an Emphasis on Tissue Engineering"** The event will be hosted by the Bioengineering Department of Ege University in Izmir, Turkey, from May 4-5, 2023. This workshop is organized within the framework of our bilateral project **(120N422)**, which is supported by NCBR (Poland) and TUBITAK.

The workshop aims to provide a platform for scientists and young researchers to exchange knowledge and ideas on breakthrough research in the field of fluidic technologies with a focus on tissue engineering applications. Discussions will be centered around topics such as biofabrication, microfluidics, supercritical fluids, biomaterials, organ-on-chips, and related therapeutics. In addition, young researchers will have the opportunity to showcase their research through poster presentations in a competitive environment. The best three poster presentations will be awarded the **"Young Researcher Excelling in Novel Fluidics"** award.

We are excited to welcome you to this scientific exchange and look forward to your valuable contributions to this meeting.

Hope to see you in Izmir

On behalf of the Organizing Commitee,

Prof. Dr. Ozlem YESIL CELIKTAS

Workshop chair,

Ege University

Organising Committee

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5th NOVEL FLUIDIC TECHNOLOGIES WORKSHOP WITH AN EMPHASIS ON TISSUE ENGINEERING

(4-5 May 2023)

	04.05.2023 (THURSDAY)
9.00-9.30	Registration
9.30-10.00	Opening session
10.00-10.45	Microfluidics-assisted biofabrication for tissue engineering (Prof. Wojciech Święszkowski)
10.45-11.15	Magnetic levitational technologies for tissue engineering applications (Assoc. Prof. Cumhur Tekin)
11.15-11.45	Addressing unmet disease modeling needs through advanced organ-on-chips (Prof. Ozlem Yesil Celiktas)
11.45-12.00	From Ultra-low volume precision dispensing to organoid isolation (MedSanTek Laboratuvar Malzemeleri SAN. ve TİC. LTD. ŞTİ.)
11.45-13.00	Lunch
13.00-13.30	Organ-on-chip platforms for reproductive medicine (Prof. Séverine Le Gac)
13.30-14.00	Optofluidic cell analyzing platforms for life science applications (Assoc. Prof. Arif Cetin)
14.00-14.30	Advanced in vitro models for the kidney (Dr. Anne Metje van Genderen)
14.30-15.00	Coffee break
15.00-15.20	Microparticles Decorated with Cell-Instructive Surface Chemistries Actively Promote Wound Healing (Zeynep Imir, PhD Candidate)
15.20-15.40	Microfluidic-assisted co-axial wet-spinning extrusion of microvascularized hydrogel fibers with low viscous bioinks. (Alessia Paradiso, PhD Candidate)
15.40-16.00	The potential of exosomes for the vaccine delivery (Ilgin Kimiz Gebologlu, PhD Candidate)
16.00-17.00	Human Organ Chips for Disease Modeling, Drug Development, and Personalized Medicine (Senior Speaker- Prof. Donald E. Ingber)
17.00-17.30	Young researcher's poster presentation

5th NOVEL FLUIDIC TECHNOLOGIES WORKSHOP WITH AN EMPHASIS ON TISSUE ENGINEERING

(4-5 May 2023)

	05.05.2023 (FRIDAY)
10.00-10.30	Microfluidic magnetic platform for isolation of biological substances (Assoc. Prof. Barbaros Cetin)
10.30-11.00	Capillary pressure barriers for spatial confinement in organ-on-a-chip devices (Assoc. Prof. Ender Yildirim)
11.30-12.00	Organoid platforms for eye research (Assoc. Prof. Sinan Guven)
12.00-13.00	Lunch
13.00-13.30	Nanoparticle-Protein Interactions and Its Role in Toxicity (Asst. Prof. Ceyda Oksel)
13.30-15.00	Pannel for eliciting clinical requirements to be implemented by organ-on-a-chips. (Prof. Oguz Yavuzgil, Assoc. Prof. Ozlem Barut Server, Assoc. Prof. Ozlem Goksel, Assoc. Prof. Ayse Guler)
15.00-15.30	Coffee break
15.30-15.50	How Lab on A Chip Systems Can Contribute To The Development Of Biosimilars? (Dilan Karabulut Bicak)
15.50-16.10	Fluidic mechano-transduction dynamics on advanced brain organoid maturation as a broad perspective for organoid intelligence (Pelin Saglam Metiner, PhD Candidate)
16.10-16.30	Protease inhibitor Nafamostat mesylate attenuates Influenza A infection in precision cut lung slice models (Dr. Ece Yildiz Ozturk)
16.30-17.00	Award ceremony and closing remarks

Invited Speakers

Microfluidic-assisted spinning for engineering skeletal muscle and skeletal muscle interfaces

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INTRODUCTION

The functional capabilities of skeletal muscle are strongly correlated with its well-arranged microstructure, consisting of parallelly aligned myotubes. In case of extensive muscle loss, the endogenous regenerative capacity is hindered by scar tissue formation, which compromises the native muscle structure, ultimately leading to severe functional impairment. To address such an issue, skeletal muscle tissue engineering (SMTE) attempts to fabricate *in vitro* bioartificial muscle tissue constructs to assist and accelerate the regeneration process. Due to the dynamic nature of muscle, SMTE strategies must employ suitable biomaterials (combined with muscle progenitors) and proper 3D architectures.[1] In light of this, 3D fiber-based strategies are gaining increasing interest for the generation of hydrogel microfibers as advanced skeletal muscle constructs.[2] In this work, we developed an innovative microfluidic-assisted wet-spinning method for the biofabrication of highly-aligned hydrogel fibers. The platform is supported by an innovative microfluidic printing head bearing a co-axial nozzle placed within a crosslinking bath micro-tank, which in turn enables the immediate gelation of extruded core/shell fibers (**Fig.1a**). Two different hydrogel formulations were optimized. Alginate was selected as a supportive biomaterial for the shell, while fibrinogen was chosen to embed skeletal muscle progenitors. After the optimization of the biofabrication parameters, the platform was validated by producing functional myo-substitutes. In addition, the microfluidic platform was adapted by including a Y-shaped channel at the core inlet for the manufacturing of muscle-tendon unit tissue-like constructs (**Fig.1b**). Indeed, to properly exert its contractile function, skeletal muscle tissue is connected to tendons to form the myotendinous junction (MTJ), which allows the transmission of forces generated by the muscle through the tendon onto the bone to produce a movement. Considering the key role of such tissue interface, it is of paramount importance to reproduce their biological and architectural complexity to enhance skeletal muscle maturation and functionality.[3] In this study, a muscle-tendon unit construct was successfully fabricated by both addressing cell alignment and the recapitulation of skeletal muscle complex interface.

RESULTS AND CONCLUSIONS

The microfluidic-assisted platform enabled the successful fabrication of core-shell fibers with a high degree of compartmentalization (**Fig.1c**). The durable and flexible support of the alginate shell allowed for the confinement of a wide variety of low-viscosity bioink cores that would otherwise be difficult to extrude using other fiber-based techniques (i.e., 3D bioprinting, hydrogel electrospinning, free-standing hydrogel molding). Moreover, by manipulating biofabrication parameters such as shell and core flow rates along with the rotational speed, it is possible to control, in certain ranges, the overall fiber diameter and the fiber diameter thickness (**Fig.1d**). To reproduce the skeletal muscle, c2c12 myoblasts were encapsulated in the core-bionk and wet-spun. At day 14, myoblasts showed a remarkable muscle differentiation demonstrated by significant myosin heavy chain (MHC) expression and an average myotube

thickness of 15 ± 3.3 µm (**Fig.1e**). The myoblast fusion allowed the addition of new myonuclei to the growing syncytium, which contributed to a sustained and harmonious muscle growth. Moreover, confocal image analysis indicated a pronounced alignment of the cells and neo-myofibers during tissue maturation along the fiber axis, confirming that the selected core/shell architecture and composition effectively guided tissue maturation. Moreover, syncytia formation in the core was observed starting from day 7, and random contractions in the myobundles were recorded between days 10 and 14.

Muscle-tendon unit-like tissue constructs were obtained by alternatively delivering two inks. Flow rates and rotational speed were optimized to produce a scaffold with a high compartmentalization degree and suitable fiber diameter; thus guaranteeing high cell alignment degree of the overall muscle-tendon unit. Finally, to successfully reproduce the biological complexity of the myotendinous junction, a multi-cellular structure was obtained.

NIH 3T3 fibroblasts and C2C12 myoblasts were labeled with green and red viable cell tracker and wet-spun in an alternate fashion. As a result, cells formed a gradient-like biomimetic pattern mimicking the tendon and muscle tissue as well as the transition zone (**Fig.1f**). Then NIH 3T3/C2C12 were cultured for up to 7 and 14 days in muscle differentiation-inducing medium, and the cells showed high viability and spreading along the microfiber direction.

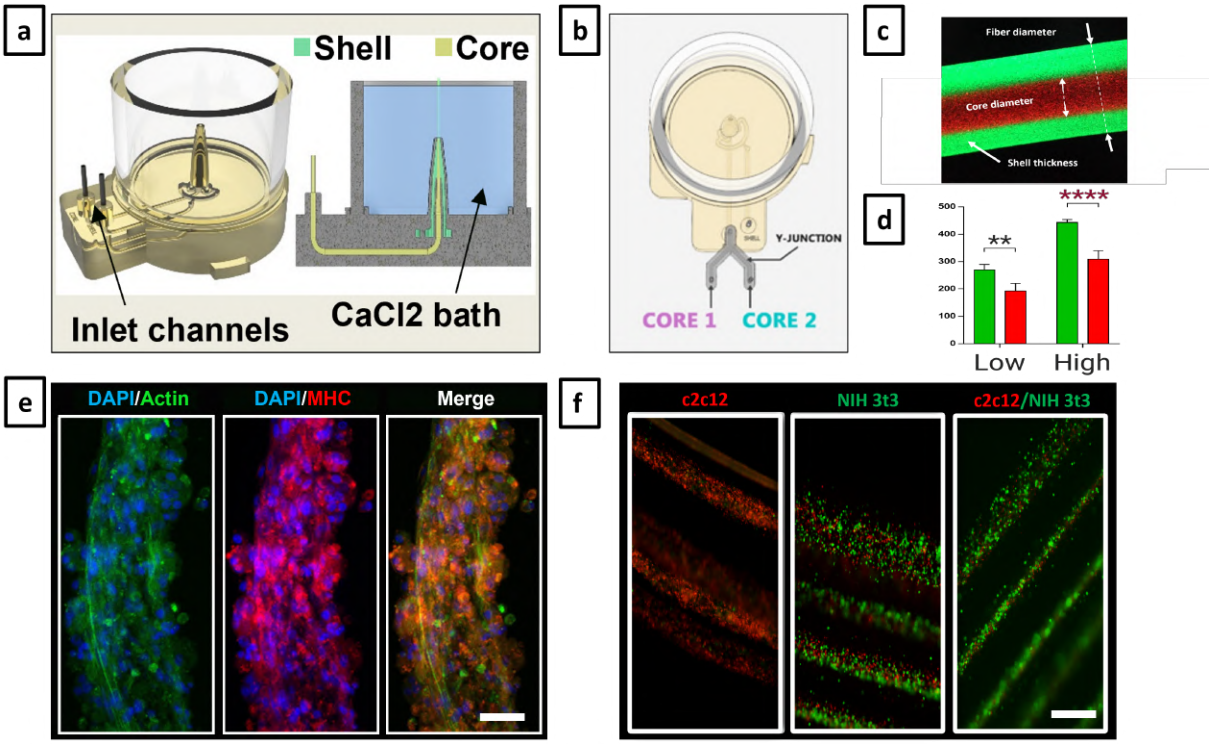

Figure 1. Microfluidic platform for the fabrication of core-shell fibers for skeletal muscle and muscle-tendon unit tissue-like constructs. **a**) microfluidic set-up for the extrusion of core/shell microfibers; **b**) Y-shaped core inlet for the delivering of two different bioinks simultaneously or in alternate fashion; **c**) core shell fiber produced with alginate-FITC (shell) and alginate-TRITC (core); **d**) fiber diameter low and high flow rate; flow shell (green) = ½ flow core (red); **e**) MHC positive myotubes after 14 days of culturing. Scale bar 50 µm; **f**) c2c12 and NIH 3T3 laden core-shell microfibers to mimic the muscle-tendon unit. Scale bar 200 µm.

ACKNOWLEDGMENTS

The Authors thank dr Joanna Idaszek from WUT Biomaterials Group for conceptual contributions in cell culture experiments. This work was financially supported by the National Centre for Research and Developments in the framework of the project "Advanced Biomaterials and Biofabrication Methods for Engineering of the Myotendinous Junctions" (grant No. PLTW/VI/3/2019), the National Science Centre Poland (NCN) within the SONATA 14 2018/31/D/ST8/03647 and PRELUDIUM 19 Project No. 2020/37/N/ST5/03272.

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Magnetic Levitational Technologies for Tissue Engineering Applications

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INTRODUCTION

Two-dimensional (2D) cell culture as a monolayer of cells is widely used for *in vivo* studies but it lacks cell-cell and cell-extracellular matrix (ECM) interactions.[1]Three-dimensional (3D) cell culture mimics cell microenvironment, cell-cell and cell-ECM interactions, and cell polarity.[2] In addition to these, 3D cell culture enables homogeneous distribution of nutrients, gases, and metabolites.[3] Magnetic force is used for cellular organization, spheroid formation or creation of cell sheets in 2D and 3D cell culture.[4] In 3D cell culture, using positive and negative magnetophoresis as a label-free method, cells are gathered in a magnetic field which is generated with permanent magnets and form cellular aggregates.[4] Magnetic levitation principle using negative magnetophoresis is a cell manipulation method depending on cell densities[5,] and cells are levitated at a specific point where a magnetic force is equal to buoyancy force.[6] Here, we will focus on magnetic levitation-based techniques for cell culture studies.

MATERIALS AND METHODS

Using a magnetic levitation platform, which includes two permanent magnets, two mirrors and 3D-printed pieces, D1 ORL UVA and MDA-MB-231 were cultured in a microcapillary channel. After the cell culture process, cells spiked in a paramagnetic medium (Gadavist) were given to a microcapillary channel, and 3D self-assembly cellular clusters were successfully formed under simulated weightlessness.[7] Adipogenic differentiation were also detected based on levitation height.[8] Then, adipocytes (7F2) were cultured and co-cultured with stem cells as scaffold-free with the same magnetic levitation platform.[9] In addition to these, the magnetic levitation platform was integrated with a holographic microscope for automated real-time single-cell analysis. The platform was used for testing the viability of D1 ORL UVA, MDA-MB-231, and U937 cell lines. The response of MDA-MB-231 cell 3D clusters to chemotherapy drug was further analyzed in the platform.[10] Moreover, to reveal the potential of the magnetic levitation technology on circulating tumor cell analysis, the magnetic levitation platform was integrated with a polydimethylsiloxane-based microfluidic channel for sorting cancer cells. The sorted cells with high viability could be used for further cell culture studies. Furthermore, to fabricate large self-assembled 3D cell structures and their different combinations, the magnetic levitation technology was applied in a platform composed of a single axial ring magnet and a tube.[11] We are also working on the high throughput formation of spheroids using magnetic levitation technology under microfluidic flows. We showed the formation of microsphere clusters in a microfluidic channel with two ring magnets underneath (Figure 1).

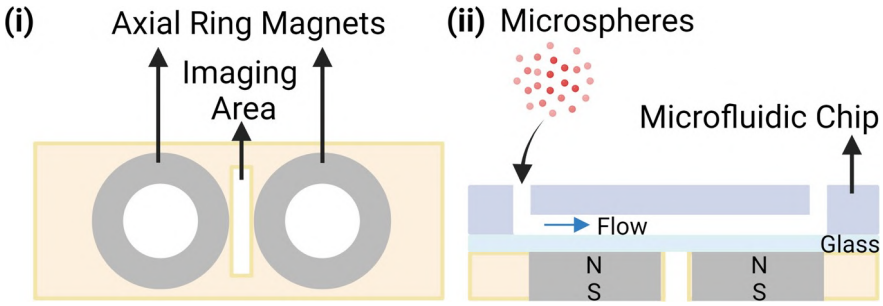

Figure 1. Illustration of axial ring magnet platform for spheroid formation. **(i)** 3D-printed magnet holder. **(ii)** Polydimethylsiloxane -based microfluidic chip placed on ring magnets.

RESULTS AND CONCLUSIONS

Using the magnetic levitation principle, the different cell lines were cultured in a simulated microgravity environment, and the levitated cells were gathered over time to form 3D cellular clusters in a scaffold-free manner. D1 ORL UVA and MDA-MB-231 cells also formed biphasic assembly *in situ* with different loading strategies.[7] Furthermore, adipocytes, which were co-cultured with stem cells, provided an opportunity to form a multilayered adipose tissue model.[9] The cell viability and cellular response to the drug were easily demonstrated based on levitation height using a holographic microscopy-integrated magnetic levitation platform.[10] Moreover, a 3D living cellular structure was formed in a tube using a single-ring magnet.[11] Under a microfluidic flow, polymer microspheres were also clustered between two ring magnets (Figure 2). The principle has a high potential to fabricate spheroids. In conclusion, magnetic levitation-based technologies could provide label-free, scaffold-free, high-throughput, and automated 3D cell culture. These clusters could be used efficiently for drug testing, and long-term analysis of microgravity.[12]

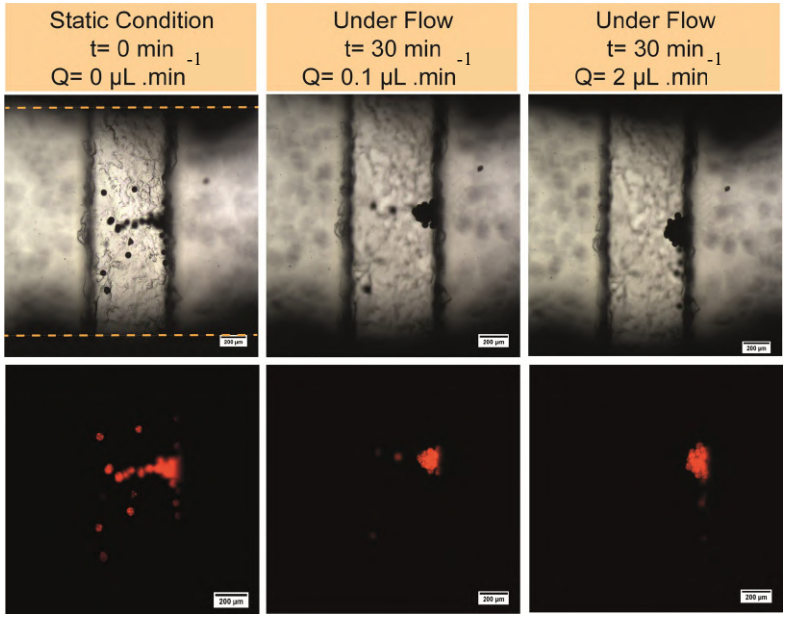

Figure 2. The images of microspheres between two ring magnets under different flow rates.

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Addressing unmet disease modeling needs through advanced organ-on-chips

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INTRODUCTION

Advances in tissue engineering, biomaterials and micro-fabrication have led to the rapid growth in organ-on-chip platforms which can mimic organ physiology in more similar conditions to those exist in human body. Organ-on-chips have demonstrated the ability to recapitulate not only human physiology but disease states[1], host-biomaterial interactions[2,3] and responses to clinically relevant drugs with a level of fidelity that is as good or better than animal models. This is due to the microfluidic culture technology, which allows for the recreation of complex tissue-tissue interfaces and mechanical forces that are present in human organs[4]. In contrast, other *in vitro* models lack this complexity and animal studies may not fully replicate human physiology.

