CENTRE FOR RESEARCH AND APPLICATIONS IN FLUIDIC TECHNOLOGIES

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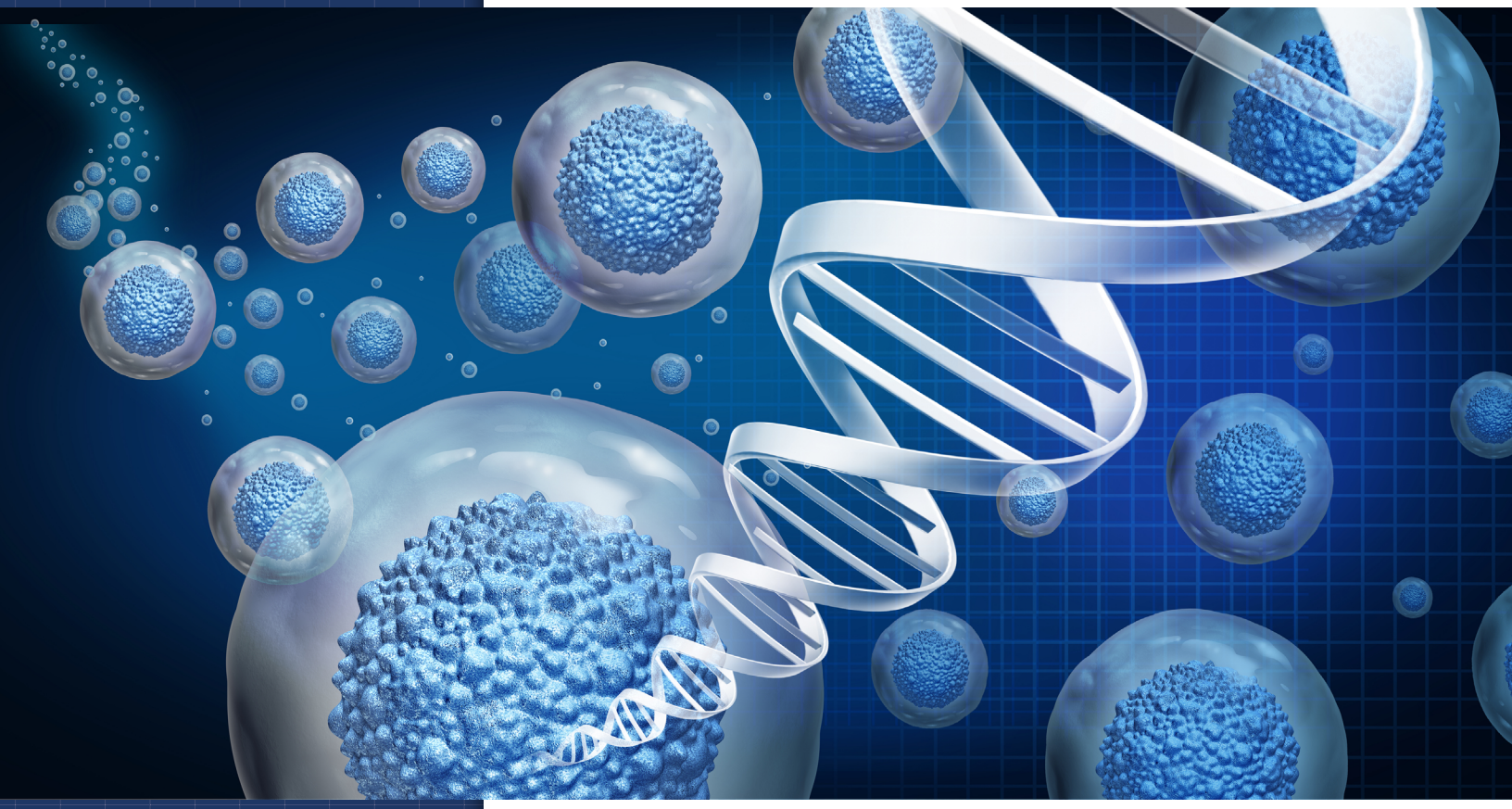
Abstract Booklet



TRAINEE PRESENTATION ABSTRACTS

Trainee Presentation #1: Convolutional neural network analysis of surface-enhanced raman spectra for the classification of cancerous single extracellular vesicles.

Carolina del Real Mata, Olivia Jeanne, Mahsa Jalali, Laura Montermini, Yao Lu,
Kevin Petrecca, Janusz Rak, Sara Mahshid.

Department of Bioengineering, McGill University; Research Institute of the McGill University Health Centre (RIMUHC); Department of Neuropathology, Montreal Neurological Institute-Hospital, McGill University.

Rationale & Objectives: Extracellular vesicles (EVs) are nanosized vesicles that have been recently appointed as a cancer biomarker. EVs are continuously shed into human biofluids such as blood by their parental cells, carrying representative material, like proteins and nucleic acids of their cells of origin. Cancerous cells derived EVs contain rich information of their cell of origin which can be used to elucidate the molecular differences with healthy EVs, however, their heterogeneity and intrinsic complexity present a challenge.

Methods: Deep learning has been successfully implemented as a tool for the analysis of complex datasets in many fields. Their application for diagnostics holds great potential to conquer complex biological data and successfully classify large datasets, such as surface-enhanced Raman spectroscopy (SERS) spectra of heterogeneous biological samples. We use a non-invasive approach, liquid biopsy, as this technology allows diagnosing or monitoring patients' diseases via the study of biomarkers; combined with our in-lab developed SERS-assisted nanostructured array, MoSERS, to collect the SERS fingerprints of single EVs. Later data is analyzed by a convolutional neural network to output information on the biomarker status.

Results: Using the MoSERS microchip, we generated a database of single-EV spectra derived from glioblastoma multiforme (GBM) cell lines, GBM patient samples, and controls. The EVs were isolated from blood samples drawn from 8 healthy individuals and 12 patients. We have used a CNN model to successfully analyze spectral data at the single-EV resolution, predicting with an 85% area under the curve the probability of having specific GBM mutations. Also, we conducted a CNN-binary study with 83% accuracy in the classification of real human samples into GBM-positive and GBM-negative groups.

Conclusions & Significance: Our study using CNN on SERS spectra has proven successful in the identification of mutations in GBM-associated cell lines, and the diagnosis of GBM in patients The validation of the MoSERS-CNN approach paves the way to study biomarkers of different cancer and demonstrate the versatility of the solution proposed.

Trainee Presentation #2: Clinical significance of circulating clonal plasma cells detected by a novel microfluidic chip in multiple myeloma.

Dongfang Ouyang, Bin Tang, Jaewon Park, Lina Hu, Jenny Hirst, Lidan You, Yonghua Li.

Department of Mechanical and Industrial Engineering, University of Toronto

Rationale & Objectives: Multiple myeloma (MM), the disorder of plasma cells, is the second most commonly seen hematological cancer. The current gold standard for MM diagnosis includes invasive bone marrow aspiration. However, it lacks the sensitivity to detect minimal residual disease, and the nonuniform distribution of clonal plasma cells within bone marrow often results in inaccurate reporting. Therefore, we need a sensitive clonal plasma cells assay for these two groups of patients to ensure timely intervention if MM occurs.

Methods: We present a novel mechanical property-based microfluidic platform to segregate cCPCs and successfully demonstrate its clinical applicability in MM. The prototype was testified by spiking human myeloma cell lines in healthy donor blood, and the key parameters such as enrichment ratio and capture efficiency were also evaluated. Next, we used our developed microfluidic platform to explore its clinical utility in comparison with existing methods of Multiparameter Flow Cytometry and Serum Protein Electrophoresis analysis. MM blood samples of different disease stages were processed through the microfluidic device.

Results: The Minimum Residual Disease test results from the 19 MM blood samples showed 89.47% of overall agreement between MFC and microfluidic chip. Through the monitoring of 2 MM patients before and after treatment, a similar fluctuation pattern was observed between the cCPCs level detected by microfluidic chip and the paraprotein level detected by serum protein electrophoresis. Furthermore, the ability of retrieving the cCPCs from the microfluidic chip was also demonstrated and the subsequent cytogenetic analysis was performed.

Conclusions & Significance: This study validates the clinical utility of microfluidic chip on the prognosis of MM. It integrated MRD detection, cCPCs recovery and cytogenetic analysis all in one platform, which provide a convenient and affordable “liquid biopsy” assay for MM patients.

Trainee Presentation #3: Rapid blood grouping powered by digital microfluidics.

Alexandros Sklavounos, Anthony Yong, Ian Swyer, Jeannie Callum, Aaron Wheeler.

Centre for Research and Applications in Fluidic Technologies (CRAFT); Department of Chemistry, University of Toronto; Institute of Biomedical Engineering, University of Toronto; Department of Laboratory Medicine and Pathobiology, University of Toronto; Department of Laboratory Medicine and Molecular Diagnostics, Sunnybrook Health Sciences Centre.

Rationale & Objectives: Blood transfusion is used replace blood loss due to surgery, injury, or an illness in more than 4.5 million patients in North America each year. Blood typing tests are performed to evaluate the compatibility of each donor's blood with the transfusion recipient, so as to ensure their body can safely accept the donated blood. In emergency scenarios, the lack of immediate pre-transfusion compatibility testing means that only products from "universal blood donors" can be transfused, leading to a chronic short supply of such products.

Methods: Since most patients have blood groups that do not necessitate the exclusive use of "universal groups", the availability of a rapid, point-of-care-test for ABO group would greatly decrease the demand for these scarce blood products. To address this issue, we have designed and built a portable instrument that can automate the entire process of blood grouping using a technology commonly known as digital microfluidics (DMF). DMF enables the manipulation of liquids (samples and reagents) in a microfluidic format and along with our AI algorithm we can reduce the sample-to-answer time from approximately an hour down to 5 minutes.