FUNDAMENTAL COMPONENTS OF ORGAN-ON-CHIPS

For thorough recapitulation of various diseases, the components comprising organ-on-chips should be considered. These platforms successfully mimicking both biological and mechanical cues can be fabricated with innovative methods such as 3D printing[5], as well as traditional methods such as photolithography, soft lithography[6], injection molding[7], hot embossing. Apart from fabrication, the biological functionality is attined by the involvement of cells from many sources and cellular organizations that mimic a particular physiology or pathology. In addition to the commonly used immortalized cell lines and primary cells, induced pluripotent stem cells have received a lot of attention in recent years because of their ability to differentiate into many different cell types. They are also promising for personalized medicine studies with the isolation from patients or healthy individuals. Due to their pluripotency, induced pluripotent stem cells are frequently used in the production of organ-like 3D constructs known as organoids, which can be generated by differentiating stem cells into several organ types such as the brain, lung, liver, intestine, and many more. Combining organoids with organ-on-chip platforms promotes organoid generation and maturation with increased cellular diversity and functionality, as well as spatio-temporal dynamics with fluid flow and shear stress (**Fig. 1**). Maturated organoids with strong *in vivo* similarity can serve as beneficial

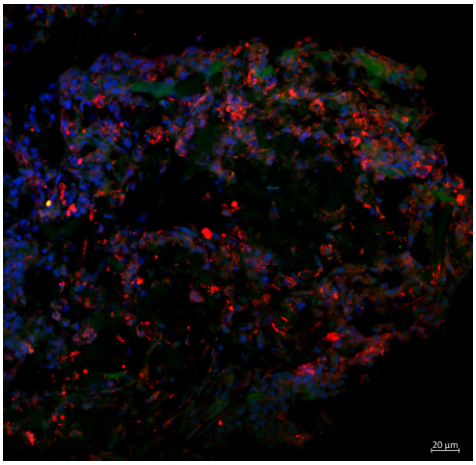

Figure 1. Cerebral organoid maturated in a microfluidic platform.

in vitro models for understanding developmental processes and disease mechanisms[8]. Spheroids, which can be generated from primary cells such as stem cells or immortalized cell lines, have shown to be useful *in vitro* models for mimicking multiple diseases as 3D cell aggregates[9]. Spheroids, in particular, that closely emulate the morphology and physiology of *in vivo* tumor patterns, are especially intriguing models for cancer research (**Fig. 2**). It is feasible to recreate the fundamental phases of metastasis such as invasion, intravasation, and extravasation by integrating the produced cancer spheroids into engineered organ-on-chip platforms. It is critical to create tumors and tumor microenvironments that best mimic *in vivo* conditions, especially in terms of invasion, and gels such as Matrigel, GelMA and alginate[10]. Additionally, decellularized matrices[11], which have gained popularity in recent years, are also being considered as potential tumor microenvironments. The fact that cancer cells leave the tumor structure and enter the vasculature or cross the vasculature and localize in a distant organ during the intravasation and extravasation processes highlights the need for materials that can effectively simulate the vascular structures in the human body. Intravasation and extravasation are extremely dynamic processes that entail cancer cell survival in fluid flow and immune cell surveillance in the blood, as well as their relocalization in a distant tissue, and organ-on-a-chip platforms are used to represent these dynamic processes. All of these processes, in addition to the functional production of the tumor structure, can be modeled on microfluidic platforms to better understand cancer mechanisms, test the efficacy of currently used drugs, and enable the development of new therapies[12]. Combining multiple organs on a single microfluidic platform, on the other hand, effectively describes the process of metastasis from one organ to another[13]. The outcomes of organ-on-chip platforms can potentially be further analyzed using bioinformatics and artificial intelligence technologies, allowing a more in-depth understanding of disease mechanisms.

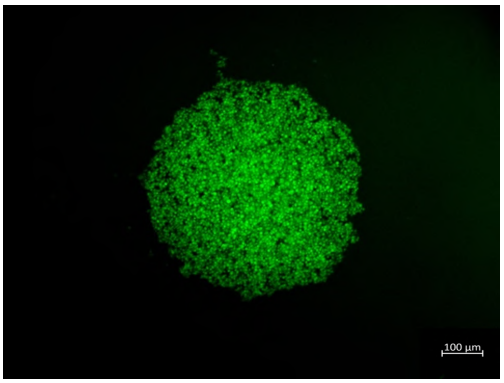

Figure 2. Breast cancer spheroid model of MCF-7 cells.

CONCLUSION

We foresee the increasing trend to continue and couple with machine learning and artificial intelligence tools to manage the huge amount of input and output in the organ-on-chip platforms. We should also keep in mind that due to the innovative and ever-changing landscape of the medical device and pharmaceutical industries, organ-on-chip research must be flexible and adaptable to the trends that are shaping these industries in order to be competitive. Positioning such a platform for success demands a firm grasp of the processes, issues, and trends in order to prepare for the risks and opportunities.

ACKNOWLEDGMENTS

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ORGAN-ON-CHIP MODELS FOR REPRODUCTIVE MEDICINE

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Assisted Reproductive Technologies (ART) are used on a daily basis to treat human fertility and in the livestock industry, while representing a promising strategy for biodiversity maintenance. Every 6th couple worldwide cannot conceive children naturally, and > 0.5 million embryos were produced *in vitro* in 2013 in the cattle industry, which demonstrates how important ART have become in our societies. Yet, *in vitro* ART procedures are sub-optimal; not only success rates for human ART stagnate around 30% but also recent evidence suggests that ART procedures may be stressful to the embryos and progeny at a later stage. Furthermore, the ever increasing infertility rates, and not only for humans, can be accounted for by exposure of individuals to chemicals, plastics, or other forms of pollution, some chemicals being known to act as endocrine disruptors.

In this context, I will present work from our group related to the development of organ-on-chip platforms for reproductive medicine: (i) for the production and culture of mammalian embryos using an oviduct-on-chip platform in an environment [1] that closely emulate interactions between the oviduct epithelium and the gametes and embryos; and (ii) to test the impact of environmental pollution could have on testis, using *ex vivo* tissues cultured in a microbioreactor under perfusion [2].

The oviduct-on-chip model comprises two fluidic compartments separated by a horizontal porous membrane; the top compartment represents the vascular system, delivering hormones and nutrients while the bottom compartment contains a bovine oviduct epithelium, grown on the porous membrane. In the latter compartment, oocytes and sperm cells are introduced, and embryos kept in-culture during the pre-implantation period, in close interactions with the epithelium. Perfusion is applied in the two compartments. The device is fabricated using PDMS (polydimethylsiloxane) and a mold produced in SU-8 using lithography. Bovine oviduct epithelial cells were isolated from oviducts from a slaughterhouse, seeded in the platform, let to attach on the membrane before perfusion was initiated in both fluidic layers. A differentiated and functional epithelium was formed, with a cuboidal-to-columnar cell morphology, ciliated cells and a tight monolayer responsive to hormonal stimulation. Bovine sperm cells introduced in the system interacted with this functional epithelium, and fertilized mature bovine oocytes on-chip. Resulting embryos were characterized at the zygote stage in terms of gene expression profiles and global methylation level, revealing that on-chip produced embryos closely resembled *in vivo* produced embryos. In contrast, embryos generated using conventional IVF presented different profiles with higher methylation rate. These results suggest that the oviduct-on-chip environment reduced the stress embryos and gametes experience *in vitro*. Current work aims at revisiting the oviduct-on-chip design and fabrication process to increase the device production yield. This second generation device will notably be used to identify which factors benefit embryo production and development in the oviduct-on-chip environment.

The testis-on-chip device comprises one culture chamber flanked on both sides by a perfusion channel, delivering nutrients and stimulatory compounds, and acting as such as a vascular-like system. A large loading port was included in the design to introduce testis tissues in the form of seminiferous tubules. The devices were also produced from PDMS,

using yet molds fabricated using 3D printing, which allowed introducing a slope in the tissue loading port. Seminiferous tubules were dissected from testis originating from gender dysphoria patients or prepubertal marmosets, and subjected to continuous perfusion directly after their insertion in the chip. Viable culture of primate (human & non-human) testis tissues for up to 10-11 days was achieved under continuous perfusion conditions, tissues exhibiting better integrity for a longer period of time than when using static and off-chip culture. Pre-pubertal marmoset tissues were stimulated to promote their endocrine function, and production of androgens (testosterone), by supplementing the culture medium with a stimulatory cocktail, and the testosterone level in the effluent was measured using ELISA off-line. An increase in hormonal activity was detected after *ca*. 4 days of on-chip culture under stimulatory conditions, showing the on-chip culture was able to support the endocrine function of the tissues. Future work will focus on exploiting this platform and assay for toxicity screening, using endocrine disruptors (phthalates and bisphenols), as well as nanoplastics.

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Optofluidic Cell Analyzing Platforms for Life Science Applications

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INTRODUCTION

Technologies integrating microfluidics and optics could enable great opportunities for life science applications. In this presentation, two platforms will be introduced for applications based on cellular analyses. The first platform is a plasmonic biosensor that could access the minute changes in the mass of a single cell within 10 minutes. The population studies in single cell precision could exhibit biophysical properties of cells and their therapeutic profiles in the presence of cancer drugs. The second technology is a cell-counting platform integrating a fast counting-algorithm enabling the count of 2000-3000 cells within only 1 minute. Our platform could yield an accuracy below 1% for cell viability, and below 5% for cell concentration. Employing a microfluidic compartment, the technology enables automated sample loading and self-cleaning of the flow-cell.

RESULTS AND CONCLUSIONS

The platform utilizes a sensitive plasmonic chip, based on periodic nanohole arrays to determine dynamic changes within the cell mass.[1] Figure 1a shows the optical setup of the optofluidic platform. A broadband white light-emitting diode source was used to illuminate the plasmonic chip. The light transmitted from the plasmonic chip was collected by an objective lens in an inverted microscope. The collected light was fiber-coupled to a multichannel spectrometer with a light-coupling scheme, including an achromatic and objective lenses, and a fiber collimator. In the multichannel spectrometer, we divided the spectral range of interest by five simultaneously working spectrometers to achieve a spectral resolution, e.g., 0.9 Å. Such high spectral resolution is critical to determine small changes in the cell mass. In order to maintain cell cultures during growth profiling measurements, we used a cell culture incubator for maintaining CO_2 level (5%), temperature (37°C) and humidity level (95%).

Figure 1b shows the growth rate profile of line and its variant resistant to difluoromethylornithine (DFMO) possessing anti-growth effect on MCF-7 cells, e.g., increasing apoptosis or decreasing tumor invasion. Figure shows the spectral variations for Wild Type (WT) MCF-7 cells under different DFMO concentrations (blue data), 0 – 10 mM. Here, the cells were seeded in the media containing DFMO 6 hours prior to the growth-profiling measurements. The anti-growth effect of DFMO on WT cells (blue data) could be clearly seen from the decrease in their growth profile. On the other hand, the data show the strong resistance of DR cells to DMFO's negative effect on cell growth, e.g., cell growth remains unchanged until DFMO = 1 mM.

Figure 1c shows the photograph of our optofluidic cell-counting platform.[2] The platform consists of three main modules. Imaging module is composed of an inverted microscopy setup providing a high-resolution cell imaging with an optical resolution of ~3.4 µm. This module employs a motorized translation stage that could autofocus on the plane within only ~8 seconds, and scans an 11 mm × 11 mm = 121 mm2 area with 12 pictures to record more than 2000 cells within only ~52 seconds. Microfluidic Module is composed of a flow cell and a

pump system for sample delivery and self-system cleaning. Pump system is composed of 4 piezo pumps that could be independently operated for different flow rates based on the solution, e.g., trypan blue – cell mixture, DI-water, PBS or trypsin. Software Module is composed of a graphical user interface (GUI) that could control the hardware components of the Imaging and Microfluidic modules, and a cell-counting algorithm that could generate viability, concentration and size data within only ~4 seconds by processing the images recorded by the camera.

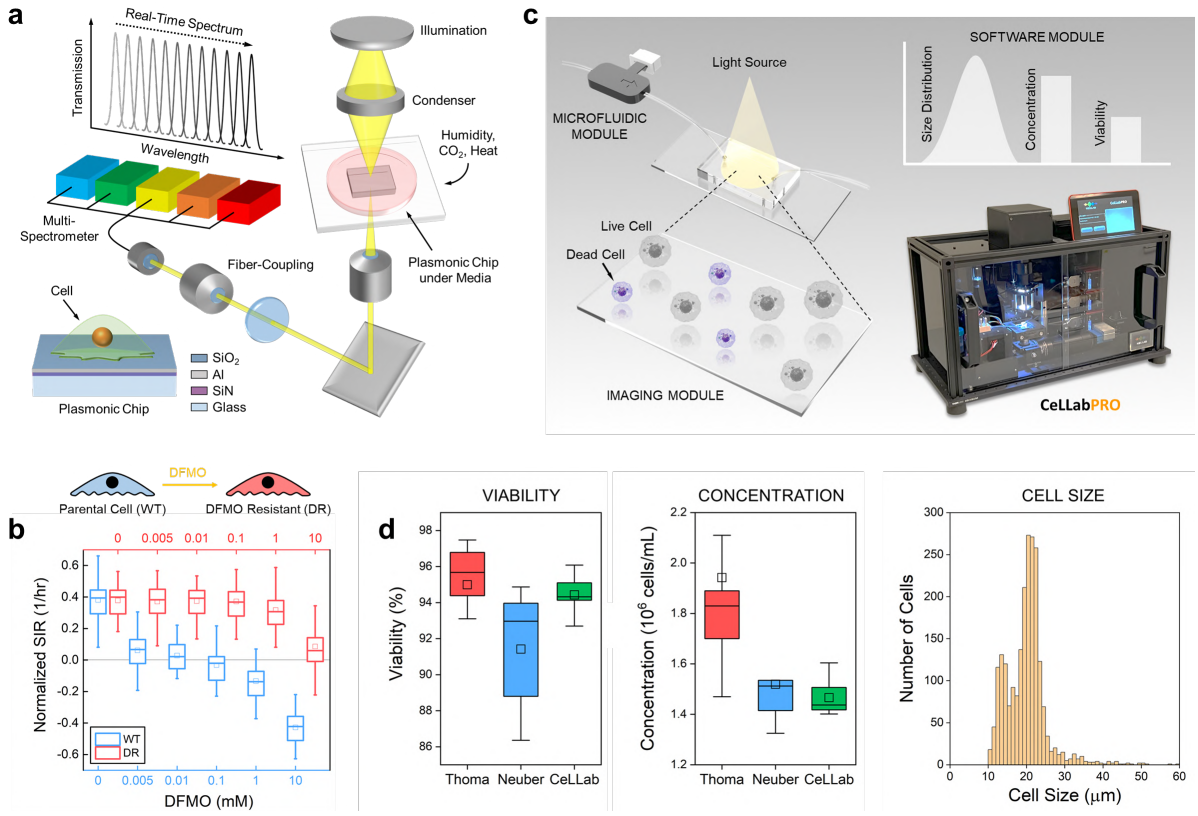

Fig.1 a Schematic illustration of the plasmonic growth rate profiler. **b** Growth profile of MCF-7 cells sensitive (blue) and resistant (red) to an anti-tumor drug with different concentrations. **c** Schematic illustration of the optofluidic cell-counting platform. **d** Cell viability, concentration and cell size results for CHO cells determined with our technology.

In order to show the accuracy and reliability of our platform, we performed cell-counting tests using Chinese hamster ovary (CHO) cells, where we compared the performance of the optofluidic platform with the classical hemocytometry performed with Thoma and Neubauer counting chambers for 10 independent tests (Figure 1d). Ability to scan higher number of cells that minimizes the operator error, our technology has lower test variation ensuring its reliability. The cultured cell line was expected to have a viability ~90%. As shown in Figure 1d, the classical hemocytometry performed with Thoma and Neubauer counting chambers yielded cell viabilities as 94.99% (red) and 91.42% (blue), respectively, while our platform determined the cell viability as 94.43% (green). More importantly, the variation for our platform was lower compared to the classical methods, e.g., standard deviation for Thoma and Neubauer counting chambers was 2.76% and 3.16%, respectively, while our platform yielded only 0.98% standard deviation. Figure 1d shows the cell concentration results obtained with the three methods. The cultured cell line was expected to have a concentration of 1 - 2 million cells/mL. Here, Thoma and Neubauer hemocytometry yielded 1.94 and 1.52 million cells/mL,

respectively, while our platform determined the concentration as 1.47 million cells/mL. Similarly, our platform provided much lower variation for cell concentration, e.g., standard deviation for 10 tests was 0.56 (red) and 0.14 (blue) million cells/mL for Thoma and Neubauer hemocytometry, while our platform yielded only 0.07 million cells/mL standard deviation, which corresponds to 4.76% percentage deviation. Figure 5C shows the size distribution of the CHO cell line, where the number of cells is 2208. CHO cells has a diameter between 4 and 50 µm, which could be clearly seen in the figure.

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Advanced *in vitro* models for the kidney

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Chronic kidney disease (CKD) affects around 13% of the world population, thereby imposing a major effect on global health. CKD often results in endstage kidney disease, for which the preferred treatment is organ transplantation including livelong immunosuppressive therapy, however, this is hampered by a shortage of donor organs. While waiting for kidney transplantation, patients need to be treated with kidney replacement therapies (KRTs) like dialysis. Current KRTs are only partially capable of replacing kidney function, leading to many different side effects such as anemia.

The kidneys each consist of about one million nephrons, the functional units of the kidney that filter approximately 180 liter of blood each day. The nephrons entail 5 main segments, starting with the glomerulus, the proximal tubule (PT), the loop of Henle, the distal tubule, ending in the collecting duct. The main functions of these nephrons are maintaining body homeostasis. The glomerulus is responsible for filtration of the blood, whereafter the tubules are responsible for excretion. KRTs replicate predominantly the glomerular function but are not able to replace the tubular functions. Therefore, novel therapies involving cell-based strategies that can further replace kidney function need to be developed to complement dialysis.

When we focus a bit more on the PT and its specific functions, we can distinguish a large variety of functional transporters located in/at the basolateral and apical side of the proximal tubular epithelial cells. These specific transport proteins contribute to the removal of endogenous waste including protein-bound uremic toxins into the pro-urine, a function that is not replaced by current KRTs. In search for better KRTs, kidney tissue engineering has received great interest as it can serve multiple purposes including the development of implantable kidney tubules and bioartificial kidney devices. Especially the combination of state-of-the-art biomaterials and kidney cells could result in systems that can facilitate uremic toxin removal from blood into the dialysate.

Our group focuses on developing (disease) models of the kidney for studying drug-drug interactions, disease modelling and drug safety testing, including work towards animals replacements and KRTs. We aim to unravel interactions between (therapeutic) molecules and biological systems. For this, humanized *in vitro* systems are being applied that functionally mimic (patients) organs, which includes the use of innovative technologies for 3-dimensional (3D) organ cultures such as microfluidics, 3D-(bio)printing and organoids. These experimental tools should aid in translating molecular interactions into therapeutic effects.

Microparticles Decorated with Cell-Instructive Surface Chemistries Actively Promote Wound Healing

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INTRODUCTION

The healing of acute wounds involves four stages - hemostasis, inflammation, proliferation, and remodeling. However, at least one of these phases is interrupted in chronic wounds which are common in people with diabetes, pressure ulcers, hardening of arteries, traumatic injury etc. As multiple cellular and molecular mechanisms are associated with wound healing, no single treatment is sufficient for complete wound healing. Therefore, various innovative treatment strategies have been explored in recent years[1].

The cellular response to biomaterial is determined by kinetic, thermodynamic and physicochemical interactions developed in the interface between biomaterial and biological system. Many studies proceed to investigate the effect of surface chemistry and physical characteristic of biomaterial on function of a range variety of cell such as human mesenchymal stem cells [2], osteoblasts[3], fibroblast[4, 5]. The characteristic of biomaterial affects cell growth, differentiation, migration and proliferation, these immune instructive chemistries of biomaterials could be used to alter macrophage phenotypes, cell attachments and wound healing[6-9].Compared to 2D wound healing solutions, 3D biomaterials become prominent because they can build a scaffold and mimic intracellular environment. Microparticles with immune instructive surface chemistries are produced via a microfluidic system which enables monodisperse particle size distribution. Fibroblasts have a critical role in wound healing due to their contribution to proliferation and the formation of granulation tissue which is fundamental for efficient wound closure.

Our proposal aims to promote the healing of diabetic wounds by positively modulating the phenotype and behavior of fibroblasts using microparticles decorated with cell-instructive surface chemistries.

RESULTS AND CONCLUSIONS

Our study introduces how monodisperse microparticles with target surface chemistries were produced and how these particles accelerate chronic wound healing in a diabetic mouse model. In that study over 300 different polymers were screened and identified according to their pro- or anti-proliferative phenotypes-inducing properties. By using polymer moieties ,which are known to have immune instructive properties, surfactants were synthesized.

Microparticles with chosen surface chemistries were produced using a microfluidic system which is based on oil in water dispersion droplet formation with the help of immune instructive polymer surfactant and a diacrylate core material as disperse phase. Surface chemistries of these particles were confirmed using Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) instrument.

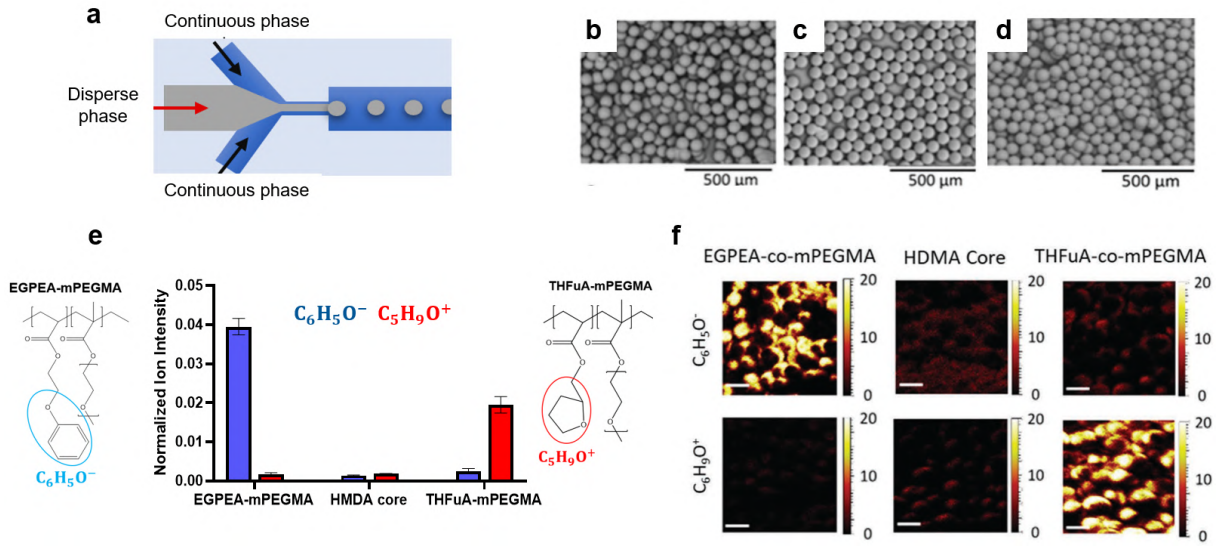

Fig.1 a The schematic of microfluidic chip with flow focusing geometry. **b** SEM image of polymer particles produced with a THFuA-co-mPEGMA surfactant with a size of 71.9 ± 2.5 µm (COV) = 3.4%). **c** SEM image of polymer particles produced with an EGPEA-co-mPEGMA surfactant with a size of 73.0 ± 1.5 µm (COV = 3.0%). **d** SEM image of polymer particles produced with no surfactant with a size of 69.4 ± 3.3 µm (COV = 4.8%). **e** Tof-SIMS data showing the identification of unique ions for EGPEA ($C_6H_5O^-$) and THFuA ($C_5H_9O^+$) on polymer microparticles Normalized ion internsities of each unique ion for particles with three different surface chemistries were shown in the graph. **f** Chemical image maps of unique ions for each particles are shown. N = 3 regions of interest used, scale bars = 100 µm. (Latif A. et al. 2022)[10]

To conclude, the microparticles derived from microfluidics, which promote cell proliferation, are successful in enhancing the formation of granulation tissue, neovascularization, and wound closure. When applied once to the wound bed, these bio-instructive microparticles accelarete wound closure in chronic wounds.

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Microfluidic-assisted co-axial wet-spinning extrusion of microvascularized hydrogel fibers with low viscous bioinks

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INTRODUCTION

Nowadays, the limited ability to properly vascularize scaffolds represents a significant drawback in developing physiologically relevant constructs for tissue engineering applications.[1] More specifically, the design and biofabrication of blood vessels at the microscale remain challenging. In fact, the capacity to produce microvessels is constrained by complications related to network complexity and size, which in turn affect nutrient and oxygen exchange, along with waste removal and mass transport limitations.
Currently, biofabrication techniques employed to engineer microvascular tissues in vitro mainly rely on extrusion-based 3D strategies (e.g., 3D bioprinting). However, they are considered time-consuming approaches and suffer issues related to resolution, viscosity, and temperature control. More recently, co-axial wet-spinning in 3D bioprinting has been rapidly emerging as an effective tool to fabricate advanced tissue constructs that can recapitulate *in vitro* organ/tissue functions.[2–4]
In this work, vessel-like 3D core-shell bundles have been rapidly fabricated using a novel microfluidic-assisted co-axial wet-spinning system to reproduce the native architecture of the microvascular network. Low viscous bioinks for core and shell compartments have been optimized and subsequently characterized to wet-spin cell-laden hydrogel microfibers. Such fibrous yarns exhibited endothelial morphologies. Hence, the material-cell interaction was investigated to validate the approach for the biofabrication of microvascular threads.
Two different hydrogel formulations were optimized. Alginate (ALG) was selected as a supportive biomaterial for the shell, while fibrinogen (FBG) was chosen for the cell-laden core to allow for the clinical translation of the constructs. Bioinks were simultaneously extruded from the co-axial nozzle immersed in a $CaCl_2$ coagulation bath to produce soft tissue-specific cell-laden (hBM-MSC/HUVEC) core-shell fibers. Then, the fibrous scaffolds were cultured for up to 21 days to evaluate cell growth and morphology.

RESULTS AND CONCLUSIONS

The system allowed for the continuous collection of wet-spun fibrous yarns on a rotating drum, which was connected to a stepper motor controlled by open-source electronics. The technology allowed for a successful compartmentalization of both core and shell structures upon optimization of the wet-spinnability window. Pre-polymer solutions of selected ALG and FBG revealed a Newtonian-like behavior (**Fig.1A**), and the hydrogel formulations were characterized in terms of elastic modulus upon compression tests (**Fig.1B**).
Fiber morphology was analyzed by scanning electron microscopy (SEM). Freeze-drying (FD) process and liquid nitrogen (LN_2) soaking were tested for drying the structure, revealing a well-preserved construct in case of FD-only and similar surface morphology for both ALG and FBG

structures as a result of low viscosities. Additional LN_2 soaking likely influenced the disruption of the core fibrin-based network, creating hollow structures.
A favorable formulation of co-culture medium was optimized to promote the heterogeneous hBM-MSC/HUVEC interplay and enhance the self-assembly of vessel-like networks for up to 21 days.[5] Afterwards, a cellular cytotoxicity test was performed to determine the impact of the co-culture medium on the cellular interaction, thus excluding plasma membrane damage. Confocal images showed that cells were mainly oriented on the fiber axis direction, forming dense and highly aligned 3D cell constructs along with robust cell spreading and elongation (**Fig.1C**). Considering the cell-cell signaling, it is also possible to speculate that cell alignment in the hBM-MSC/HUVEC-laden hydrogel fibers is most probably related to a more effective integrin-mediated focal adhesion and cytoskeleton deformation than in HUVEC-laden bundles. In conclusion, this study highlighted a novel approach to biofabricate tissue constructs for complex microvascular networks as a potential alternative to 3D bioprinted scaffolds. Along with the utilization of low viscous bioinks, the strategy successfully led to the microfluidic-assisted extrusion of highly aligned hBM-MSC/HUVEC-laden hydrogel fibers, promoting cell migration and the formation of microvessel-like structures. Further studies will investigate the functional role of the fibers in tissue engineering applications.

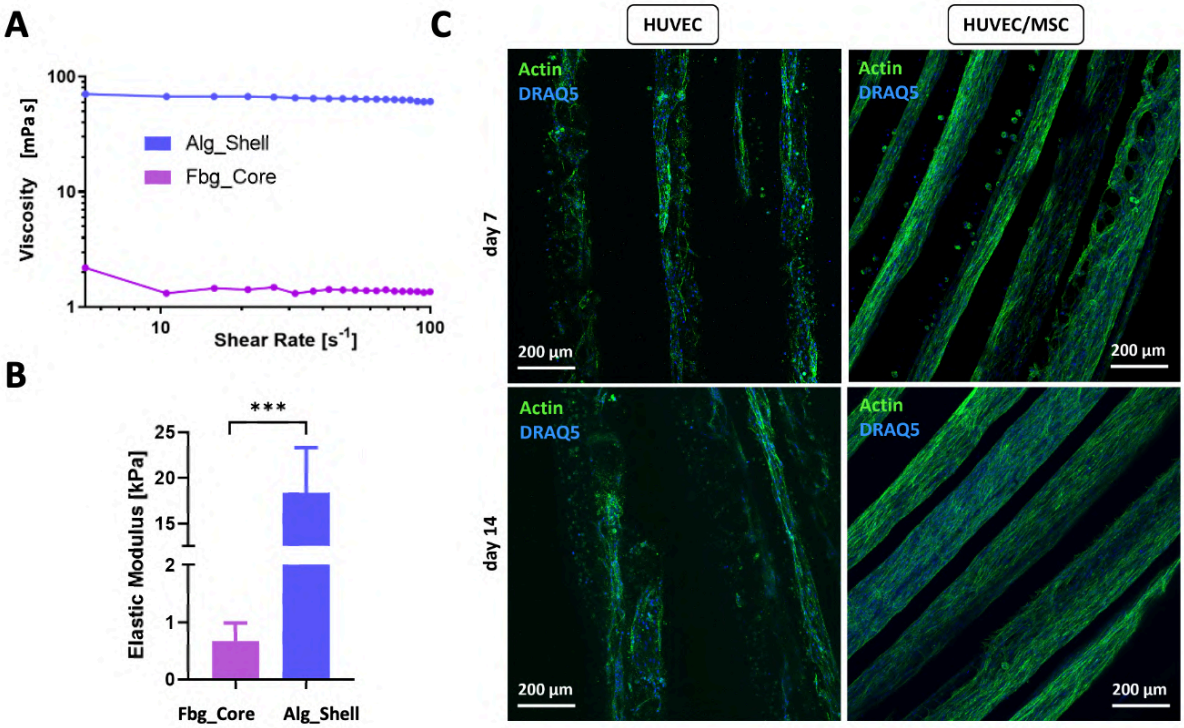

Fig.1 A) Rheological characterization of the pre-polymer formulations, displaying a Newtonina-like behaviour. B) Elastic Modulus of the hydrogels tested in compression. C) Morphology of hBM-MSC/HUVEC cells cultured into the core-shell yarns compared to HUVEC cells cultered in the same fibrous structures. Representative confocal images of actin (cytoskeleton, green) and DRAQ5 (nuclei, blue) at two different time points (i.e., day 7 and day 14, respectively).

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The potential of exosomes for the vaccine delivery

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INTRODUCTION

Nanocarriers are particles prepared from substances that can easily be uptake by the body, ideally 10-100 nm in size, that deliver the drug, vaccine or diagnostic agent to where the effect is expected and/or provide controlled release[1,2]. The use of exosomes, called natural liposomes, which are membrane nanovesicles released from prokaryotic and eukaryotic cells, as carrier systems has increased rapidly since the discovery of exosomes in the 1970s[3,4,5]. However, the major problem of exosome-based therapeutics is low yield and efficiency, and this problem can be solved by the production of exosomes in a large-scale[3]. The production of exosomes on a large scale in a controlled bioreactor, their separation, and their characterization as potential carrier systems are the goals of this work. Exosomes can be obtained in large numbers in a short time with this method. This may benefit commercial applicability by considering their potential application areas in the biopharmaceutical industries.

RESULTS AND CONCLUSIONS

THP-1 (human monocyte) cells were adapted to a chemically defined medium (FBCD, Florabio) that does not contain any substances derived from animals because the biopharmaceutical industry is the target application area for these exosomes. Thus, unwanted animal originated exosomes are also avoided. After that, production conditions were optimized in erlenmeyer flasks and spinner flasks before cells which were adapted to serum-free medium were grown in a stirred tank bioreactor (STR)[6]. Exosomes were isolated using the ultrafiltration technique, which is more suitable for large-scale production. Isolated exosomes were characterized in size, homogeneity, and protein concentration. Detailed characterization studies of the exosomes are ongoing.

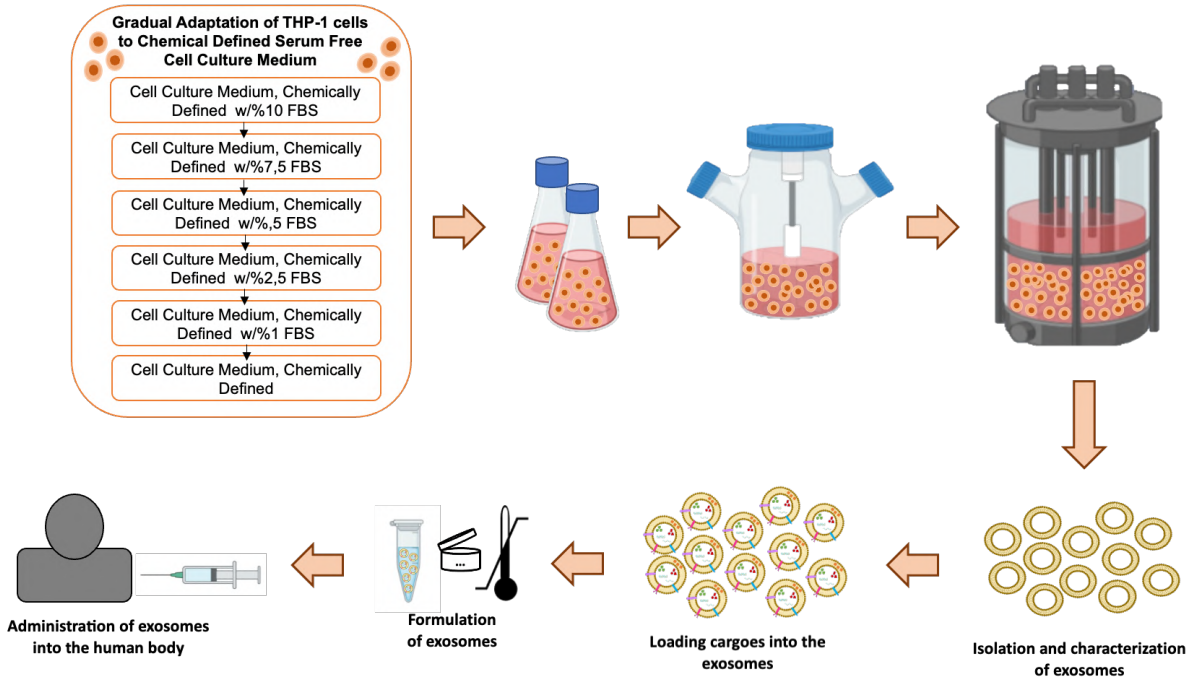

Figure A schematic illustration of large-scale production in a controlled bioreactor, isolation, and characterization of exosomes (Kimiz-Gebologlu et al., 2022; Kimiz-Gebologlu and Oncel, 2022)

In conclusion, a production platform and an appropriate isolation method have been optimized for the production of exosomes, which are expected to be used increasingly as a transport system in the biopharmaceutical industry, in a short time.

ACKNOWLEDGMENTS

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Human Organ Chips for Disease Modeling, Drug Development, and Personalized Medicine

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Failure of animal models to predict therapeutic responses in humans is a major problem that also brings into question their use for basic research. In this presentation, I will describe Organ-on-a-chip (Organ Chip) microfluidic devices lined with living human tissues that form tissue-tissue interfaces, reconstitute vascular perfusion and organotypic mechanical cues, integrate immune cells, contain living microbiome, and recapitulate organ-level physiology and pathophysiology with high fidelity. Work will be presented describing how single human Organ Chips and multi-organ human Body-on-Chips systems have been used to model complex diseases and rare genetic disorders, study host-microbiome interactions, quantitatively predict drug pharmacokinetic and pharmacodynamic parameters, recapitulate whole body inter-organ physiology, and reproduce human clinical responses to drugs, radiation, toxins, and infectious pathogens. We also have used human Organ Chips to gain new insight into mechanisms of host immunity to viral infections and to develop new therapeutics for potential pandemic respiratory viruses, including influenza and SARS-CoV-2. My message is that the possibility that human Organ Chips can be used in lieu of animal models for drug development and as living avatars for personalized medicine is coming ever closer to becoming a reality.

Microfluidic magnetic platform for isolation of biological substances

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INTRODUCTION

Biological substances such as DNA/RNA, protein, enzymes, exosomes, drug molecules, cells, and microbiological organisms are perhaps the most important components for a simple diagnosis[1]. In the current technology, the traditional bench-top macroscopic systems (*i.e.*, high-pressure liquid chromatography HPLC)[2] or commercial systems(*i.e.*, gravity columns, spin columns)[3] are used for the isolation and/or purification of these valuable biological substances. Compared to other chromatographic systems such as packed microfluidic columns or monoliths; we proposed a new system that is highly cost-effective, does not rely on bulky equipment such as specialized high-pressure pumps, and provides fast analysis with more efficient operation by eliminating the diffusion barriers during purification.

In this study, we present a novel miniaturized device as a 3D-printed microfluidic magnetic platform to isolate biological substances specifically designed for manipulating magnetic microparticles in a microfluidic chip. The novel design enables the movement of the magnetic particles in the same or opposite directions with the flow or suspending them in a continuous flow. The proposed miniaturized devices provide excellent features such as reducing amounts of required samples having fast analysis, relying on relatively less sophisticated equipment, low-cost fabrication, and having the potential to be portable for point-of-care testing[4].

MATERIALS and METHODS

The platform was 3D-printed and assembled with permanent magnets on a brushless motor moving compartment (in Fig 1-A). The compact microfluidic system and PDMS-based microfluidic chip isolate biological substances such as fish sperm DNA, human placenta DNA, and pathogenic bacteria (in Fig 1-B). COMSOL Multiphysics was used to develop a 3D time-dependent model to evaluate the rotational effect of the magnets. The free tetrahedral mesh structure was employed (in Fig 1-C). Each magnet was modeled by using its remanent flux density. Then, the

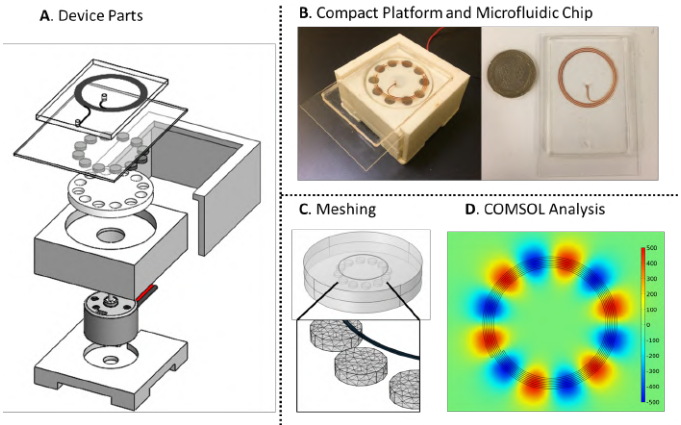

Figure 1. (A) a schematic illustration of 3D-printed magnetic microfluidic platform, (B) platform and PDMS microfluidic chip, (C) COMSOL Multiphysics meshing model, and (D) analysis

generated magnetic flux density in the channel was determined by Gauss' Law for magnetism (in Fig 1-D).

The superparamagnetic silica microbeads were synthesized using a multi-step polymerization technique and loaded in PDMS based-microfluidic chip (in Fig 2-A). The microbeads were used to isolate fish sperm DNA (Sigma-D1626), human placenta DNA (Sigma-D3035), and Streptococcus pneumonia.

RESULTS AND CONCLUSIONS

Fish and human placenta DNA was purified in the microfluidic platform (Fig 2-B). First, microbeads were washed with adsorption buffer (6M Guanidium HCL- Tris Buffer pH:6.0). Then, a known amount of human placenta DNA sample (0.6mg to 250mg) was loaded with adsorption buffer. Then, microbeads were washed with 4:1 (v:v) isopropanol/water. In the final step, DNA was collected using elution buffer (10mM tris buffer pH:9). The adsorbed DNA concentration (QDNA [ng DNA/mg dry particles]) was analyzed by Nanodrop (Thermo Fisher Scientific ND 2000, USA). The optimum adsorption and desorption flow rate were determined as 10µl/min and 20 µl/min, respectively (Fig 2-C).

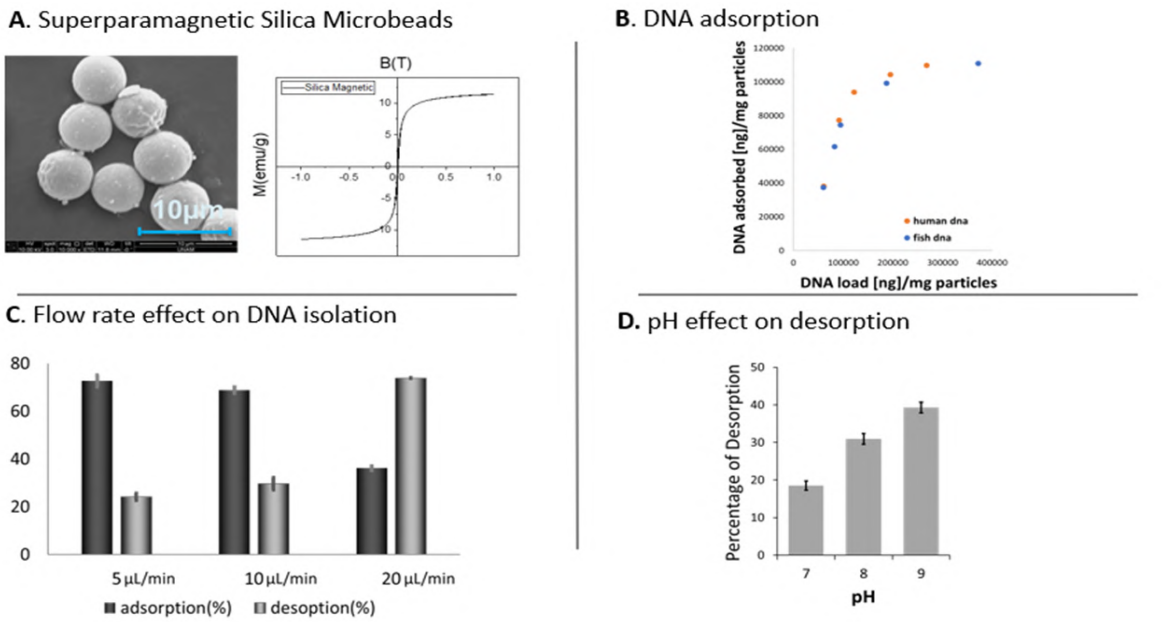

Figure 2. (A) Superparamagnetic silica microbeads, (B) DNA adsorption curve, (C) Flow rate effect on DNA isolation (D) pH effect on desorption

The elution performance is highly dependent on the pH of the elution buffer. The best desorption (elution) performance was 40% for adsorbed DNA on the particle at pH 9 (Fi g2-D).

The novel platform exhibited superior performance in adsorption capacity and operation time compared to the systems using identical particles and buffers[5, 6] . This new system seems to be a promising alternative to the available technologies and can be further implemented for the isolation/purification of Streptococcus pneumonia with aptamer modification of the surface of the magnetic particles.