Results: We begun by developing ABO and RhD blood typing tests that can be run on a single, easy-to-use cartridge. In a study performed at Sunnybrook Health Sciences Center, an untrained person used our system to determine the ABO and Rhesus blood type of 24 samples with 100% concordance to the standard lab test. Currently, we are conducting a larger study (n>200 samples) that includes scarce and obscure samples that are anticipated to challenge our system. In addition, we have recently expanded our portfolio of available targets to over 10 analytes including a complete Rhesus panel and other important antigens (e.g., M, N, S groups) which will allow us to better determine the donor-recipient compatibility.

Conclusions & Significance: To our knowledge, this is the first microfluidic-powered and fully automated blood typing system with a portable footprint. With these promising preliminary results, we hope to deploy our systems at the points of need (e.g., trauma settings), which will eliminate the wait time needed for batch analysis thus improving both the caregiver and patient experience.

Trainee Presentation #4: Photocrosslinkable liver extracellular matrix-based hydrogels for 3D bioprinting of liver microenvironment models.

Nima Tabatabaei Rezaei, Hitendra Kumar, Simon S. Park, and Keekyoung Kim.

Department of Mechanical and Manufacturing Engineering, University of Calgary; Department of Pathology and Laboratory Medicine, Cumming School of Medicine, University of Calgary; Department of Biomedical Engineering, University of Calgary.

Rationale & Objectives: The liver is a vital organ that plays major roles in balancing biochemical environments in the human body. To recapitulate the native cellular microenvironment, utilizing decellularized extracellular matrix (ECM) for in vitro tissue generation has shown promising results. Due to tissue-specific ECM's crucial role in governing cellular dynamics, tissue integrity, and function, utilizing decellularized ECM to fabricate the bioinks would significantly influence the cell behavior.

Methods: In this study, we used detergent-based protocol to produce decellularized porcine liver ECM. Through this process, ionic and nonionic detergents were utilized to remove the cell properly, followed by solubilizing and functionalizing with methacrylic anhydride steps to generate photocrosslinkable methacrylated liver dECM (LdECMMA) hydrogels. Then mechanical and physical properties were evaluated for each composition of the bioink.

Results: Firstly, we successfully formulated stable and fast crosslinkable LdECMMA-GelMA hydrogel bioink. The decellularization protocol showed proper cell removal obtained from DNA quantification. The compressive modulus, pore size and swelling ratio of the hydrogels were directly dependent on the LdECMMA concentration in the hybrid hydrogel, thereby demonstrating the tuneability of the mechanical and physical properties of these hydrogels. Human hepatoma HepG2 cells were encapsulated in the hybrid hydrogels and cytocompatibility of the hydrogels was demonstrated in one week of culture, showing the influence of dECM presence in the bioink.

Conclusions & Significance: In summary, the LdECM-based hydrogel system provides a simple, rapid photocrosslinkable platform, which can potentially be used as a scaffold to simulate native liver ECM for liver disease research, drug studies and cancer metastasis modeling.

Trainee Presentation #5: Shape-adaptable printhead enables single-step conformal bioprinting of tissue sheets onto physiologically curved surfaces.

Sushant Singh, Lihua Wei, Ehsan Samiei, Teodor Veres, Axel Guenther.

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Rationale & Objectives: Advances in bioprinting technology have allowed formation of spatially organized biomaterial and tissue structures. Stationary and handheld extrusion, inkjet, and stereolithography based approaches promote in vitro and in situ tissue formation in a contactless manner or via contact with rigid printhead. However, current approaches remain inadequate in several regards, including the consistent deposition onto physiological surfaces without the need for prior scanning, the shape fidelity in mm-thick deposited filaments and slow printing speed.

Methods: We developed a shape-adaptable printhead that conforms to physiologically curved surfaces. The printhead contains three feature layers. The bottom two fluidic layers accommodate separate microchannel networks that deposit at the exit an inch-wide biomaterial sheet covered with a cross-linker onto a physiological surface. Curvature sensors integrated within the fluidic layer and a soft robotic actuation layer attached on top allow the printhead to adapt to surface curvature radii as small as 10mm. Further, to facilitate the consistent and faithful deposition on physiological substrates, we synthesized a modular bioink with tailored shear-thinning behavior by jamming 100mm-diameter gelatin microgels and cells.

Results: To demonstrate shape fidelity regardless of substrate orientation we demonstrated highly uniform 1mm-thickness tissues onto flat, inclined, and curved deposition surfaces. The printed tissue construct offers a suitable environment for embedded cells to migrate, proliferate, and differentiate, with minor tissue contraction. As a case study, we in-situ deposited a bilayer skin construct (consisting of human dermal fibroblasts and keratinocytes) on a custom-designed culture platform that allows long-term in vitro culture of curved tissues at air-liquid interface. The deposited bilayer skin construct maintained its curved topology during culture, with uniform proliferation and granulation in the epidermal layer.