ACKNOWLEDGMENTS

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Capillary Pressure Barriers for Spatial Confinement in Organ-on-a-chip Devices

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INTRODUCTION

Organ-on-a-chip systems have been investigated for more than a decade and are becoming more pronounced especially as potential alternatives to animal testing in drug development. Fundamentally the devices are composed of microfluidic channels to provide perfusion of the culture medium and compartment in which the cells can be cultured. The compartment can either be separated from the perfusion channel via a membrane so that the cells can be directly cultured on the membrane, or via pillars so that the cells suspended in a gel, acting as the extracellular matrix, are confined in the chamber on one side of the pillar. In the second arrangement, the pillars act as capillary pressure barriers preventing overflow of the cell-laden gel through the perfusion channel while pipetting the gel in the chip before it gets cured. In this study, numerical and experimental investigation of different capillary pressure barriers that can be utilized in organ-on-a-chip systems are presented.

RESULTS AND CONCLUSION

Phaseguides

Phaseguides, which are defined as shallow ridges patterned at the bottom of a microfluidic structure, were originally utilized for bubble free priming of even dead-end microfluidic chambers [1]. The operation of the phaseguides rely on capillary pinning phenomenon. Accordingly, when a capillary meniscus faces an acute change in the geometry, such as the 90° bend at the front face of the phaseguide (Figure 1a), the meniscus gets pinned at the location of the acute change, namely the phaseguide. As it is pinned, the meniscus stretches until the apparent contact angle equals the contact angle of the liquid on the structural material of the channel walls. The phaseguides were then successfully implemented by the company MIMETAS B.V. in their organ-on-a-chip platforms, where a cell carrying gel (indicated by 1 on Figure 1a) is pinned at the phaseguide until it gets cured, then the medium (indicated by 2 on Figure 1a) is provided through the neighboring channel [2]. On the other hand, the meniscus can breach a phaseguide mainly because of the corner filling effect, which states that the apparent contact angle on a wedge is smaller than the advancing contact angle of the liquid measured on the structural material [3]. For this reason, the meniscus tends to advance along all corners including the ones at the intersection of the front face of the phaseguide and the channel side walls (Figure 1a). This may cause the meniscus to breach the phaseguide leaving the barrier non-functional. We have presented the details of this principle in ref. [4]. To resolve the corner filling problem, we propose to utilize filleted phaseguides, where the intersections of the front face of the phaseguide with the channel side walls were rounded so that the meniscus cannot advance down on the front face of the phaseguide due to corner filling effect (Figure 1b). To observe the effect of the fillet, we have built a numerical model

based on Surface Evolver, a free software for solving geometry of a meniscus under surface tension and other effects by minimizing the energy of the liquid surface [5]. However, the software does not include a graphical user interface thus the initial geometry has to be defined manually in a datafile. To simplify the process, we have integrated Surface Evolver with FreeCAD, a free software for computer aided design, allowing the user to define the geometry directly on CAD environment thus simplifying the workflow (Figure 1c). The results prove that the corner filling can be prevented by rounding the corners on the phaseguide (Figure 1c).

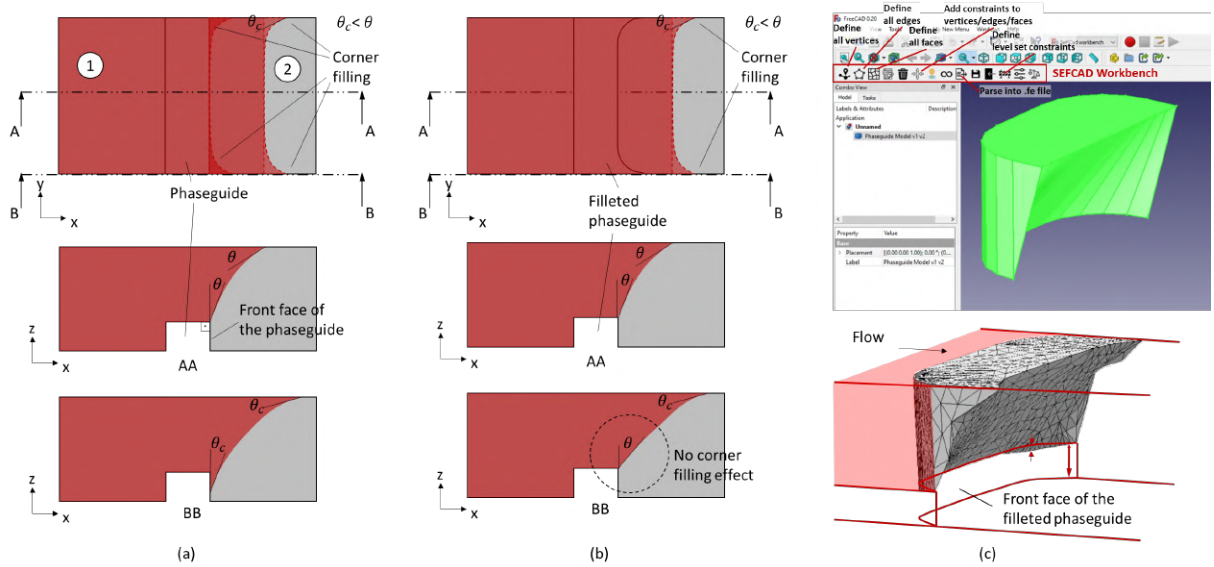

Fig.1 a A schematic illustration of the phaseguide showing the corner filling effect. **b** Schematic illustration of filleted phaseguide to solve the corner filling problem. **c** Use of FreeCAD to define the initial geometry of the meniscus sitting on the filleted phaseguide and the solution obtained by using Surface Evolver.

Micro-wells on a channel

Although originally the phaseguides are ridges at the bottom of a microchannel, a similar change in the channel geometry can be implemented by posing micro-wells at one side of the microchannel such that the intersection of the well and the channel wall results in an acute change in the channel geometry similar to phaseguides. On the other hand, the angle of the acute bend can now be larger than the typical 90º bend in phaseguides (Figure 2a). We have examined the behavior of the capillary flow over such capillary pressure barrier by using COMSOL Multiphysics (Figure 2b). The results show that, over a critical pressure, an aqueous liquid overflows the corner as illustrated in Figure 2b. This behavior suggested that the structures can be used to confine cell-laden gel in the micro-wells, which can then be perfused for 3D culturing. For this purpose, cell-laden gel could be first pipetted into the channel. The gel would breach the barrier at the intersection of the well and the channel wall and then fill the well entirely. The excess gel remaining in the channel can then be vacuumed out again by using the pipette as the vacuum source. After waiting sufficiently long for curing of the gel, the culture medium could be infused through the channel (Figure 2c). The micro-wells were fabricated on polymethyl methacrylate (PMMA) by micromilling and sealed by solvent assisted thermal bonding. The device was tested for cytotoxicity assay on MCF7 cells. Red Fluorescent Protein (RFP)-MCF-7 cells were loaded in the device suspended in a collagen matrix. The cytotoxicity of doxorubicin was tested by a live-dead assays utilizing Hoechst 33342 (blue fluorescence) and Propidium iodide (red fluorescence). The assay was continued for 48 hours. The results indicate the reduction in viability of the cells treated by doxorubicin as expected, which proved the feasibility of the device (Figure 2d).

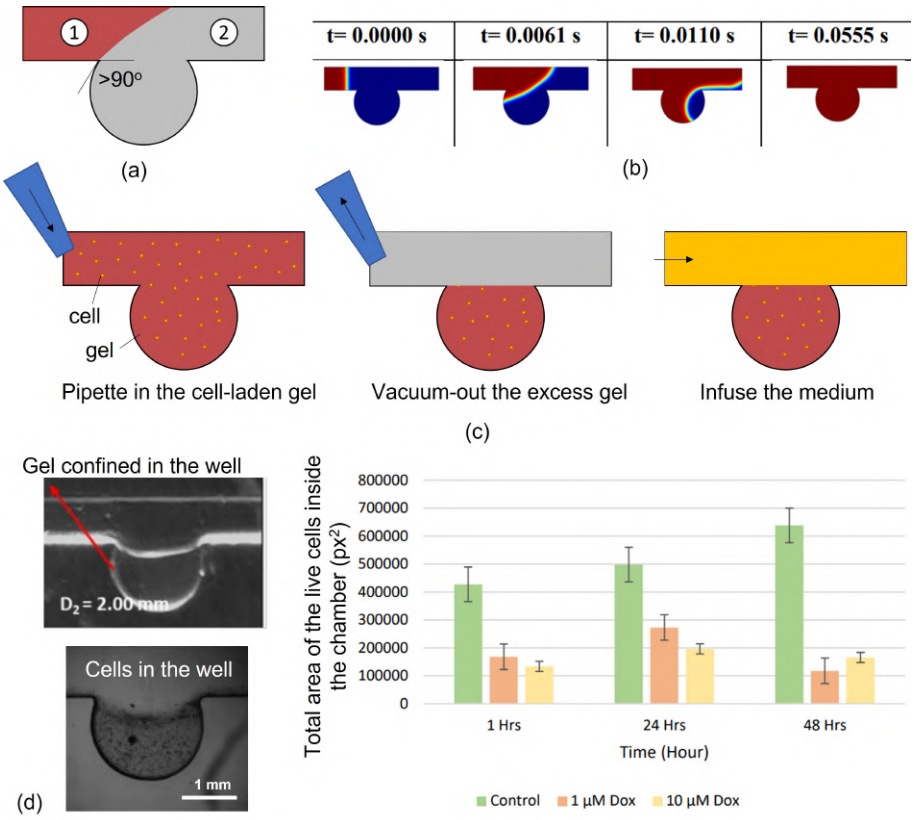

Fig.2 a A schematic illustration of the capillary pressure barrier formed by the intersection of the micro well with the channel. **b** COMSOL Multiphysics simulations showing the timelapse surface plot of the aqueous phase filling the well. **c** Operation steps of the device. **d** Fabricated device with gel confined in the well. Cell viability in time under different concentrations of doxorubicin.

ACKNOWLEDGMENTS

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Organoid platforms for eye research

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Ophthalmic diseases originating from trauma, inherited genetic background or as result of other systemic diseases affects a large portion of the human population. Advances in cell-based therapies promise to improve the vision and patient comfort. Today novel regenerative medicine approaches in ophthalmology have been significantly influenced by induced pluripotent stem cells (iPSCs) and their potential in ocular disease modeling and treatment of ocular diseases. iPSCs have been successfully demonstrated to form organoids, organ mimicries recapitulating cellular phenotypes and functions of organs/tissues *in vitro*. Anterior and posterior eye compartments have been addressed utilizing organoid technologies to elaborate the development of human eye and generate disease models. Cornea, retina, and lacrimal gland organoids have been generated from iPSCs and efforts to use them in associated diseases attracts tremendous interest.

Organ-on-a-chip (OoC) platforms mimicking native microphysiological environments have been established that allow cell differentiation and tissue growth rather than using research animals to improve the *in vitro* models. Such tools facilitate investigation of cellular mechanotransduction, tissue morphogenesis and advanced control of other physiological factors affecting *in vitro* performance of organoids. As growing interest of pharma industry on bioengineered OoCs, organoid models in eye research become more attractive.

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Nanoparticle-Protein Interactions and Its Role in Toxicity

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INTRODUCTION

Nanotechnology has led to the development of advanced products, devices, and systems with novel properties and broad applications. The novel physical and chemical properties of nanoparticles (NPs) relative to their bulk forms make them very useful for diverse commercial applications but also strongly effects their behavior in biological and environmental systems. Unlike small organic molecules with well-known structures, investigating dynamic properties of nanostructured materials is technically challenging, requiring a deeper understanding of the complexities of environmental and biological interactions.

There is ample evidence in the literature that the surfaces of NPs are immediately coated by a layer of adsorbed proteins, so-called protein corona, upon introduction to biological fluids, and that various factors play a role in this process.[1-5] In our recent paper, we provided a detailed discussion on how the protein corona is formed and how the structure of adsorbed protein layer changes as a function of intrinsic NP characteristics, extrinsic environmental parameters and time. [6] The protein layer the surface of NPs constitutes a new nanobio-interface where dynamic interactions between NPs and biological systems such as cells take place. The corona masks the original surface characteristics of pristine NPs and modulates key biological processes such as cellular uptake, biodistribution, and biological activity.[4, 7, 8] Therefore, understanding and controlling NP protein corona is critically important to optimize *in vivo* applications of nanobiotechnologies.

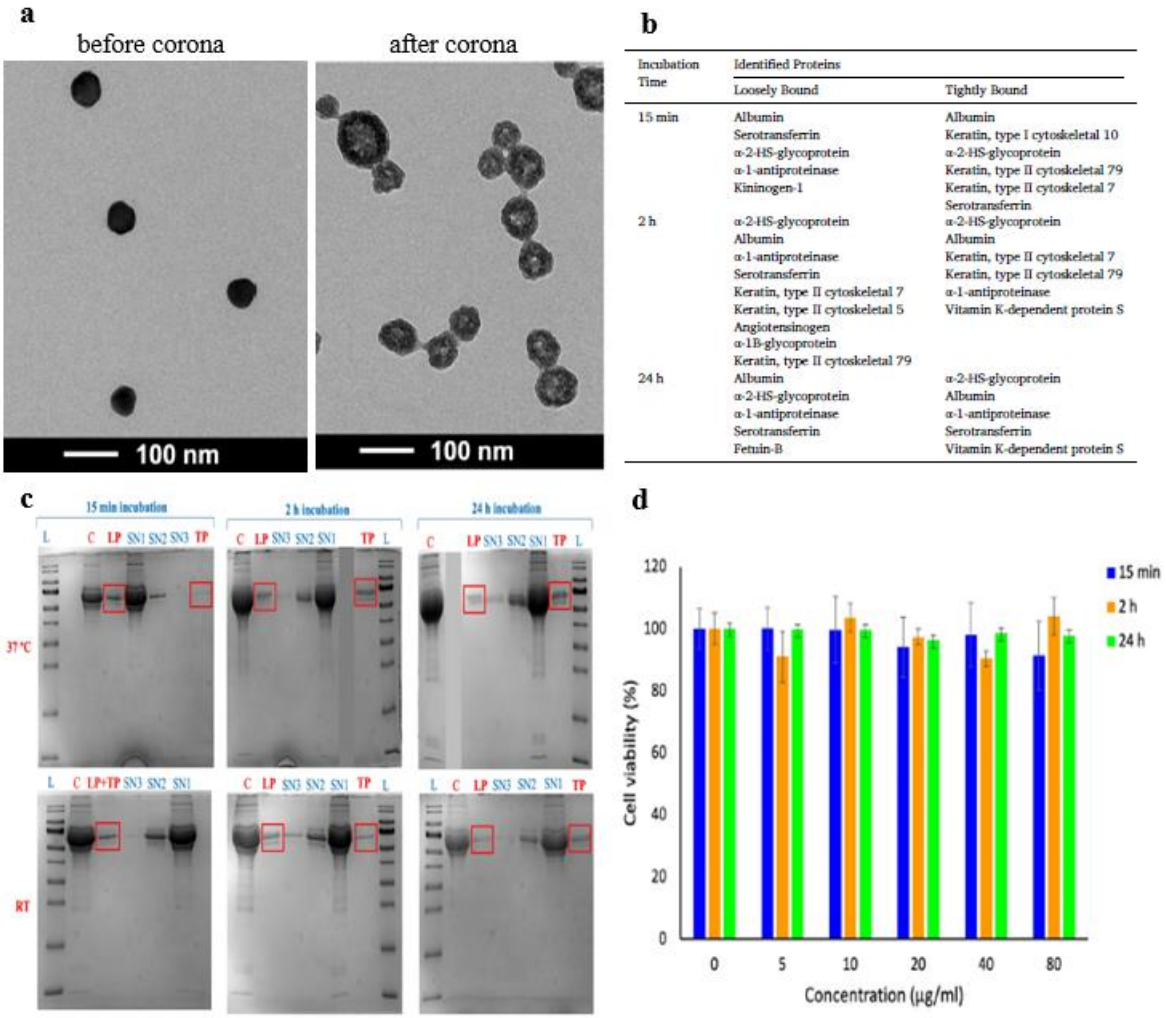
In this study, we explored the formation of the silver nanoparticle protein corona on exposure to cell culture media containing 10 % fetal bovine serum supplemented Dulbecco's Modified Eagle's medium. Sodium dodecyl-sulfate polyacrylamide gel electrophoresis and liquid chromatography-mass spectrometry/mass spectrometry analysis were used to monitor how different parameters such as incubation time, heating duration, cell culture medium, incubation temperature, and the number of washes affect the nanoparticle–protein corona complex. silver nanoparticles with and without bound proteins were characterized by electron microscopy, dynamic light scattering, and ultraviolet-visible-near-IR spectroscopy. The tetrazolium-based MTT assay was used to determine viability of A549 human lung adenocarcinoma cells treated with silver nanoparticles. Characterization of the nanoparticles before and after protein binding provided insights into their changing morphology on corona formation.

RESULTS AND CONCLUSIONS

AgNPs were characterized in terms of size, morphology, and surface properties before and after protein corona formation. TEM images revealed that protein adsorption caused an increase of 25 nm in AgNP radius (from 50 to 75 nm). However, it caused much larger aggregated structures with a mean hydrodynamic diameter of 287 nm, confirming protein-

induced bridging of AgNPs. The UV-Vis-NIR absorption maximum of AgNPs was red shifted by about 13 nm after incubation in cell culture medium. A decrease in the peak height of absorbance curve was observed with increasing incubation time. The zeta potential of AgNPs in aqueous solution was increased to more negative values afterprotein corona formation suggesting the adsorption of high surface charge serum proteins on AgNP surfaces at longer incubation times.

Two major protein bands observed in all SDS-PAGE samples were at 65 and 60 kDa, which potentially correspond to the size of bovine serum albumin and α-2-HSglycoprotein (or α-1-antiproteinase), respectively. After separating bound proteins by SDS-PAGE, the extracted peptides were subjected to LC-MS/MS analysis for sequence identification followed by protein sequence database search using Mascot software. LCMS/ MS analysis and Mascot database searching results confirmed that serum albumin, α-2-HS-glycoprotein, serotransferin, α-1-antiproteinase, and keratins were the major proteins identified on AgNP surfaces in varying abundances across different incubation times.



Incubation Time	Identified Proteins	
	Loosely Bound	Tightly Bound
15 min	Albumin	Albumin
	Serotransferrin	Keratin, type I cytoskeletal 10
	α-2-HS-glycoprotein	α-2-HS-glycoprotein
	α-1-antiproteinase	Keratin, type II cytoskeletal 79
	Kininogen-1	Keratin, type II cytoskeletal 7
		Serotransferrin
2 h	α-2-HS-glycoprotein	α-2-HS-glycoprotein
	Albumin	Albumin
	α-1-antiproteinase	Keratin, type II cytoskeletal 7
	Serotransferrin	Keratin, type II cytoskeletal 79
	Keratin, type II cytoskeletal 7	α-1-antiproteinase
	Keratin, type II cytoskeletal 5	Vitamin K-dependent protein S
	Angiotensinogen	
	α-1B-glycoprotein	
	Keratin, type II cytoskeletal 79	
24 h	Albumin	α-2-HS-glycoprotein
	α-2-HS-glycoprotein	Albumin
	α-1-antiproteinase	α-1-antiproteinase
	Serotransferrin	Serotransferrin
	Fetuin-B	Vitamin K-dependent protein S

Fig.1 a TEM images of AgNPs and AgNP-protein corona complex following 2 h incubation in 10 % FBS supplemented cell culture medium **b** List of most abundant proteins identified by LC-MS/MS and Mascot search after 15 min, 2 h and 24 h incubation in 10 % FBS supplemented cell culture medium. **c** SDS-PAGE protein binding patterns of AgNPs as a function of incubation time (15 min, 2 h and 24 h) and incubation temperature **d** Viability of human lung adenocarcinoma A549 cells after incubation (15 min, 2 h and 24 h) with varying doses of AgNPs. Each measurement was repeated three times.[9]

We observed slight differences in cell viability responses after 15 min, 2 h and 24 h incubation with AgNPs. This could be partly attributed to the changes in protein corona patterns and surface properties of AgNPs exposed to cell culture media for different periods, providing them with distinct biological identity. We detected ROS in A549 cells treated with AgNPs using a fluorescent dye-based approach and observed an about 1.6 fold increase in fluorescence (> 20 μg/mL), compared with the control. Despite having a high ROS-generating capacity, AgNPs did not induce significant cytotoxicity in A549 cell line, possibly because of their new biological identity provided by the protein corona.

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How Lab On A Chip Systems Can Contribute To The Development Of Biosimilars?

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INTRODUCTION

Biosimilars are biotherapeutic products with similar efficacy, safety, and quality to a licensed bio-originator. Biosimilar molecule process development, phase I and phase III clinical trial period take approximately 10 years. After laborious and high-cost work, a biosimilar molecule can be approved by a biosimilar regulatory agency if all the critical attributes are assessed very well as well as clinical trials. Pharma companies seek alternative solutions to decrease the required cost and time to reach the market.

Microfluidic systems and Organ A Chip solutions could be great alternatives to reduce cost and time effort by contributing clinical trials and scale down model process development.

FDA's recent decision about animal testing and utilization of PD biomarkers in clinical studies for biosimilar molecules strengthened the idea of using alternative way outs for clinical trials such as cell based assay, 3D printed organ chips, microphysical systems, computer modeling, and human based trials.

Biosimilar process development, process validation, and characterization studies should be performed with scale down models. In this point usage of microreactors could be efficient in terms of reducing cost and saving time. Currently, scale down model formation of 2000 L scale bioreactor into 2 L bioreactor system is available. On the other hand the number of studies the microreactors as scale down model process is increasing. Application of microreactors on high-throughput experimentation for process design characterization and validation studies under quality by design approach could be great alternatives for biotechnology companies.

RESULTS AND CONCLUSIONS

Creating reliable, reproducible, and standardized systems as strong candidates for animal testing will change the roles in the pharmaceutical industry.
Although OOACs are able to emulate various organ functions, standardization seem to be a major challenge. It is known that there are already established standards, such as ISO 100991-2009 on the vocabulary of micro process engineering for OOAC definitions, CEN /ISO on medical devices for OOAC sterilization and packaging.
To be a reliable and promising candidate for animal testing and clinical trials for biosimilar molecules, there is a long regulatory milestone. Even if they do not completely replace clinical trials, they can at least reduce costs and shorten the drug development period by reducing the number of patients studied.

ACKNOWLEDGMENTS

The authors acknowledge İLKO ARGEM Biotechnology Center Team Members.

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Fluidic mechano-transduction dynamics on advanced brain organoid maturation as a broad perspective for organoid intelligence

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INTRODUCTION

A bioengineered whole and region-specific brain organoids recapitulate the *in vivo* conditions in a physiological, molecular, and functional manner of healthy and diseased brain[1,2]. Well designed and automated fluidic systems allow the direct differentiation, study temporal variables, and grow organoids in long term with reproducibility, stability, durability and sustainability to improve organoid intelligence (OI)-on-a-dish studies[1,3,4,5]. OI describes an emerging multidisciplinary field working to develop biological computing using brain organoids and brain-machine interface technologies, which aims to leverage the extraordinary biological processing power of the brain[4]. Various mechano-transduction signals such as shear stress, cyclic stretching, hydrostatic pressure, compression, and fluid distribution provided by programmable fluidic dynamic, effect multiple biological processes including morphogenesis, spatio-temporal cellular organization, differentiation, and migration[1,5]. Although they employe for generation of physiologically functional brain organoids but are still not clearly understood. Here, we demonstrated two different engineering approaches, a newly designed microfluidic platform and rotary cell culture system (RCCS) that provide controlled dynamic laminar flow with lower shear stress conditions to improve advanced identity of high-quality brain organoid maturation.

RESULTS AND CONCLUSIONS

Further matured organoids have reached 95% harvestability with non-variable organoid sizes, rich cellular diversity (CD31+/b-cathenin+ endothelial like cells, CD11b+/IBA+ microglia, MAP2+/NEUN+ mature neurons, GFAP+/S100b+ astrocytes and MBP+/Olig2+ oligodendrocytes), structural morphogenesis (cortical plate, ventricular zone, subventricular zone, preplate stracture), expanted neuronal identity (Gabaergic, glutamatergic, progenitor-mature, forbrain, hindbrain neurons) and prolonged survivability (Ki-67+ and TUNEL-proliferative cells on around day 120) in **Figure 1**.

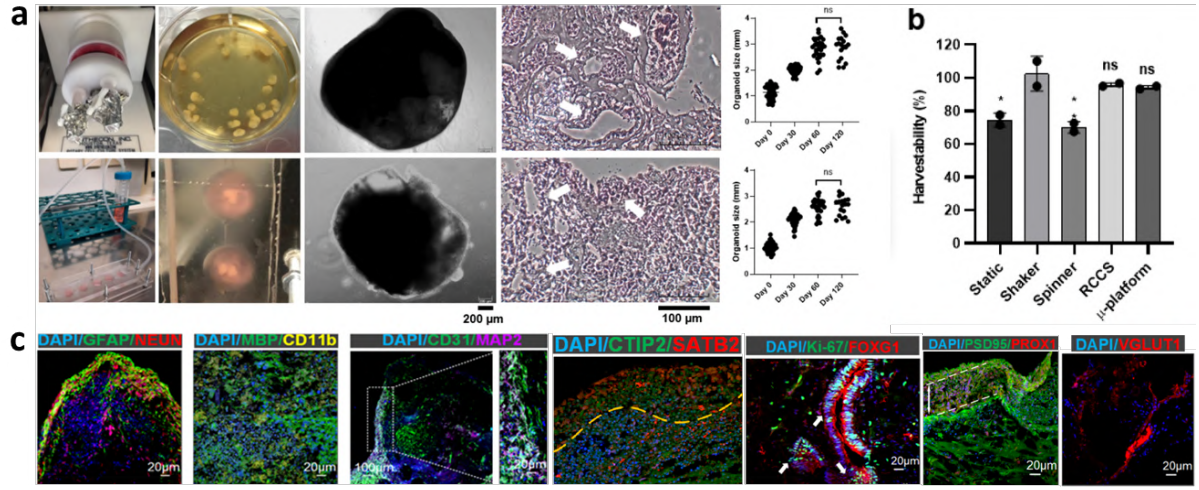

Fig.1 a Cerebral organoid maturation process in RCCS and µ-platform systems. **b** Percentage of organoid harvestability results. **c** Immunofluorescence staining of specific neural/glial/endothelial cells and maturation specific markers (GFAP, NEUN, MBP, CD11b, CD31, MAP2, CTIP2, SATB2, Ki-47, FOXG1, PSD95, PROX1, VGLUT1) at 120 days of cerebral organoids.

Combining of advanced organoid maturation systems with spatiotemporal chemical and electrophysiological signaling tools is a state-of-the-art technology to accelerate OI in the near future as a new frontier in biocomputing.

ACKNOWLEDGMENTS

Financial support provided by The Scientific and Technological Research Council of Turkey (TUBITAK) under grant number 119M578 is highly appreciated. Also, presenting first author gratefully acknowledges the TUBITAK 2211-A National Graduate Scholarship Program and 2214-A International Doctoral Research Fellowship Program.

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Evaluation of Nafamostat Mesylate on the Organotypic Lung Tissue Culture Model in the Management of Viral Infections

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INTRODUCTION

Antiviral strategies targeting host systems in viral infections may have both therapeutic and prophylactic potential and may help reduce the development of virus resistance[1]. Nafamostat Mesylate (NM) is a synthetic serine protease inhibitor used in the treatment of acute inflammatory diseases such as intravascular coagulation, shock and pancreatitis[2]. Along with various therapeutic effects of NM, *in vitro* studies have shown suppressive effects on SARS-CoV-2 and other seasonal influenza infections with the ability of NM to inhibit serine protease, which causes the development of airway inflammation[3]. In drug discovery and safety studies, the organotypic lung tissue culture model, which preserves *in vivo* microenvironmental conditions, lung physiology, functionality, and cellular relationships, has been shown to be a reliable, convenient and cost-effective method for the evaluation of antiviral agents in preclinical infection treatments in studies for the evaluation of diseases[4]. The model is a potential solution for translational models as it provides the opportunity to examine multiple regions of the lung. It also eliminates disadvantages such as high cost in *in vivo* cultures and ethical concerns in animal experiments[5]. Infection models prepared with *ex vivo* precision-cut lung tissue slices (PCLS) have a critical importance in identifying potential therapeutics that can suppress viral replication to prevent or alleviate viral infections that cause recurrent epidemics and global pandemics, and to accelerate their transition to the clinic[6]. Within the scope of the study, NM was evaluated in *ex vivo* organotypic lung tissue culture model by comparison with *in vitro* Calu-3 human lung airway epithelial cell culture.

RESULTS AND CONCLUSIONS

In this context, characterization tests were performed to evaluate the therapeutic and antiviral activities of NM. The safety doses of NM were determined as 10 µM for *in vitro* culture and 22 µM for *ex vivo* PCLS model that reduce virus infectivity while protecting cell/tissue viability and total protein content. After NM drug administration at safe and effective dose; it has been shown that cell death and proinflammatory responses due to virus infectivity are reduced. In order investigate the immune response against viral infection and NM treatment, gene expression levels of specific pro-inflammatory, anti-inflammatory and cell surface

markers were investigated. The significant inflammatory response has been shown to support the 3D *ex vivo* organotypic infection model established by H1N1-infected PCLSs. It was concluded that the effect of NM in reducing virus penetration is promising, and the use of the *ex vivo* organotypic lung tissue culture as a preclinical model is very suitable for screening candidate therapeutics in viral infections.

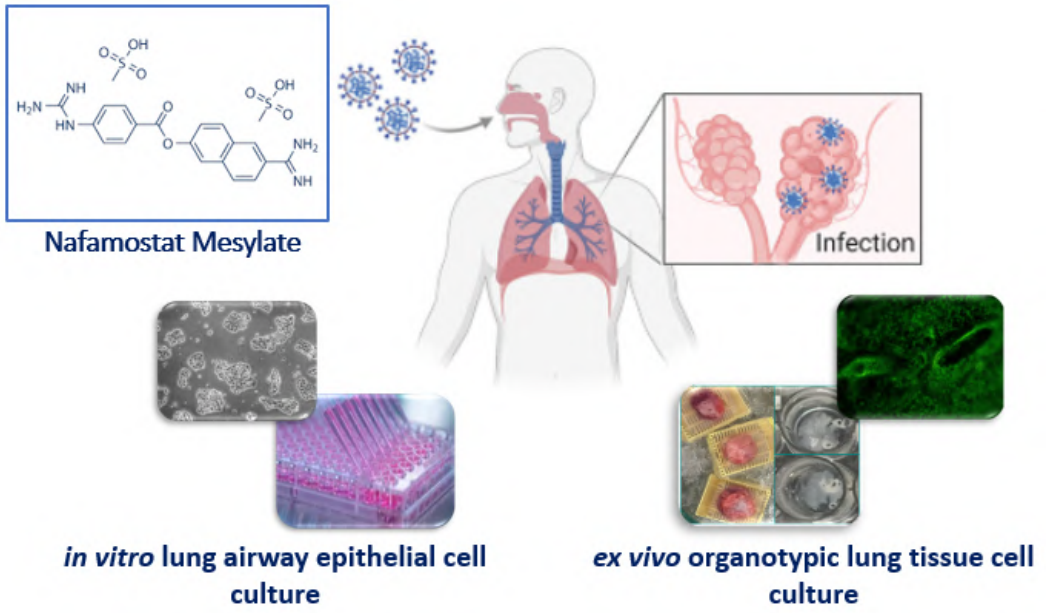

Fig.1 A schematic illustration of *in vitro* lung airway epithelial cell culture and *ex vivo* organotypic lung tissue cell culture as preclinical infection model.

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Panel For Eliciting Clinical Requirements to Be Implemented by Organ-On-Chips

Organ-on-Chip Systems and Current Applications

Animal models, which have constituted the basis of preclinical studies, have been used for many years to develop new drugs and methods for the treatment of human diseases. However, the inability of animals to fully mimic human physiology, the lengthy drug development process, and high failure rates have directed researchers towards alternative approaches. One such example of three-dimensional (3D) in vitro cell culture systems developed to meet this need is organ-on-a-chip (OoC) technology.

OoC systems designed to simulate tissue and organ-level physiology are microfluidic devices consisting of perfused channels that provide favorable environmental conditions for live cells[1,2]. The foundations of OoC technology were laid with the emergence of microelectromechanical systems (MEMS) in the 1960s, and significant progress has been made in the 2000s with the development of bioengineering tools such as soft lithography and 3D bioprinting. The development of the first tissue chip, the lung-on-a-chip, marked a pivotal moment in the field of tissue engineering[3]. In this model with two parallel microchannels, researchers used a PDMS membrane to mimic the alveolar-capillary barrier. PDMS, which is commonly used in OoC systems, is coated with ECM to better mimic the cellular microenvironment. Human alveolar epithelial and pulmonary endothelial cells were respectively seeded on top and bottom of the membrane, and a continuous flow was applied within the channels. Moreover, the dynamic structure of the lung has been simulated by applying a vacuum through the two channels located on the sides. In another study published, researchers were able to model cardiac tissue using the 3D printing method[4]. The device primarily consists of consoles that contain embedded sensors and a tissue-guiding layer, electrical interconnections, and eight independent wells. Researchers using PDMS in the tissue-guiding layer were able to support the formation of heart tissue by utilizing neonatal rat ventricular myocytes (NRVMs) and human-induced pluripotent stem cell-derived cardiomyocytes (hiPS-CMs). Furthermore, by applying the drug isoproterenol, which increases heart rate when ingested, to this system under *in vitro* conditions, researchers have observed the same result at the cellular level. The brain is another organ that has been studied at the chip level, and the maturation of organoids to mimic physiological conditions has recently become a prominent focus of researchs[5]. A recent study, investigated the effect of alpha-synucleins on the blood-brain barrier in Parkinson's disease[6]. In the study focused on the substantia nigra region, cells such as astrocytes, pericytes, and neurons were seeded into the brain channel, while endothelial cells were seeded into the vascular channel. These channels were separated by a porous PDMS membrane, and after permeability analysis, Parkinson's pathology was induced, resulting in observed damage to the membrane structure. Subsequently, it was demonstrated that injecting the disaccharide trehalose into the vascular channel led to significant passage into the brain channel and improved the membrane structure. Real-time measurements can also be obtained through sensors integrated into brain-on-a-chip platforms[7]. In addition, OoC platforms are also used to mimic eye diseases. In another study, retina organoids (ROs) generated from human induced pluripotent stem (hiPS) cells were combined with a chip platform[8]. The main goal was to enable ROs, which are devoid of vascularization, to access nutrients carried by the blood in a manner like the *in vivo* environment. In the chip system, retinal pigment epithelium cells were seeded on a porous membrane separating the perfused channel at the bottom and the tissue chamber above. Subsequently, the ROs were

placed in the top tissue chamber, and it was observed that the photoreceptors interacted with the RPE cells on the chip via phagocytosis. This study successfully modeled a complex organ such as the retina, which contains many different types of cells, and demonstrated its potential as a model for finding suitable treatment methods for eye diseases in the future.

The "Panel for eliciting clinical requirements to be implemented by organ-on-chips" moderated by Prof. Dr. Ozlem Yesil Celiktas, brought together four distinguished clinicians from different specialties: Cardiologist Prof. Dr. Oguz Yavuzgil, Ophthalmologist Assoc. Prof. Ozlem Barut Selver, Pulmonologist Assoc. Prof. Ozlem Goksel, and Neurologist Assoc. Prof. Ayse Guler. During this panel, the clinicians shared their perspectives on the clinical needs and requirements in their respective fields and discussed how organ-on-chip technology could be used to address these needs. They also provided insights into the current state-of-the-art in organ-on-chip technology, the challenges in developing robust and reliable models, and the potential impact of these models on advancing our understanding and treatment of diseases.

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Poster Presentations for “Young Researchers Excelling In Novel Fluidics” Awards

No	Presenting Author	Poster Name
P.1	Beyzanur Ozogul	Hydrodynamic Cavitation on a Chip: A Tool for Anticancer Activity Improvement
P.2	Ece Nur Ertung	Comparison of Production Modes for Adalimumab Biosimilar
P.3	Ercil Toyran, Farzad R.Talabazar	The Local characterization of cavitating flow fluctuation in HC on a chip
P.4	Rabia Mercimek, Unal Akar, Morteza Ghorbani, Beyzanur Ozogul	A novel Clot-on-Chip Model Based on Complex Multiphase Flow
P.5	Sena Yanasik, Yagmur Filiz	Engineered Microfluidic Platform for Cerebral Organoid Maturation
P.6	Yagmur Arslan	Extracellular matrix Hydrogel Derived from decellularized liver tissue for recapitulation of the liver-specific microenvironment
P.7	Ozgur Can Gumus	Isogeometric Boundary Element Formulation for Deformable Particles
P.8	Reyhan Coban	Electrically Conductive Biomaterials: Production and Impact on Proliferation in Motor Neuron-Like Cells

Biyomimetik Mikrosistemler

Hydrodynamic Cavitation on a Chip: A Tool for Anticancer Activity Improvement

Beyzanur Özogul*, İlayda Namlı*, Zeynep Karavelioglu*, Sevilay Burcu Sahin*, Seyedali Sarraf, Araz Aghdam, Rahmetullah Varol, Abdurrahim Yılmaz, Dila Bozkaya, Havva Yagci Acar, Huseyin Uvet, Sibel Cetinel, Ozlem Kutlu, Morteza Ghorbani, Ali Koşar

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Hydrodynamic cavitation-on-a-chip (HCOC) and its prospective applications have sparked great attention in recent years because of advancements in lab-on-a-chip technologies. In this work, we propose using HC as an adjuvant to produce discrete zones of cellular damage and improve the anticancer activity of Doxorubicin (DOX). In addition, after the DOX treatment, HC was added to the confluent cell monolayer. It was discovered that the combination of DOX and HC has a stronger anticancer action on cancer cells than DOX alone. Using carbon dots (CDs), the effect of HC on cell permeabilization was also demonstrated. Finally, the cell stiffness parameter, which has been linked to cell proliferation, migration, and metastasis, was investigated using cancer and normal cells exposed to HC. The HCOC has the benefit of concurrently establishing well-defined zones of bio-responses upon HC exposure, accomplishing cell lysis and molecule delivery by permeabilization by giving spatial control. Ultimately, micro-scale hydrodynamic cavitation is a promising method for increasing the therapeutic efficacy of anticancer drugs.

INTRODUCTION

Cavitation is a phase change event caused by low local pressures that involve the rapid development and collapse of vapor bubbles inside a medium. While extensive research on hydrodynamic cavitation (HC) has been conducted in recent years, current findings on the effect of HC on cellular entities are insufficient to fully assess the interaction between cavitation physics and biophysical response. In this study, we investigated the biophysical effect of HC on confluent cell monolayers using an HC-on-a-chip device (HCOC) (cascade parallel multi-microchannel device). The HCOC device enables spatial control of HC exposure, resulting in a regulated bio-response of cells. We studied the areas as cell responses to HC exposure. The SEM (Scanning Electron Microscopy) technology was used to examine the changes in cell morphology caused by cavitation in detail. Calcein AM/PI staining was used to analyze dead and living cells in the cavitation zone. Additionally, CDs (carbon dots) were used to show the effect of HC on cell permeability, while an acousto-holographic microscope was used to examine changes in the biomechanical structures of cells after HC exposure. The effects of HC alone and in conjunction with DOX on cancer and non-cancerous cells were also studied. The preliminary data show that when anticancer medicines are combined with HC, their anticancer potency is enhanced. We believe the findings show that micro-scale HC has a significant potential for integrating several cellular responses at the same time, indicating its utility in biological research.

MATERIAL METHODS

SH-SY5Y, MCF-7, HUVEC, A549, and BEAS-2B cells were used in cell culture studies. Cells were counted using a Thoma chamber after trypan-blue staining. Cavitation experiments were performed on confluent cells. The inlet pressure was set at 758 kPa. Each sample was treated with HC for 3 seconds. The used microfluidic device has one inlet and eight parallel micro-orifices, with an open wall serving as the device's outlet, where the fluid stream departs. The impact of HC and DOX therapy on cell growth was assessed using cytotoxicity analysis. SEM analysis was conducted after gold-palladium coating at an acceleration voltage of 2 kV. Dead/living cells were analyzed using Calcein AM and PI solutions, followed by fluorescence microscopy. Cellular uptake without HC exposure was assessed at various time points. Elasticity modulus and cell stiffness were measured using an acousto-holographic microscope. Immunocytochemistry analysis was performed on A549 and BEAS-2B cells using DAPI and a confocal microscope. Statistical analyses were performed using one-way ANOVA and Newman-Keuls multiple comparison test.

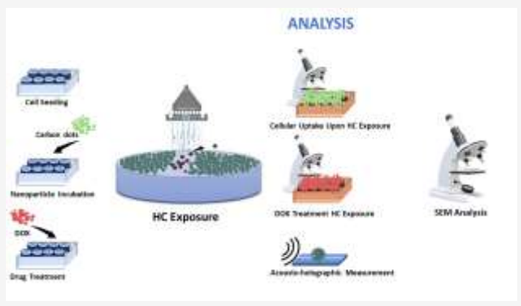

Figure 1: A diagram summarizing the experimental methods before and after HC exposure.

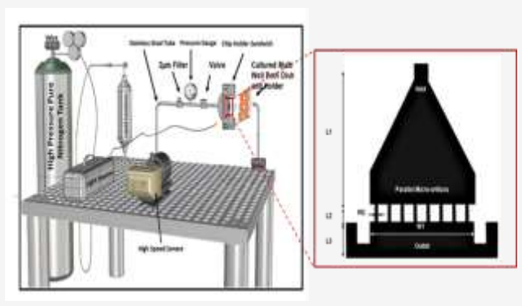

Figure 2: Experimental setup

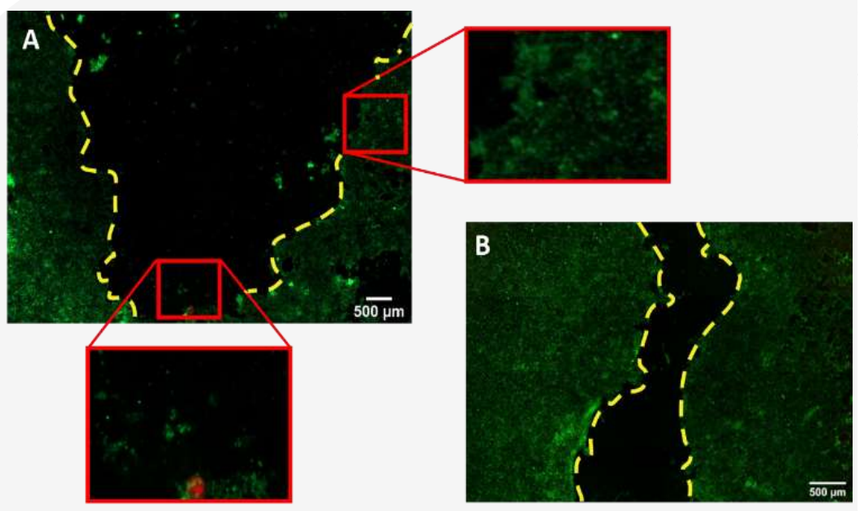

Figure 3: Fluorescent images of the A) A549 cells and B) BEAS-2B cells after Calcein AM-PI staining following HC exposure. The different regions appear based on cell attachment upon HC exposure. Most of the detached cells are washed with the help of HC. Some cells keep their attachment, but not their viability. The green color represents the viable cells, while the red color refers to dead cells.