Conclusions & Significance: We present a shape-adaptable printhead that allows conformal deposition of biomaterial sheet onto arbitrarily oriented and curved surfaces. Rheological behavior of jammed bioink allows deposited construct to maintain fidelity on arbitrary surfaces. Week-long culture of the construct at air-liquid interface demonstrates a physiologically curved bi-layer in vitro skin.

Trainee Presentation #6: MANufacturing on the GO (MANGO): a portable device for low-cost cell-free protein biomanufacturing.

Quinn Matthews, Pouriya Bayat, Severino Jefferson Ribeiro da Silva, Andy Wu, Fahim Masum, Mohammad Simchi, Lauren Cranmer, Idorenyin Iwe, Justin Vigar, Ryan Fobel, Soham Jorapur, Livia YuXiu Guo, Seray Cicek, Aidan Tinafar, Soheil Talebi, David Sinton, Keith Pardee.

Graduate Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto; Department of Mechanical and Industrial Engineering, Faculty of Applied Science and Engineering, University of Toronto.

Rationale & Objectives: Recombinant proteins are of extreme importance globally, comprising therapeutics, diagnostics, research tools, and more. Currently, recombinant proteins are largely synthesized using cell-based expression in well-resourced laboratories, then distributed globally – often refrigerated – along sometimes fragile supply chains. This poses a challenge for low-resource and remote communities, and results in a slow-to-respond system when facing outbreaks. We aim to build a device for simple, portable, and automated recombinant protein expression.

Methods: Low-cost cell-free protein synthesis reactions were prepared in-house for protein expression. An automated fluid control system (controlled by a raspberry pi microcontroller) was devised to enable synthesis and purification of the expressed protein by transferring fluids through CNC-milled channels in an acrylic plate and an affinity purification column. Temperature was controlled by a low-cost chicken egg incubator. A 3D-printed enclosure was designed to house these elements. Expressed proteins were validated through a variety of functional assays.

Results: We report the fabrication of MANufactring on the GO (MANGO), a low-cost (USD $1.3k) device for the automated manufacturing of recombinant proteins. Leveraging an automated fluid control system enables the synthesis and purification of milligram quantities of protein from freeze-dried cell-free reaction systems with no need for a highly-skilled operator. To showcase the applications of MANGO, we manufacture proteins used in two common diagnostic assays: first, a nanobody targeting SARS-CoV-2 for enzyme-linked immunosorbent assays (ELISA), and second, the enzymes required to carry out loop-mediated amplification (LAMP) for the detection of SARS-CoV-2 and the Malaria-causing parasite Plasmodium.

Conclusions & Significance: Protein-based inputs that underlie the detection/treatment of disease are often unavailable for remote and low- and mid-income communities due to cost, shipping constraints, and lack of infrastructure. By providing a low-cost and open-source platform for on-demand portable protein biomanufacturing, we aim to help democratize global access to research and healthcare.

Trainee Presentation #7: Modeling the progression of placental transport from early to late stage pregnancy by tuning trophoblast differentiation and vascularization.

Sonya Kouthouridis, Alexander Sotra, Sandeep Raha, Boyang Zhang.

Department of Chemical Engineering, McMaster University; School of Biomedical Engineering, McMaster University; Department of Pediatrics, McMaster University.

Rationale & Objectives: During pregnancy, the placenta is the main site of nutrient transport to the fetus. The early-stage placental barrier is characterized by a lack of fetal circulation and by a thick trophoblastic barrier, whereas the later-stage placenta consists of vascularized chorionic villi encased in a thin, fused trophoblast layer, ideal for nutrient transport. In this work, we show how trophoblast differentiation and placental vascularization can be modulated to create predictive models of early- and late-stage placental transport.

Methods: Human placental stem cells (PSCs) were differentiated and characterized (gene expression, hCG secretion and immunofluorescence) before being seeded onto the center well of our lab's proprietary IFlowPlate device, which consists of three interconnected 384-wells whose central chamber is cast with a biopolymer gel. Cytokine analysis was performed to assess secretion of pregnancy-related inflammatory cytokines. To create a vascularized barrier model, the hydrogel was loaded with human endothelial and fibroblast cells which self-assembled into a perfusable vascular network, mimicking fetal circulation in the placenta. Barrier function of these models was assessed using dextran and insulin permeability assays.

Results: PSC differentiation resulted in a thinner, fused trophoblast layer, as well as an increase in hCG secretion, barrier permeability and apical-basal polar secretion of certain inflammatory cytokines, which are consistent with in vivo findings. Further, gene expression confirmed this shift towards a differentiated trophoblast subtype. The vascularization of our barrier model resulted in a size-dependent change in dextran and insulin permeability, highlighting the role of vascular perfusion in placental permeability.

Conclusions & Significance: Transport of harmful exogenous substances through the placenta can cause pregnancy complications and, occasionally, death. We have shown that trophoblast differentiation and vascularization can be modulated to model barrier function of the placenta at different stages. Thus, our model could be used as a predictive measure to assess fetal toxicity of such substances.