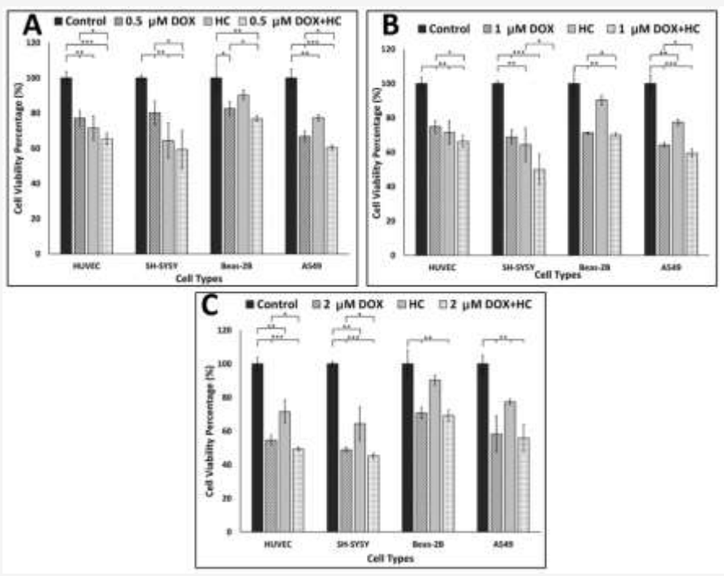

Figure 4: Cell viability profiles of HUVEC, SH-SY5Y, BEAS-2B ,and A549 cells. Cell viability is shown for cases without any treatment and exposure (control), with treatment with different concentrations of DOX. [A) 05 mM, B) 1 mM, C) 2 mM], upon HC exposure and after different concentrations of DOX [A) 05 mM, B) 1 mM, C) 2 mM] combination with HC exposure.

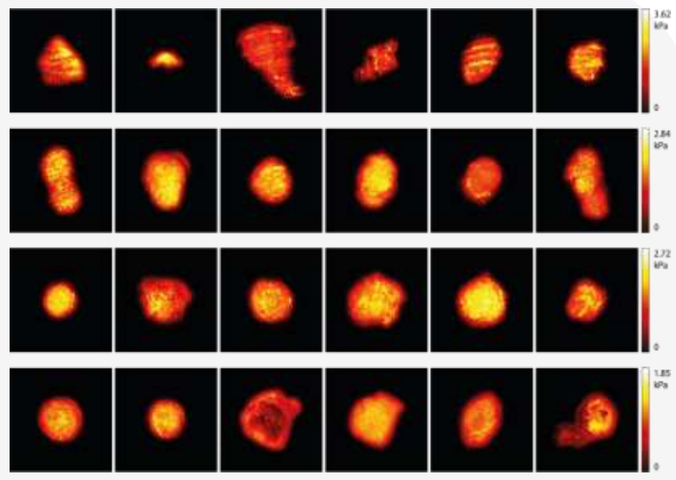

Figure 5: Acousto-holographic stiffness distribution of the cells from top to bottom rows A549 control cells, HC-treated A549 cells, BEAS-2B control cells, and HC-treated BEAS-2B cells.

CONCLUSION

In vitro research shows that micro-scale HC is a potential strategy for studying various cellular responses and increasing the chemotherapeutic effectiveness of the anticancer medication DOX. Within the realm of experimental research, the cell proliferation assay demonstrates that cancer cells treated with the combination of DOX + HC have more pronounced anticancer activity than cells treated with DOX alone. We also showed that on cancer cells, HC has a stronger cytotoxic effect than on normal cells. Following cavitation exposure, an indistinct number of dead cells are discovered near the cavitation zone. Furthermore, the use of CDs has demonstrated that HC has greater anticancer activity by increasing the DOX penetration of cancer cells. Finally, changes in cell stiffness associated with cell proliferation, migration, and metastasis following HC treatment are investigated.

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Biyomimetik Mikrosistemler

Comparison of Production Modes for Adalimumab Biosimilar

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ABSTRACT

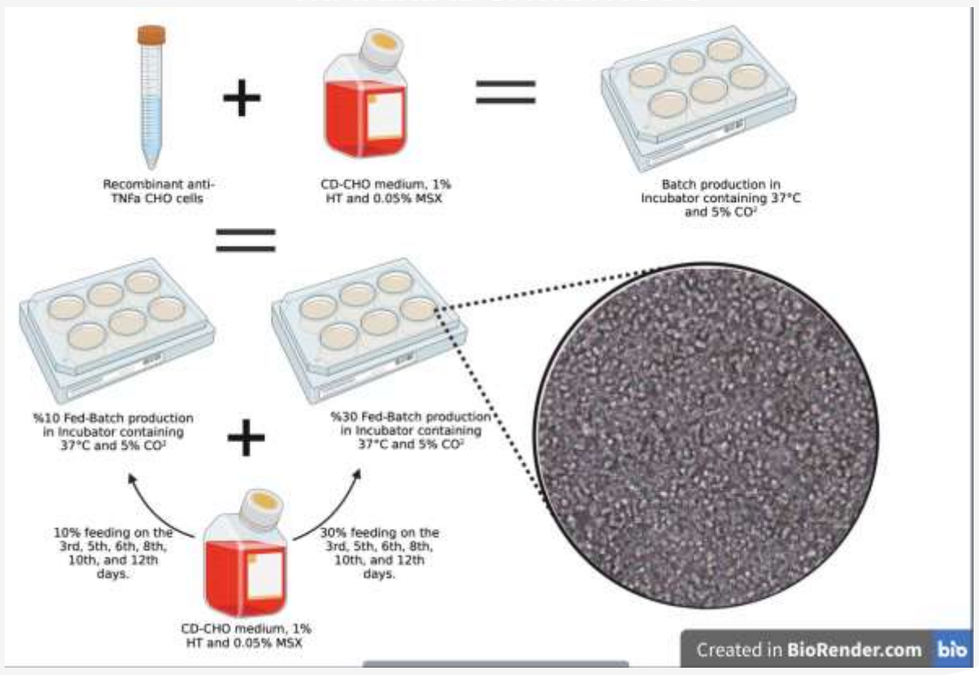
In the developing and changing world economy, where biotechnology is at the center, it is very important for Turkey to enter and take place in the biosimilar market. In this respect, considering the high demand in the world, it is necessary to determine the most efficient way of the production of biotherapeutics. The aim of this study is to determine the most efficient way for the production of monoclonal antibody (Adalimumab, Humira®) which specifically binds to anti-tumor necrosis factor alpha (TNF-α) by comparing and optimizing the production modes at the laboratory scale. For this purpose, as the first step batch and two feeding strategies (10% and 30%) of fed-batch production modes were carried out by using TNF-α producer recombinant Chinese Hamster Ovary (rCHO) cells. Following this study, the perfusion mode will also be tested, and finally regards metabolite analysis and antibody titer to compare the efficiency of all production modes. Thus, the most efficient method for the production of this biosimilar, which is licensed in Turkey but not commercially produced in our country, will be selected and its applicability to the industry will be demonstrated.

Keywords: Biosimilars, Recombinant Chinese Hamster Ovary (rCHO) cells, Adalimumab, production modes

INTRODUCTION

In the biomedical market, particularly in the production of human therapeutic proteins, the most commonly used mammalian expression system is rCHO cells (1). Adalimumab (trade name Humira®) is a monoclonal antibody which specifically fully human IgG1 that binds to TNF-α and neutralizes its activity, also produced from rCHO cells. Adalimumab is currently used in the treatment of rheumatoid arthritis, ankylosing spondylitis, Crohn's disease, juvenile idiopathic arthritis, psoriatic arthritis, and psoriasis (2). Batch and fed-batch production modes are commonly used in bioprocesses, while perfusion is less preferred than other modes. However, perfusion is a production mode that increases volumetric productivity and product quality by increasing cell concentration through continuous feeding of fresh media and removal of toxic byproducts (3). Especially in biotherapeutics, different production modes can also affect the properties of the product in terms of its structural, biochemical, and functional aspects. Therefore, the choice of production mode has directly related the quality and quantity (4).

MATERIAL & METHODS



RESULTS

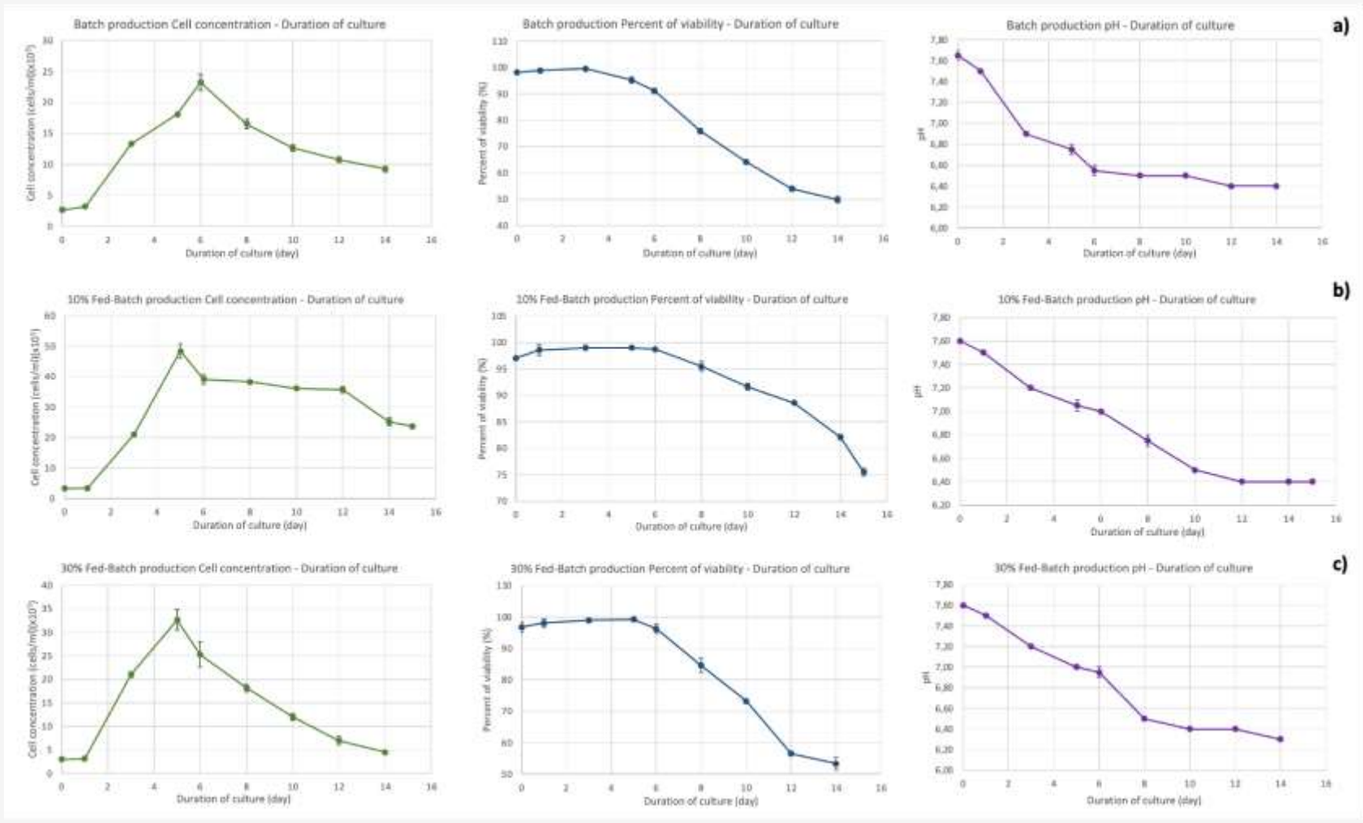


Figure 1: Growth kinetics graphs of rCHO cells in different production modes;
a) Batch, b) 10% Fed-Batch, c) 30% Fed-Batch

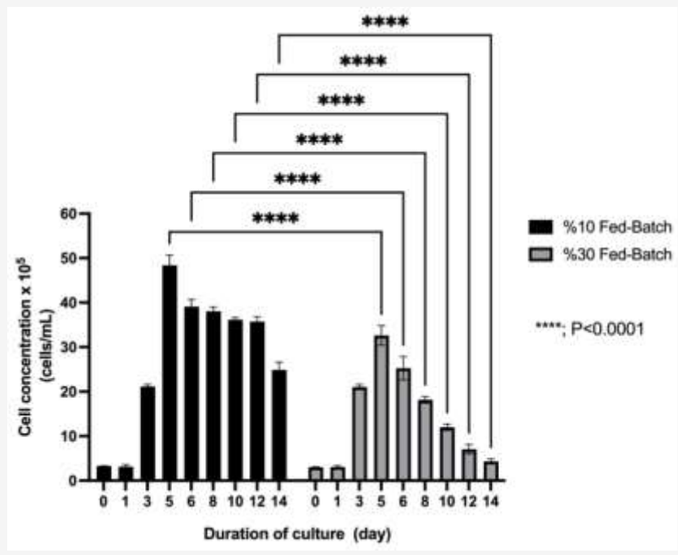


Figure 2: Comparison of 10% fed-batch and 30% fed-batch productions using ANOVA test.

CONCLUSION

As a result, comparing the growth kinetics of batch, 10% fed-batch, and 30% fed-batch productions, it is determined that the cell concentration throughout the production is lower in batch production than in both 10% and 30% fed-batch productions. For fed-batch experiments, the 30% production set has shown a rapid decrease in cell concentration and lost its cell viability compared to the 10%. Especially in the 10% production set, it was observed that cells remained in the stationary phase for a long time and maintained their viability at maximum levels. By considering the scientific information that monoclonal antibodies are a secondary metabolite and these are more efficiently produced in the stationary phase, therefore these results indicate that the 10% fed-batch production is the most efficient feeding strategy and production mode compared to the other two production sets.

ACKNOWLEDGEMENT

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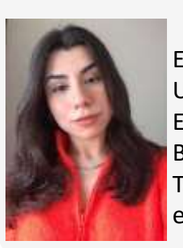
CONTACT



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Biyomimetik Mikrosistemler

The Local Characterization of Cavitating Flow Fluctuation in HC on a Chip

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ABSTRACT

Hydrodynamic Cavitation (HC) is a phase change phenomenon occurring when the flowing liquid pressure drops suddenly below the vapor saturation pressure. This phenomenon in microfluidics leads to the development of the HC-on-a-chip concept which have been mostly investigated via high-speed visualization systems to date. However, along with the advanced visualization methods, there is a need for auxiliary methods to locally characterize the cavitation phenomenon on microscale. In this study, we propose a new acoustic noise and vibration measurement method for HC on a chip concept measurements.

Keywords: Hydrodynamic Cavitation-on-chip, Microfluidics, Noise measurement, PVDF Sensor

INTRODUCTION

Cavitation is the formation and collapse of vapor cavities due to a sudden pressure drop in liquid, which causes noise and vibrations that can erode surfaces. Hydrodynamic cavitation has implications in various fields, including biomedical engineering [1], with different behavior when it is formed on the microscale. High-speed visualization techniques coupled with lighting equipment are usually used to detect cavitating flow patterns, but this is challenging in microchannels due to the scale effects, inefficient illumination, and microchannel opaqueness [2]. To address this challenge, a new auxiliary measurement technique based on noise measurement due to bubble collapse is proposed in this study, which can detect microscale cavitation initiation, evaluate flow patterns, and could detect tiny bubbles that are not visible with visualization techniques.

MATERIAL METHODS

The glass wafer assists in visualizing the fluid flow inside the channels. The experiments were conducted by applying different inlet pressures. The inlet pressure varied from 1 to 7 MPa, while the outlet pressure at the microchannel was fixed to 0.1 MPa. The volumetric flow rate increased with the change in the inlet pressure. During the measurement, a 128-µm thick PVDF piezoelectric membrane was adhered to the microchip using a double-sided adhesive. The data obtained from the PVDF sensor were analyzed on MATLAB using Fast Fourier Transform (FFT) and Wavelet transform techniques together with the image processing of the images record via camera at 70,000 fps.

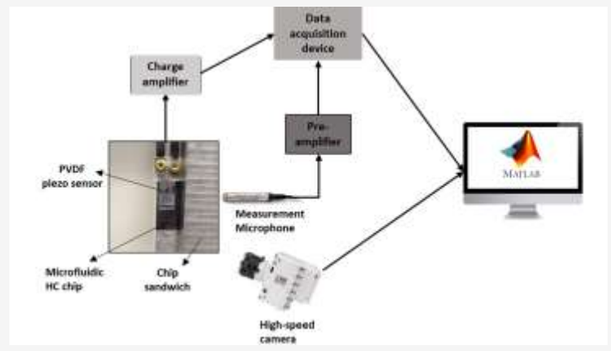

Fig. 1 Measurement setup

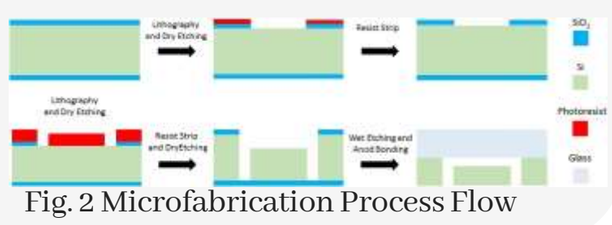

Fig. 2 Microfabrication Process Flow

RESULTS

Power Spectral Density(PSD) analysis(fig.3) shows the change in the intensity of cavitation noise in the frequency domain. At the cavitation inception point, an increase of about 6 dB is seen in the 8khz-24khz frequency range. Beyond inception, the cavitation intensity increases with the increase in pressure, and this increase appears consecutively between 4kHz and 24kHz and reaches the highest level when the cavitation is fully developed. Wavelet analysis(fig.4) shows the intensity change in the frequency domain with respect to time. With time-dependent frequency analysis, the time of the cavitation inception can be detected with the increase at high frequencies. As the cavitation intensity increases, the spikes at high frequencies (10kHz-24kHz) become stronger over time.

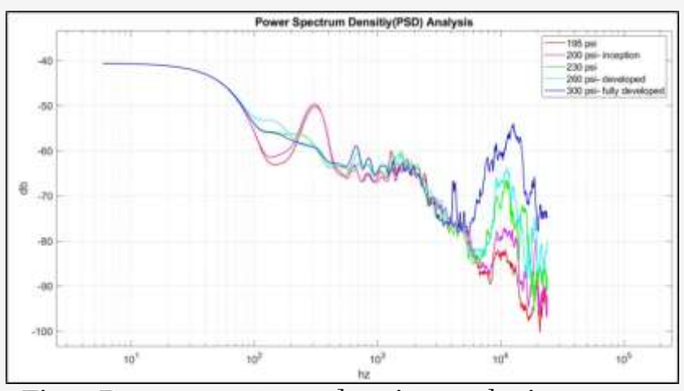

Fig. 3 Power spectrum density analysis

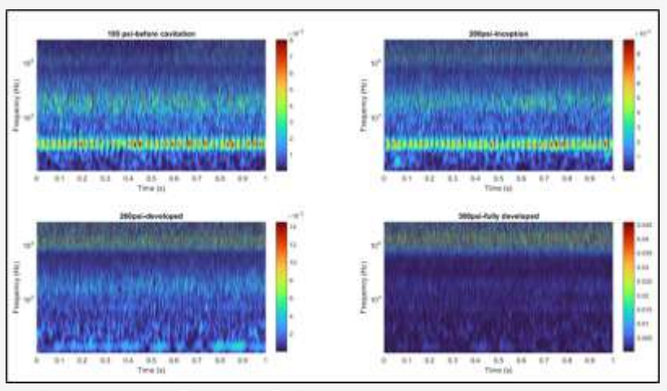

Fig. 4 Wavelet analyis

CONCLUSION

The proposed measurement method can detect the inception and evolution of cavitation, which can be used alongside high-speed visualization methods for the HC-on-a-chip concept. The results show that a sudden increase at high frequencies was observed at the first stages of the cavitation bubbles formation. Furthermore, with the development of cavitation, the high frequencies increase consecutively with the increase in flowrate and reach a maximum in fully developed cavitating flow.

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Biyomimetik Mikrosistemler

A Novel Clot-on-Chip Model Based on Complex Multiphase Flow

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Excessive clotting can lead to dangerous disorders, which is treated with chemical medications with adverse effects and limitations. Microfluidic advancements enable easier operation and employment of complex flow in organ on a chip studies. This study presents a novel concept based on the generation of excessive microbubbles in the field of adverse pressure inside a single microchannel. The flowing fluid of different qualities is directed to the targets i.e., clot, in this approach and a model for efficient clot studies in the micro domain is suggested.