Trainee Presentation #8: Use of vascularized heart-on-a-chip platform to identify HUVEC-EVs as an anti-inflammatory treatment for SARS-COV-2 acute myocarditis.

Rick Lu, Naimeh Rafatian, Yimu Zhao, Karl Wagner, Bo Li, Carol Lee, Erika Beroncal, Eryn Churcher, Ana Andreazza, Agonisto Pierro, Claudia Dos Santos, Milica Radisic.

Institute of Biomedical Engineering, University of Toronto; Toronto General Hospital Research Institute, UHN; Hospital of Sick Children; Department of Pharmacology and Toxicology, University of Toronto; St. Michael's Hospital, Unity Health Toronto; Department of Chemical Engineering and Applied Chemistry, University of Toronto.

Rationale & Objectives: Myocarditis is characterized by inflammation of the heart, which results in poor heart function. Interest in viral-associated myocarditis peaked during the COVID-19 pandemic, yet reliable biomarkers for the early detection and treatment of myocarditis are an unmet clinical need. This motivates the need to develop an advanced model that enables the synergistic integration of endothelial cells, immune cells, and functional cardiac tissues to deepen the understanding of SARS-CoV-2-induced acute myocarditis in vitro settings.

Methods: To mimic SARS-CoV-2 infection under more physiologically relevant conditions, we repurposed a pre-established vascularized heart-on-a-chip system to capture the complex cascade of viral infection and subsequent myocardial inflammation in the presence of circulating immune cells.

Results: We showed that immune cells infiltrated to cardiac tissue (CT) and that stimulates the release of cytokines, which leads to electromechanical dysfunction of CT. Myocardial inflammation also caused the release of ccf-mtDNA in both an in vitro CT and in COVID-19 patients, demonstrating that fragmentation of myocardial mitochondria is an important pathophysiological mechanism leading to myocarditis. Using this model, we showed the cardioprotective effects of HUVEC-EVs in alleviating SARS-CoV-2-induced myocardial dysfunction. Our results illustrated HUVEC-EVs suppressed inflammatory responses by suppressing NF-kB and IFN activation, which in turn mitigated mitochondrial stress by inhibiting the NLRP3 inflammasome.

Conclusions & Significance: Collectively, our study suggests that miRNAs may serve as promising immunomodulatory agents and that the transfer of anti-inflammatory miRNAs helps maintain mitochondrial energetics.

Trainee Presentation #9: Development of flexing joint-on-a-chip device for compression of osteoarthritic cartilage explants.

Lauren Banh, Ka Kit Cheung, Kevin Perera, Kebin Li, Byeong-Ui Moon, Edmond Young, Sowmya Viswanathan.

Centre for Research and Applications in Fluidic Technologies (CRAFT); Department of Mechanical & Industrial Engineering, University of Toronto; Osteoarthritis Research Program, Division of Orthopedic Surgery, Schroeder Arthritis Institute, University Health Network; Krembil Research Institute, University Health Network; Institute of Biomedical Engineering, University of Toronto; Medical Devices Research Centre; National Research Council of Canada; Division of Hematology, Department of Medicine, University of Toronto.

Rationale & Objectives: Osteoarthritis (OA) is a whole-joint disease that largely damages the cartilage but has no known cure. Many disease modifying drug candidates have failed in clinical trials due to the failure to address multifactorial disease parameters; preclinical models with multi-tissue interactions can help bridge this translational gap. Our objective is to develop a "joint-on-a-chip" (JOC) model using advanced organ-on-a-chip technology for drug testing. Here, we developed our first chip module, a flexing JOC (flexJOC) that compresses cartilage explants.

Methods: The flexJOC has 3 layers: (1) flexure spring layer with mounted paddles, connected to a (2) motor-actuated carriage layer that applies axial force to 6 explants in (3) parallel microwells. Desired compressive strains are achieved by adjustable screws that define the minimum and maximum position of the paddle travel. Layers were CNC micromilled from 3 mm polymethylmethacrylate sheets, aligned with locating pins, and bonded by cyanoacrylate adhesive. Full depth cartilage explants (d=1.5 mm) acquired from end-stage knee OA patients during joint replacement surgery were subjected to physiological and hyperphysiological loading conditions (10% and 30% compression, 1 Hz, 3 hr/day cyclic compression for 1 week).

Results: The resulting flexJOC mechanism moves in response to the electric servomotor and applies a trapezoidal compression profile. This corresponds to a simplified pattern of typical stress in the knee joint during walking, but the motor controller can be programmed to apply other loading profiles (e.g., triangular, sinusoidal). Preliminary results show that cartilage explants under physiological and hyperphysiological compression result in no changes in gene expression relative to static unloaded conditions. Future experiments will involve monitoring the explants under a microscope over the experimental timeline to analyze the motion profile and optimize the loading protocol for patient explants.

Conclusions & Significance: We developed a novel method for applying mechanical stimulation to cartilage explants. In the future, this device will be integrated into our multilayered JOC platform to model the OA joint. Eventually, the combined JOC platform will be used as a research tool to test novel OA therapeutics.

POSTER ABSTRACTS

Poster #1: Automated digital microfluidic platform for COVID-19 immunity detection in saliva.

Gilberto Camacho, Sathishkumar Narayanaswamy, Alexandros Sklavounos, Mohammed Abdullah, Anthony Yong, Joshua Dahmer, Aaron Wheeler.

Centre for Research and Applications in Fluidic Technologies (CRAFT); Institute for Biomedical Engineering, University of Toronto; Department of Chemistry, University of Toronto; Donnelly Centre for Cellular and Biomolecular Research, University of Toronto.

Rationale & Objectives: In a post-pandemic era, the capacity to test and monitor immune status against any antigen quantitatively is becoming more and more important for personalized medicine. Gold standard detection techniques such as ELISA have been implemented. Despite having high sensitivity, its time-response nevertheless is slow and they lack of portability, requiring laboratory conditions to handle this immunoassays. In this work, a Digital Microfluidic platform was utilized to perform an automated magnetic-beads ELISA immunoassay for COVID immunity detection.

Methods: A sandwich ELISA was developed, were streptavidin magnetic-beads were coated with biotinylated COVID-Spike protein (S1 domain), following by capturing human antibodies IgA, IgM and IgG supplemented in pre-COVID saliva samples. After that, a conjugation was performed with either anti-IgA, anti-IgM or anti-IgG (respectively) all conjugated with HRP enzyme. Finally, Luminol-H2O2 was utilized as substrate to generate a chemiluminescence signal.

Everything was performed in a fully automated DMF platform (MR-Box) which contains the components required (magnetics lens for washing procedure; optical sensor for chemiluminescence detection) to perform the ELISA laboratory steps.

Results: The DMF-system is capable to detect chemiluminescence signals in around 50 minutes for IgA, IgM and IgG, of 10, 50 and 50 ng/mL, respectively, which are well below their clinically relevant range in saliva (IgA = 1.275 µg/mL, IgG = 0.602 µg/mL). This demonstrates its robust capability to become one of the main player point-of-care technologies in the near future.

Conclusions & Significance: This work demonstrates the capability to run a fully automated ELISA immunoassay for COVID immunity sensing, in a Digital Microfluidic platform, achieving significantly low limits of detection in the range on ng/mL, below to the clinical relevant range. This could potentially allow the users to detect their immunity levels with a broad spectrum of time.

Poster #2: A high-throughput crypt-patterned colon model with perfusable vasculature to recapitulate inflammatory bowel disease and for drug testing.

Alexander Sotra and Boyang Zhang.

School of Biomedical Engineering, McMaster University; Department of Chemical Engineering, McMaster University.

Rationale & Objectives: Traditional preclinical drug screening utilizes animal models and 2D transwell cultures that bottleneck successful drug discovery from clinical trials. High throughput multi-well based tissue models (organ-on-a-chip) can bridge the gap to yield more predictive drug screening data. The objective of our work is to create geometrically engineered colon tissues with perfusable vasculature in a 384 well plate form factor. We apply our model to study chemotherapeutic screening against colon cancer tissue and inflammatory disease modelling.

Methods: Within the IFlowPlateTM, Fibrin hydrogels are casted and stamped with a custom device to yield a 16-crypt array. Caco-2 cells are then seeded atop the patterned gel to form a monolayer. With vasculature, human umbilical vein endothelial cells (HUVECs) and lung fibroblasts are co-embedded within the hydrogel before stamping. A second HUVEC seeding in the inlet and outlet wells form a perfusable microvascular bed (MVB). Chemotherapeutics are apically incorporated in increasing doses and a barrier permeability assay using fluorescent dextran measures tissue damage. TNF**α** and IFNɣ are apically delivered to induce an inflammatory disease condition and quantified using a human proinflammatory cytokine multiplex assay.

Results: We show that Caco-2 cells form a patterned monolayer with 16 crypts in 0.21 mm2 of culture area and a depth of 700 µm. Mature barrier formation is quantified with fluorescent dextran at 65 kDa and 4 kDa. A 5-fold decrease in barrier permeability is observed by day 8 of culture indicating barrier function and monolayer maturation. A chemotherapeutic drug dose response indicated loss of barrier function at increasingly high doses on crypt patterned Caco-2 monolayers. Confocal imaging of vascularized crypt patterned Caco-2 monolayers reveals MVB interaction with crypt tissue. Vascularized tissues exhibit unique cytokine release profiles compared to avascular controls for 15 assayed cytokines.

Conclusions & Significance: Our model serves as a proof-of-concept for architecturally guided tissue formation with advanced co-culture capabilities in a high throughput custom well plate. The study sets a basis for more sophisticated in-vitro disease modelling and drug screening on colon tissue. We plan to include primary tissue and demonstrate automated tissue fabrication in future work.

Poster #3: Application of vacuum thermoforming for rapid manufacturing of GLAnCE platform with improved optical properties.

Aleksandra Fomina, Adam Tam, Nila Wu, Jennifer Lam, Nancy Li, Alison McGuigan.