INTRODUCTION

Venous thromboembolism affects 1-2 in 1,000 people annually and is a leading cause of vascular mortality. Current treatments have drawbacks including high failure, recurrence rates, and the risk of distant embolism. Microscale cavitation has been researched for its ability to ablate diseased tissue. It is a physical phenomenon generated by the liquid being forced to flow at high speeds in a limited channel or nozzle causing bubbles to form and burst, producing shock waves and pressure gradients. In this study, the aim is to understand the influence of microbubbles on the clot using the microfluidic device for the first time. The findings of this work will contribute to the development of novel technologies for drug-free catheter-directed thrombolysis prototypes, as well as improve knowledge of cavitation induced thrombolysis at the micro-scale.

MATERIAL METHODS

The experiments were conducted employing singular-channel chips, whose configuration is depicted in Figure 1. The chip design encompasses an inlet and outlet radius of 900 µm, an inlet channel measuring 3750 µm in length and 900 µm in width, a microchannel spanning 100 µm in length and 300 µm in width, as well as an extended channel measuring 3750 µm in length and 900 µm in width, coupled with reservoirs that are connected to this channel. To execute cavitation, the experimental apparatus comprises a high-pressure nitrogen tank (Linde Gaz, Gebze, Kocaeli), a container for liquid (Swagelok, Erbusco BS, Italy), stainless steel pipes (Swagelok, Erbusco BS, Italy), and a sandwich.

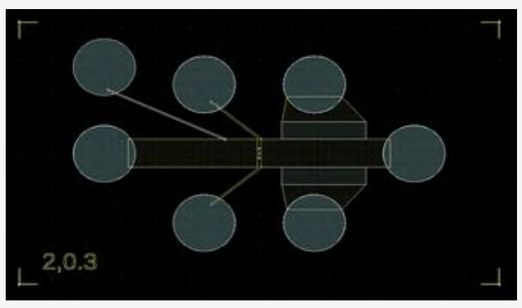

Figure 1: single channel chhip design

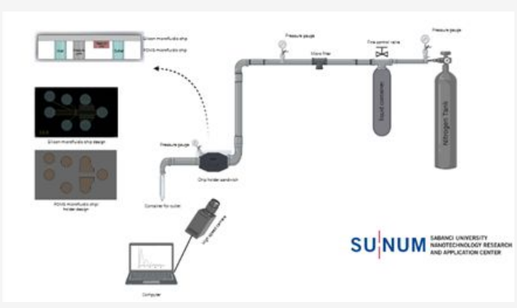

Figure 2: Experimental setup shema

RESULTS

Based on the initial outcomes, we have demonstrated that sheet cavities are appeared at the upstream pressure of 100 psi, as depicted in Figure 3. As the pressure increases, the cavity length inside the sheet cavity increases stably without the shedding of the cavity clouds or other vortices, as demonstrated in Figure 4. These findings constitute a promising foundation for the development of the concept and utilize the complex flow in the disintegration of clots.

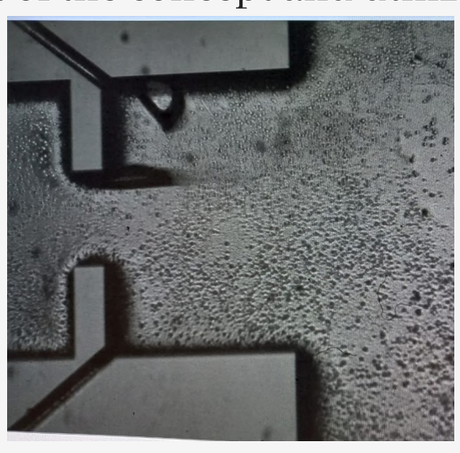

Figure 3: 100 PSI

Figure 4: 155 PSI

CONCLUSION

In this study, a single channel chip designed to examine the effect of cavitation on clot is discussed. In the chip designed for this purpose, it was first tested whether cavitation occurred which is the principle aim to perform the clot experiments in the next step. By optimizing the system, the cavitation was successfully visualized, data for the physical activity and initial pressures of the cavitation were obtained.

Engineered Microfluidic Platform for Cerebral Organoid Maturation

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ABSTRACT

Organoids are three-dimensional structures that are derived from stem cells and have the potential to mimic the complexity and functionality of *in vivo* organs. Induced pluripotent stem cells (iPSCs) have been used to generate organoids that are specific to various tissues, including cerebral organoids, which mimic the developmental processes of the human brain. Microfluidic platforms have been developed as a promising tool for cerebral organoid generation and maturation and these platforms provide a controlled microenvironment that can simulate the dynamic systems of the human brain. By combining the advantages of cerebral organoids and microfluidic systems, more accurate models of the human brain can be developed and advance our understanding of neurodevelopmental and neurological diseases to support disease modeling and drug discovery. This study demonstrates the potential of microfluidic platforms in cerebral organoid maturation, including reproducibility, hydrodynamic forces, astrocyte, microglia, oligodendrocyte, neuron and endothelial cell diversity, brain morphogenesis, viability and functionality.

Keywords: Cerebral organoid maturation, microfluidic platform, computational fluid dynamics simulation, cellular diversity, functionality

INTRODUCTION

In vitro models that accurately replicate the human brain are essential for researching neurodevelopmental and neurological diseases and developing novel treatments. Cerebral organoids, generated from iPSCs due to their self-organizing ability and diverse cell differentiation, are a promising option to mimic the human brain (Kim et al., 2020). While cerebral organoids hold great potential for modeling the human brain, their maturation process is complex and microfluidic systems have been developed as promising tools. These systems provide a controlled microenvironment that mimics the physiological conditions of the human brain and allows for the observation of neurodevelopmental processes in real-time (Cho et al., 2021). Microfluidic systems also offer great reproducibility, morphological similarity, and harvestability, making them a highly appealing choice for the maturation of cerebral organoids. The combination of cerebral organoids and microfluidic systems presents a powerful tool for disease modeling and drug discovery, as well as for advancing our understanding of the human brain (Seiler et al., 2022).

MATERIALS & METHODS

For the generation of cerebral organoids, iPSCs induced to form embryoid bodies. Following neuroectoderm and neuroepithelium formation, neuroepithelial tissues were embedded in a Matrigel droplet and generation steps was completed with expanded neuroepithelium structure (Lancaster and Knoblich, 2014). After 15-day generation, cerebral organoids were transferred to PDMS-based microfluidic platform and maturation was provided with a dynamic laminar flow system for 120 days. Maturation dynamics were evaluated with computational fluid dynamics simulation and maturated cerebral organoids were characterized in terms of cellular diversity, brain morphogenesis, viability and functionality (Saglam-Metiner et al., 2023).

Figure 1. Cerebral organoid maturation in microfluidic platform

RESULTS

When the shear stress profiles were evaluated at different initial flow conditions by creating the 3D geometry of the microfluidic platform **(Fig 1a)**, the maximum and minimum shear stresses on the microfluidic platform were observed as 7.72×10^{-3} and 5.13×10^{-7} Pa at 100 µl/min (**Fig 1b**). The characteristics of gravity-driven laminar flow allowed for more homogenous shear stress distributions to be seen on the organoids in the microfluidic platform (**Fig 1c**).

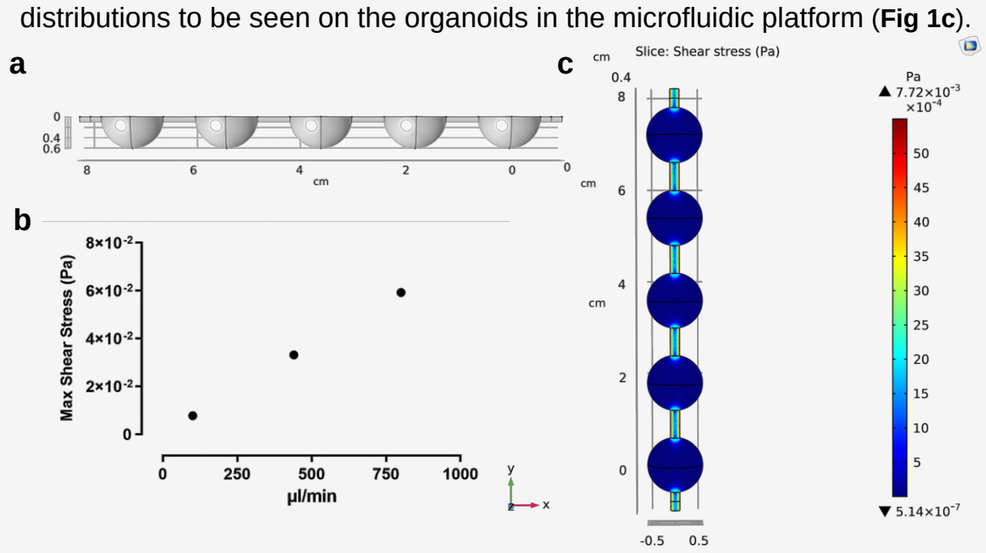

Figure 2. 3D geometry of the microfluidic platform (a), maximum shear stress values for different initial flow conditions in the microfludic platform (b), shear stress simulation for 100 µl/min flow rate in the microfludic platform (c).

Cerebral organoids were grown to an average size of 3 mm on day 120. Various cell types were observed within the organoids including CD11b+ microglia, MBP+ oligodendrocytes, GFAP+ astrocytes, NEUN+ neurons, and CD31+ endothelial cells (**Fig 3**). CTIP2+ cells in the upper layers upregulated b-catenin levels indicated spatial organization of the cortical plate (**Fig 4**), while the presence of Ki-67+ cells demonstrated viability and proliferation, GABA-A+ and VGLUT1+ cells highlighted further maturation (**Fig 3**, Saglam-Metiner et al., 2023).

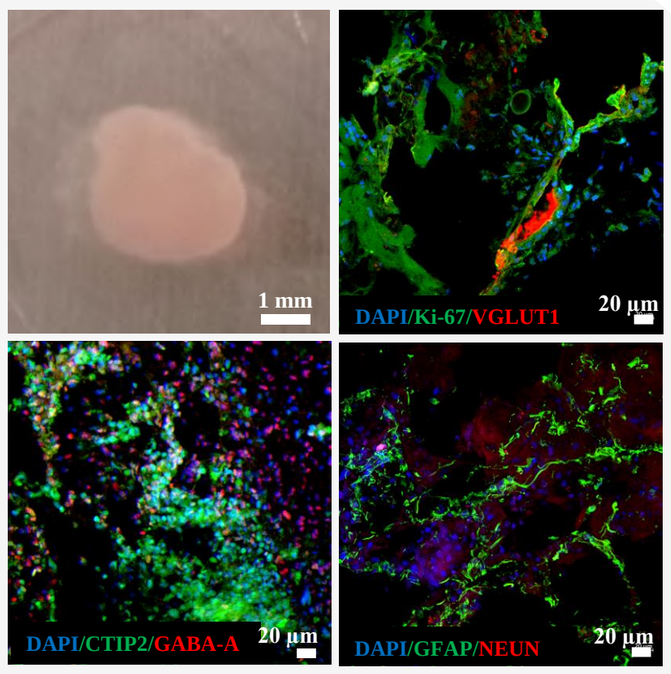

Figure 3. Immunofluorescence staining of specific markers at 120 days of cerebral organoids matured in microfluidic platform (scale bars = 20 µm for 25× magnification images)

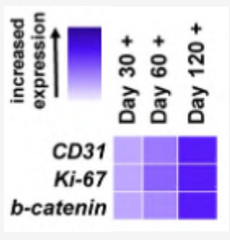

Figure 4. qRT-PCR analysis for CD31, Ki-67, and β-catenin gene expression of cerebral organoids matured in microfluidic platform on day +30, day +60, and day +120.

CONCLUSION

Results suggest that gravity-driven laminar flow in the microfluidic platform will recapitulate the characteristics of human embryonic cortical development, providing enhanced cellular diversity, spatial brain organization, viability and functionality of cerebral organoids. Cerebral organoids matured in microfluidic platform represent a powerful tool for studying the human brain and developing new treatments for neurodevelopmental and neurological diseases.

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Biomimetic Microsystems

EXTRACELLULAR MATRIX HYDROGEL DERIVED FROM DECELLULARIZED LIVER TISSUE FOR RECAPITULATION OF THE LIVER-SPECIFIC MICROENVIRONMENT

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ABSTRACT

Decellularized extracellular matrix (dECM)-based biomaterials have become popular in tissue engineering applications for constructing 3D *in vitro* liver models. The dECM is obtained through various methods that remove cellular components while retaining the structure, protein composition, and mechanical properties of native tissue. dECM hydrogels can closely recapitulate the ECM of native tissue and facilitate the adhesion, growth, proliferation, differentiation, and migration of cells. This study employed a detergent-based decellularization protocol for the porcine liver to fabricate a biomimetic hydrogel to recapitulate the liver microenvironment.

INTRODUCTION

Nowadays, tissue engineering strategies have given rise to a preference for dECM-based biomaterials in the construction of 3D *in vitro* liver models. The dECM is procured through various physical, chemical, and biological decellularization methods, which eliminates cellular components from the native tissue while retaining the tissue's structure, protein composition, and mechanical properties [1]. dECM hydrogels have the ability to closely recapitulate the ECM of the tissue and support the adhesion, proliferation, differentiation, and migration of cells. Such biomaterials comprise structural proteins such as collagens and elastin, proteoglycans, and adhesion proteins like laminin, fibronectin, integrin [2].

MATERIALS & METHODS

To emulate the 3D microenvironment of liver tissue, a detergent-based decellularization protocol was employed for the porcine liver. The resulting ECM was then processed through acid-pepsin digestion to create a pre-gel form (Fig 1A). Subsequently, the pre-gel solution was subjected to physiological conditions, which facilitated its conversion into a 3D hydrogel form. To assess the biocompatibility of the developed hydrogel, the liver hepatocyte cell line (HUH-7) was embedded within the ECM hydrogel, and qualitative and quantitative viability analyses were performed.

RESULTS

- After four-day detergent-based decellularization, the porcine liver was effectively decellularized (%99), with an amount of dsDNA less than 50 ng/mg (Fig 1C).
- SEM images reveal that dl-ECM with a high level of porosity and fibril proteins, as well as an absence of cellular materials compared to the native liver (Fig 1B).
- When HUH-7s were embedded in the dl-ECM hydrogel, their viability was significantly higher compared to GelMA, a widely used hydrogel to mimic 3D microenvironment both on day 1 and day 5 (Fig 1E-D).

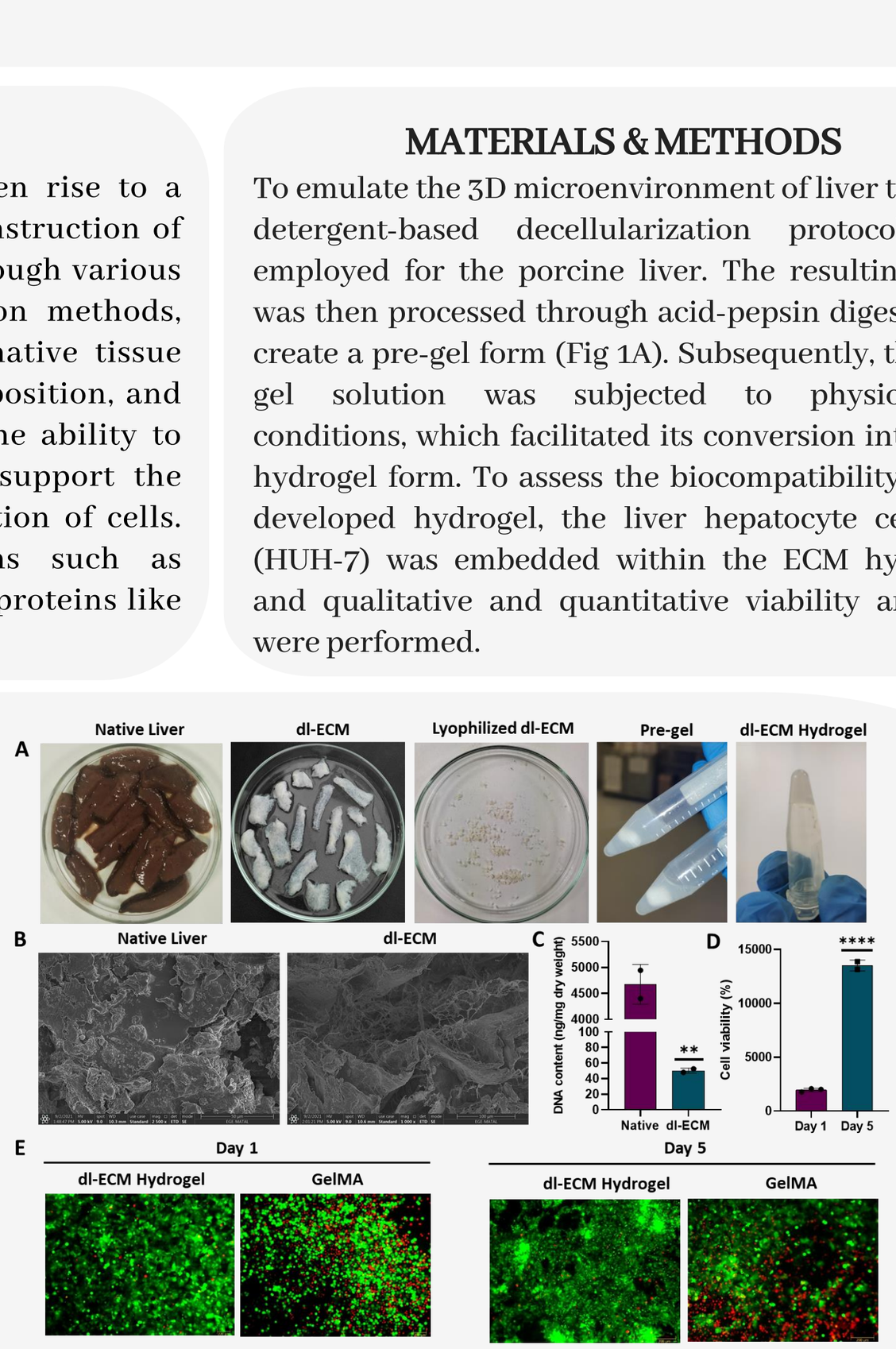

Figure 1. (A) Liver ECM hydrogel formulation process, **(B)** SEM images and **(C)** DNA content analysis of both native and decellularized liver tissue, **(D)** Cell viability (%) and **(E)** Live/Dead images of HUH-7 cells embedded in dl-ECM hydrogel both on day 1 and day 5 .

CONCLUSION

These findings suggest that the developed decellularization methodology successfully removed cellular components from porcine liver tissue, resulting in a biomimetic hydrogel that recapitulates liver-specific microenvironment with desirable ECM protein content, which has potential applications in tissue engineering applications.

ACKNOWLEDGEMENT

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Isogeometric Boundary Element Formulation for Deformable Particles

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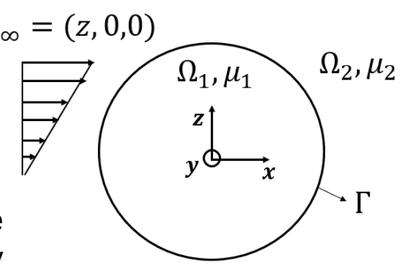
Boundary Element Modeling (BEM)

BEM maps partial differential equations (PDEs) onto surfaces and interfaces of the geometry which reduces the dimensionality of the problem by one.

Employing fundamental solutions of PDEs make it a semi-analytic and highly accurate technique.

Fundamental solutions of Stokes equations are available which govern the incompressible, low-Reynolds number flows both internal and external to the interface.

$$\begin{cases} -\nabla p_i + \mu_i \nabla^2 \boldsymbol{u}_i = 0 \\ \nabla \cdot \boldsymbol{u}_i = 0, \end{cases} \quad \text{on } \Omega_i, \text{ with } i = 1,2$$



u_i, p_i, and μ_i prescribe the velocity, pressure and the viscosity of the i^{th} phase. $\lambda = \mu_2/\mu_1$ denotes the viscosity ratio. The boundary conditions are:

$$\begin{cases} (\Pi_1 - \Pi_2) \cdot \boldsymbol{n}(\boldsymbol{x}) = \boldsymbol{f}(\boldsymbol{x}) \\ \boldsymbol{u}_1 = \boldsymbol{u}_2 \end{cases} \quad \text{on } \Gamma \quad \text{where} \quad \boldsymbol{f}(\boldsymbol{x}) = \frac{2}{Ca} \kappa(\boldsymbol{x}) \boldsymbol{n}(\boldsymbol{x})$$

The boundary integral equation for the velocity of a point x_0 positioned on the interface Γ can be expressed as follows:

$$(1+\lambda) u_j(\boldsymbol{x}_o) = 2u_j^\infty(\boldsymbol{x}_o) - \frac{1}{4\pi\mu_1} \int_\Gamma f_i(\mathrm{x}) G_{ij}(\boldsymbol{x}, \boldsymbol{x}_\mathrm{o}) \mathrm{d}\Gamma + \frac{1-\lambda}{4\pi} \int_\Gamma u_\mathrm{i}(\boldsymbol{x}) T_{ijk}(\boldsymbol{x}, \boldsymbol{x}_\mathrm{o}) n_k(\boldsymbol{x}) \mathrm{d}\Gamma$$

Fundamental solutions: $G_{ij}(\boldsymbol{x}, \boldsymbol{x}_0) = \frac{\delta_{ij}}{r} + \frac{\hat{x}_i \hat{x}_j}{r^3}$

$$T_{ijk}(\boldsymbol{x}, \boldsymbol{x}_0) = -6 \frac{\hat{x}_i \hat{x}_j \hat{x}_k}{r^5}$$

Isogeometric Formulation

The isogeometric analysis proposed by Hughes *et. al.* (2005) closes the gap between CAD engineering analysis models by using the same parametric functions, generally in the form of B-splines, used to create geometry to approximate the unknown field variables.