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Rationale & Objectives: GLAnCE (Gels for Live Analysis of Compartmentalized Environments) is an in vitro platform that incorporates microchannels with thin cell-containing microgels which allow longitudinal image-based monitoring of tumor cell dynamics and therapy response. The major limitation of GLAnCE is that image acquisition can be completed at low magnifications only (up to x10) due to the thickness of the hot-embossed bottom component. This limits GLAnCE optical properties, prohibiting the investigation of tumor cell dynamics at a single-cell resolution.

Methods: We employ vacuum thermoforming, an in-house microfabrication technique, to enable rapid manufacturing of the GLAnCE bottom component from thin polystyrene (PS) films. Using a desktop thermoformer and a 3-by-3 prototype of GLAnCE positive aluminum mold, we optimize thermoformer heating temperature, time, mold preheating temperature, position, and number of vacuum holes in the mold. We apply methods for polydimethylsiloxane casting and bright-field imaging to characterize thermoformed film thickness and microchannel definition. We scale up the process to a 12-by-8 version, validate thermoformed films in the current GLAnCE assembly and cell seeding workflow, and perform image acquisition using confocal microscopy.

Results: The thermoformer heating temperature of 120°C (greater than PS glass transition temperature) and the time of 4 minutes are the optimal conditions to produce the greatest mean reduction in thickness of PS films. As determined from mean angle measurements of microchannel side walls relative to bottom, the most precise microchannel definition is achieved by preheating the mold to 190°C and positioning 0.5 mm vacuum holes 0.2 mm away from microchannels. Upscaled thermoformed PS films are compatible with the current workflow for GLAnCE assembly and cell seeding and show improved optical properties, allowing imaging of fluorescent cells in 3D at up to 63x magnification.

Conclusions & Significance: Thermoformed GLAnCE platform offers a unique opportunity to monitor cell dynamics longitudinally at a single-cell and subcellular resolution. We envision the application of this platform for studies on single-cell dynamics of modeled tumor microenvironments to provide insights into the drivers of disease progression and phenotypic drug screening and discovery.

Poster #4: Monodisperse elastomeric particle jamming for use in self-healing biomaterials.

Jennifer Kieda, Kaitlyn Ramsay, Richard Jiang, Milica Radisic.

Department of Biomedical Engineering, University of Toronto; Department of Chemical Engineering, University of Toronto; Toronto General Hospital Research Institute.

Rationale & Objectives: The polymer, POMaC, shows promise across a variety of tissue engineering fields. Currently, its widespread use is bottlenecked by the methodology by which these polymer particles are generated. Here, we show the use of a plug-and-play droplet microcapillary device capable of generating monodisperse POMAC particles in a high throughput manner for use in tissue engineering.

Methods: The modular microcapillary platform is a coaxial assembly of three sizes of glass and/or PTFE capillaries held together and aligned using a junction box cast from 3D printed moulds. Two syringe pumps were set at a controlled rate, where the inner phase consisted of POMaC, and the outer phase consisted of 1% w/v PVA solution. These particles are collected in a bath of PVA, placed into a confined circular mold, and crosslinked using a UVP crosslinker to create a jammed material. The PVA is then aspirated from around the droplets and Phosphate-Buffered Saline (PBS) is added. Self-healing tests are conducted by pulling apart and reattaching the jammed material.

Results: Using two controllable syringe pumps, when the dispersed POMaC and continuous PVA-based solutions come into contact at the coaxial junction, high throughput monodisperse POMaC particles are formed. Moreover, preliminary experiments have shown that these droplets are capable of self-healing which is defined as a successive adhesion and re-adhesion of the droplets. In conjunction with electrostatic attraction factors, the structure of the hydrogel drives the development of new bonds via reconstructive covalent dangling side chains or non-covalent hydrogen bonding.

Conclusions & Significance: Our new method for POMaC particle generation allows us to tailor bespoke particles which are capable self-healing and for use in the generation of permeable bioinks. We project that our work will enable the production of a new generation of new biocompatible bioinks for use in medicine, engineering, and biochemistry.

Poster #5: Optimization of microcontact printing for immobilizing biomolecules in diagnostic device manufacturing processes.

Lubna Najm, Amid Shakeri, Liane Ladouceur, Tohid F. Didar.

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Rationale & Objectives: Microcontact printing (μCP) can be used to create devices for measuring biomolecules in human serum. Clinically, these devices aid in diagnosing diseases at their early onset, like cancer and other immune-based diseases. μCP is cost effective, non-invasive, and customizable for different cytokines or proteins. However, achieving high repeatability and reproducibility is a longstanding limitation of μCP, making it difficult to implement in industry. As such, the objective of this project is to optimize and propose a novel μCP printing protocol.