A univariate B-spline can be defined by a non-decreasing knot vector:

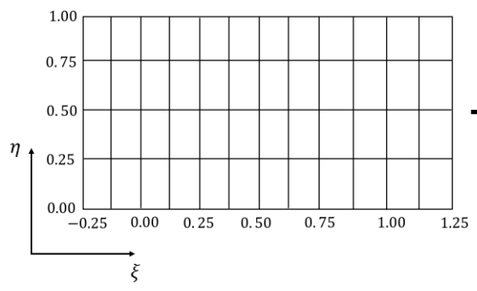
$$\Xi = \left[\xi_1, \xi_2, \cdots, \xi_{n+p+1}\right]$$

where $\xi_i \in \mathbb{R}$ is the i^{th} knot defined in parameter domain, n is the number of basis functions and p is the order of the B-spline

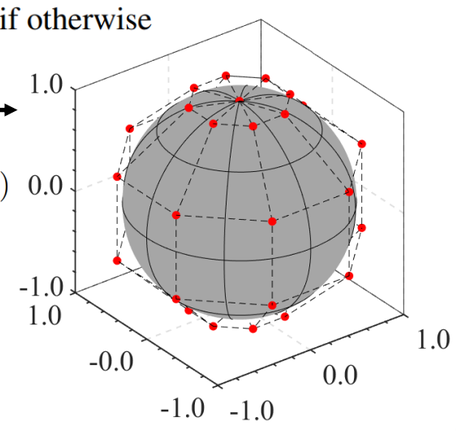
B-spline functions with $p = 0$: $N_{(i,0)}(\xi) = \begin{cases} 1, & \xi_i \le \xi < \xi_{i+1} \\ 0, & \text{otherwise} \end{cases}$

Higher order B-splines: $N_{(i,p)}(\xi) = \tau_{(i,p)} N_{(i,p-1)}(\xi) - \tau_{(i+1,p)} N_{(i+1,p-1)}(\xi)$

$$\tau_{(i,p)}(\xi) = \begin{cases} \dfrac{\xi - \xi_i}{\xi_{i+p} - \xi_i} & \text{if } \xi_{(i+p)} \neq \xi_i \\ 0 & \text{if otherwise} \end{cases}$$



$$\boldsymbol{x}(\xi, t) = \sum_{i=1}^{n} N_{(i,p)}(\xi) \boldsymbol{P}_i(t)$$

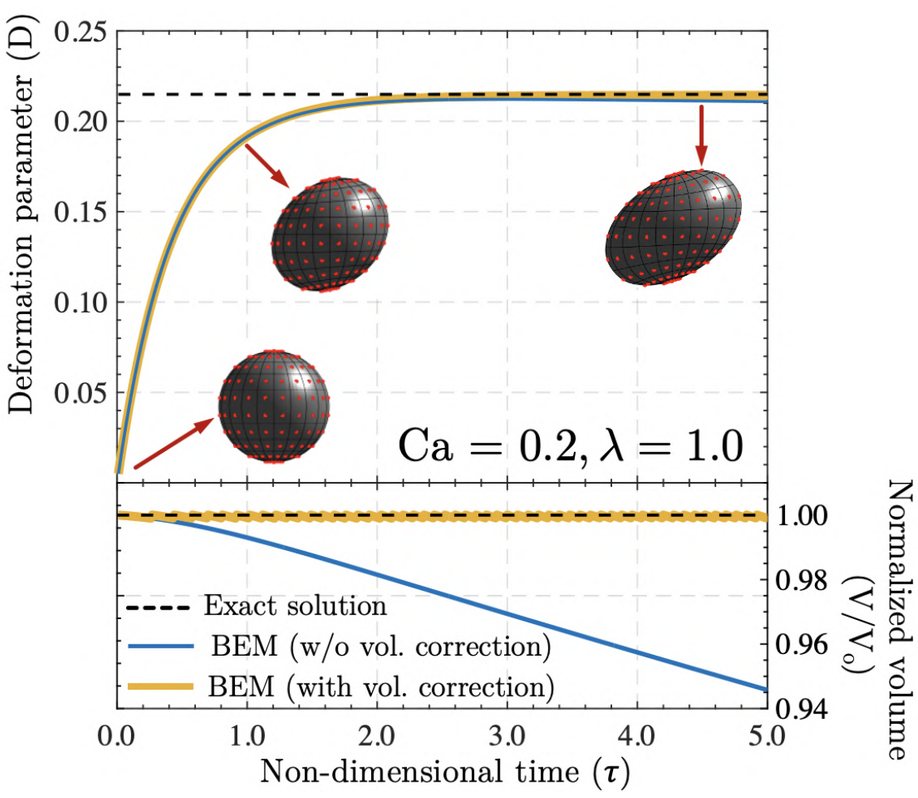


The velocity field is represented by B-spline functions:

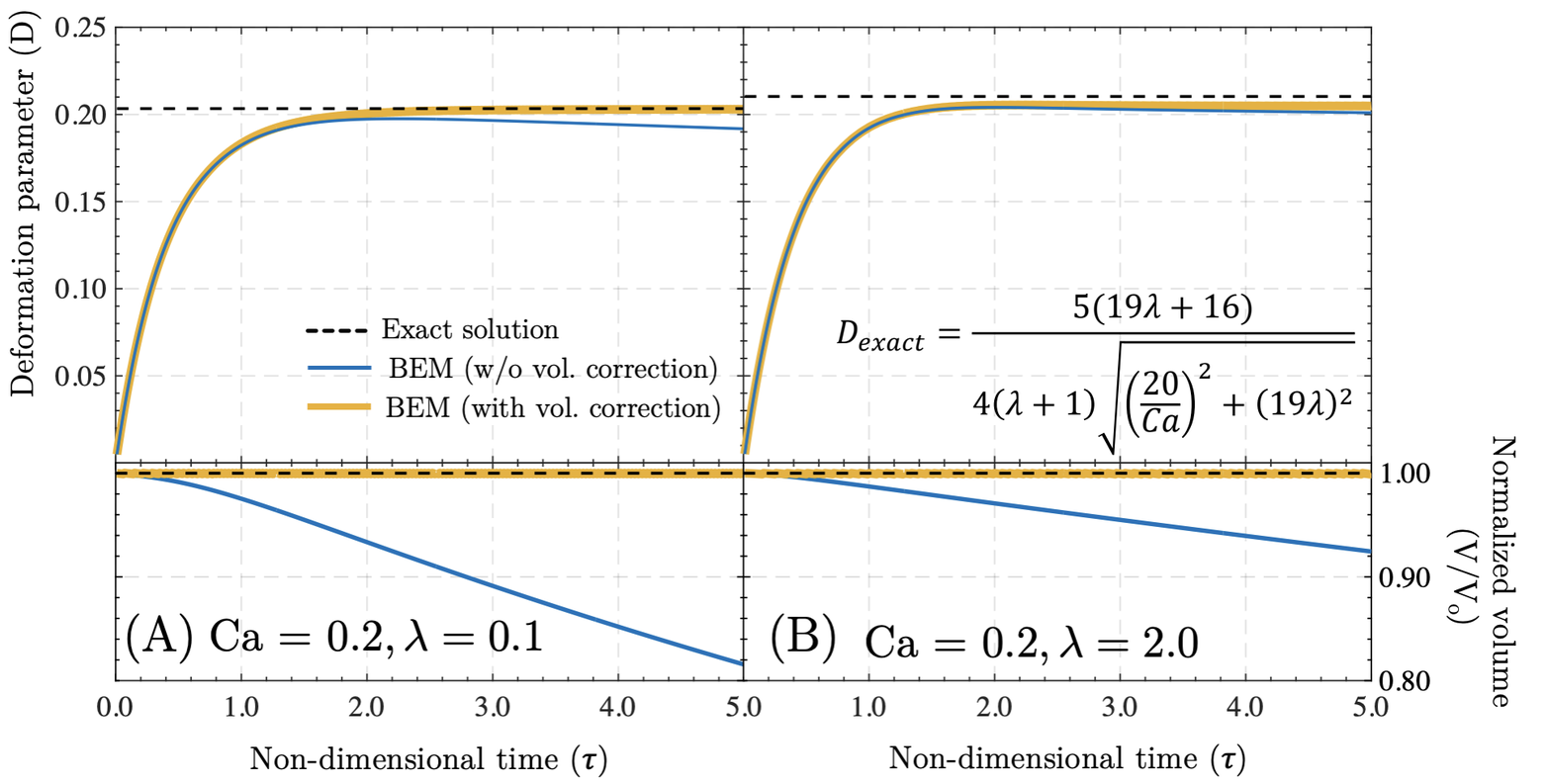
$$\boldsymbol{u}(\xi, t) = \sum_{i=1}^{n} N_{(i,p)}(\xi) \boldsymbol{U}_i(t)$$

Volume Correction Algorithm

Inaccuracies in the calculation of layer potentials lead to unphysical volume change. A non-linear optimization scheme is implemented to keep the volume change within a prescribed tolerance.



Volume correction reduces the error from 1.9% to 0.2%.



Employing volume correction becomes essential for low-viscosity ratio analyses where the amount of volume change increases dramatically.

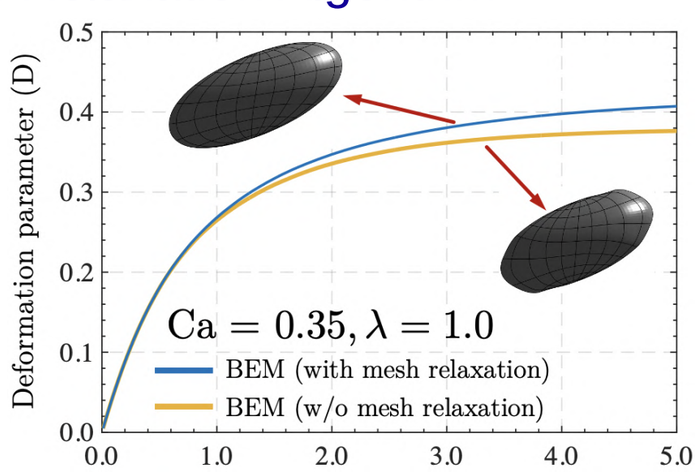
Mesh Relaxation Algorithm

The distances between adjacent nodes may change and cause a distorted mesh/instabilities, hence it might be desirable to concentrate nodes around high curvature zones.

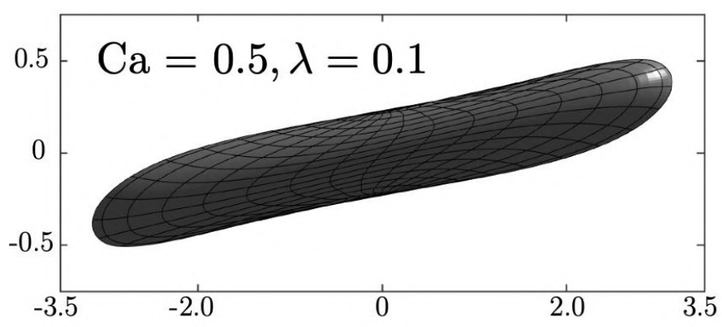
A mesh relaxation algorithm proposed by Siqueira *et al.* (2017) has been implemented where virtual time steps are applied between each physical time step with the following tangential field:

$$\boldsymbol{\Pi}(\boldsymbol{x}_i) = \frac{N_\Delta^{3/2}}{1+\lambda} [\boldsymbol{I} - \boldsymbol{n}(\boldsymbol{x}_i)\boldsymbol{n}(\boldsymbol{x}_i)] (C_{r1} + C_{r2} |\kappa_j(\boldsymbol{x})|^{3/2}) S_j^e (\boldsymbol{x}_j - \boldsymbol{x}_i)$$

where C_{r1} and C_{r2} are control parameters, S_j^e element area and N_Δ is the number of elements



An extreme case:



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ELECTRICALLY CONDUCTIVE BIOMATERIALS: PRODUCTION AND IMPACT ON PROLIFERATION IN MOTOR NEURON-LIKE CELLS

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ABSTRACT

Motor neurons represent a diverse assemblage of cellular entities that function to synthesize and integrate signals emanating from the brain and sensory systems to regulate voluntary and involuntary movements, the absence of which would result in an insurmountable impediment to complex life processes in the event of any damage. The extent of proliferation exhibited by motor neuron-like cells is subject to an array of diverse environmental factors. Hence, the proficient derivation of motor neuron-like cellular cultures *in vitro* serves as a pivotal aspect in comprehending motor neuron development, differentiated cellular phenotypes, modeling disorders *in vitro*, and developing cell replacement strategies for such disorders. To this end, a platform has been conceived that is founded on the notion of generating proliferation-enhancing electrical stimulation in motor neuron-like cells, with the intent of promoting neural-like cellular activation and augmenting cellular proliferation.

Keywords: Biomaterial, neuron, electrical stimulation, cell proliferation

INTRODUCTION

With the rapid advancement of technology, a plethora of synthetic materials and devices are being engineered for the purpose of disease diagnosis, treatment, and drug characterization experiments in modern medicine. Among these, organic thin films, particularly polymers, represent a compelling class of biomaterials due to their remarkable flexibility in accommodating a diverse range of chemical groups onto their surface, thus enabling control over tissue-biomaterial interactions (Figure 1). In particular, the application of electrical stimulation in conjunction with electroactive scaffolds has emerged as a promising strategy for promoting healing by enhancing cell adhesion and proliferation, as well as modulating cellular differentiation (Vendra et. al. 2011).

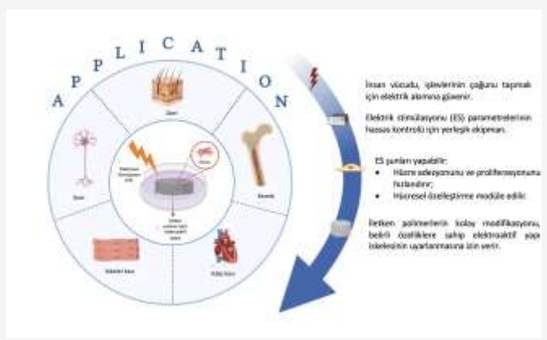

Figure 1. Illustration about advantages of conductive polymeric-based electroactive scaffold and their electrical stimulation for various tissue engineering applications (Marsudi et al., 2021).

METHODS

1. Synthesis and Characterization of Biomaterial

a solvent pouring method was employed for the production of PVDF/CNF-based biomaterial, a strategy that is widely recognized for its effectiveness in generating high-quality biomaterials. To obtain a comprehensive understanding of the physicochemical properties of the PVDF/CNF-based biomaterial, four distinct tests were conducted, including an assessment of its electrical conductivity, a contact angle test, and imaging analyses performed utilizing both scanning electron microscopy (SEM) and atomic force microscopy.

2. Platform Design and Fabrication

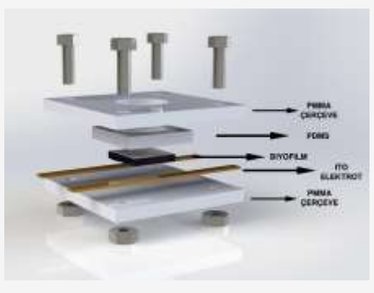

Figure 2. Platform design and layers

3. Cell Culture and Electrical Stimulation

In order to explore the impact of electrical stimulation on neuron cells cultivated on the PVDF/CNF-based biomaterial, two distinct forms of stimulation were employed, consisting of 100, 150, and 200 mV AC (50 Hz) and DC (10 µs). Following an 8-hour duration of electrical stimulation, the viability and proliferation of the cells were evaluated using a viability test.

RESULTS

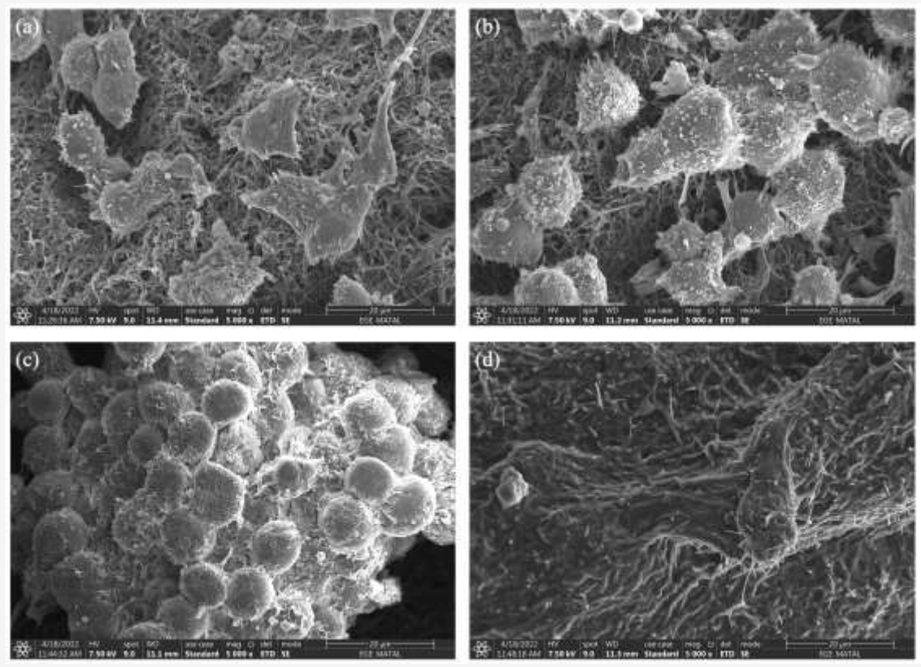

Figure 3. 5000x scanning electron microscopy (SEM) image of the biomaterial. (a) neuron cells cultured on the smooth surface of protein-modified biomaterial, (b) neuron cells cultured on the rough surface of protein-modified biomaterial, (d) neuron cells cultured on the rough surface of without protein-modified biomaterial, (d) naked view of the biomaterial

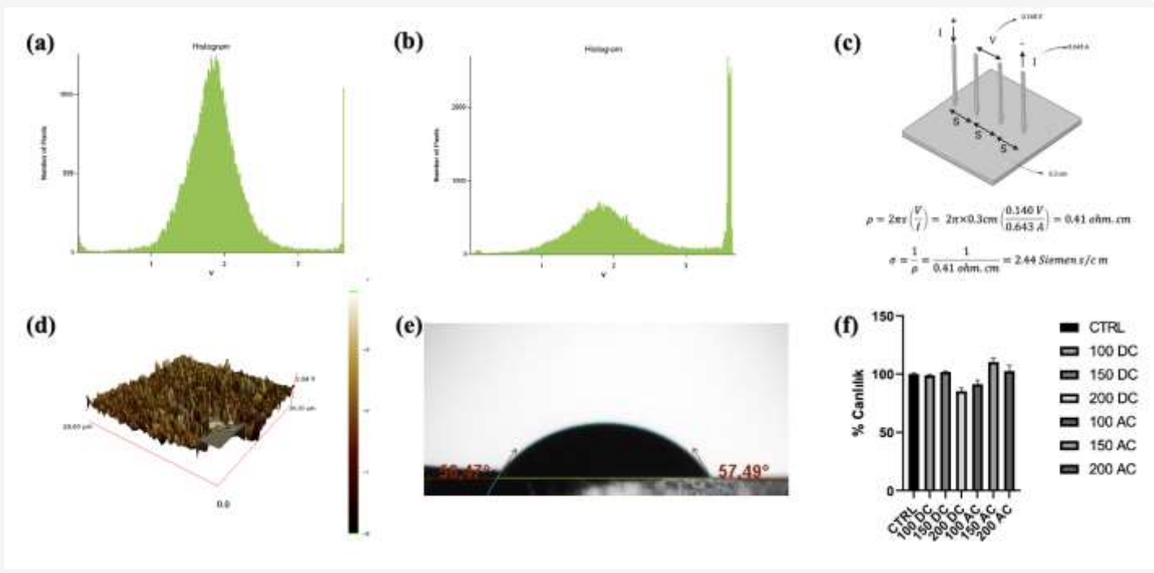

Figure 4. A crafted histogram graph displaying the peaks observed on the (a) rough surface and (b) smooth of the biomaterial, obtained through atomic force microscopy (AFM) analysis, (c) Schematic description of the four-point probe technique, in which the electrical conductivity of the biomaterial is measured, (d) AFM analysis 3D image, (f) Contact angle analysis of the biomaterial (d) Viability analysis of cells cultured by electrical stimulation

CONCLUSION

Whilst the PVDF/CNF-based film structure, fabricated in this project with the aim of stimulating the proliferation of motor neuron-like cells through electrical stimulation, has been synthesized and characterized before (Figure 3 and 4), its potential utility in biomedical and tissue engineering applications cannot be overstated. Through the adoption of a cutting-edge bioengineering approach, this study envisions the implementation of a novel, non-invasive and label-free method for the detection of *in vitro* motor neuron-like cell proliferation (Figure 4). The development of this innovative film presents a promising avenue for the advancement of *in vitro* disease modeling and cell replacement therapies, thereby serving as a beacon of hope for individuals suffering from a myriad of debilitating disorders.

ACKNOWLEDGEMENT

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