Methods: μCP involves the use of a predesigned stamp in contact with a substrate to transfer and immobilize molecules, such as capture antibodies (CAb), in a specialized pattern. In our protocol, the solution of CAb is applied as droplets onto defined regions of the stamp, unlike conventional μCP, which spreads solution along the entire stamp surface. After incubation of the droplets, excess solution is removed, and the stamps are dried in a humidity-controlled environment. This is followed by application of the stamp to the substrate with a weight to facilitate the immobilization of the CAb. These CAb become the basis for an enzyme-linked immunosorbent assay (ELISA) to detect the target biomolecule of disease.

Results: Optimization for this novel protocol has been completed using a labelled, inexpensive biomolecule, bovine serum albumin – fluorescein isothiocyanate (BSA-FITC). External environmental factors, solution components, droplet volume, droplet application and removal, drying time after droplet removal, and amount of weight applied have all been tested and optimized. Currently, this optimized novel protocol has achieved industry standard levels of repeatability and reproducibility, with reduced variation between results and high signal intensity compared to conventional μCP. Regardless, challenges exist in perfecting the protocol manually, and as such, opportunities arise for automation before moving on to ELISA.

Conclusions & Significance: Our findings show high reproducibility and repeatability, making our μCP approach promising in terms of scaling manufacturing processes for these diagnostic devices in industry. There is potential to create an automated streamlined protocol as well as allow for detection of multiple target biomolecules with one device, which is significant for diagnostic capabilities.

Poster #6: Universal aqueous two phase system microfluidic platform for solid, hollow, and droplet filled fibers.

Niloofar Ghasemzaie, Morteza Jeyhani, Scott S. H. Tsai.

Department of Biomedical Engineering, Toronto Metropolitan University; Department of Mechanical and Industrial Engineering, Toronto Metropolitan University.

Rationale & Objectives: Engineers often draw inspiration from nature. Researchers have developed micro- and nanofibers based on connective tissues in the human body. Electrospinning and microfluidics are the most common techniques for making nano- or microscale fibers. Compared to electrospinning, microfluidic fabrication is relatively safe, allowing sensitive materials such as enzymes, proteins, drugs, and cells to be loaded into the fibers. This project aims to create solid fibers, hollow fibers, and droplet-filled fibers with the same microfluidic platform.

Methods: We combined microfluidic spinning with aqueous two-phase systems (ATPS) to produce different types of fibers using only one device. Here, the alginate fibers were obtained through crosslinking with barium chloride. As a starting point, ATPS provides an aqueous environment that is compatible with biological materials, and by controlling only the inlet pressures, we are able to produce hollow and droplet-filled fibers. Changing the inlet pressure yields a phase diagram, indicating what type of fiber can be achieved at each pressure. Furthermore, each type of fiber was examined for how pressure changes affected its diameter.

Results: Our microfluidic device has four inlets. The ATPS is created using dextran and polyethylene glycol, to form an ATPS of dextran-rich (DEX) and polyethylene glycol-rich (PEG) phases. PEG, DEX, alginate, and barium chloride concentrations were optimized. By merely adjusting the inlet pressures, we were able to obtain all three types of fibers. Through controlling the pressures, we controlled the diameter of the fiber in solid fibers, the shell and core diameters in hollow fibers, and the size of the droplets and fiber diameters in hollow fibers.

Conclusions & Significance: In the end, all three forms of fibers were able to be produced using the same platform. It was possible to manipulate fibers and droplet sizes easily by adjusting the inlet pressures. The next step would be to coat alginate fibers with chitosan and incorporate drugs into the droplets.

Poster #7: The rapid measurement of yield stress of whole blood using an inexpensive and portable microfluidic platform.

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Rational & Objectives: The yield stress is a characteristic stress that demarcates the solid-like and liquid-like behaviour of materials. The low yield stress that blood possesses is significantly higher for blood of the patients suffering from sickle cell, systemic sclerosis, and many other cardiovascular diseases. However, the existing rheometers that can measure low yield stress of blood are exorbitantly expensive, require high volume of blood, and are less likely be portable, thereby making them unfit in clinical setting.

Methods: We propose using a microfluidic technique for rapid measurement of low yield stress of the order of a few mPa that is inexpensive and portable. We use a camera that records a tiny, continuous blood flow through a microfluidic extensional flow device (MEFD). The design of the MEFD is such that it allows the formation of a non-moving, solid island of blood due to its yielding behaviour in a prescribed range of flow rates. We measure the size of the solid island through the video recording of the flow and employ an empirical relationship to calculate the yield stress.

Results: We first characterized the MEFD using a model yield stress fluid (carbopol gel) to obtain a characterization curve for the measurement of an unknown yield stress. Further, the characterization curve was implemented in the measurement of the yield stress of blood obtained from three donors at various volume fractions of red blood cells (hematocrit) in the range between 20 and 70 mPa. We compared the yield stress measured using our method with rheometer measurements as well as the literature data, and they showed a fair agreement. The yield stress of blood was found to be larger for blood with higher hematocrit.

Conclusions & Significance: We have developed an inexpensive and portable microfluidic technique that can perform rapid measurement of the yield stress of blood as low as a few mPa. This technique can be used to distinguish between healthy and diseased blood through the measurement of the yield stress.

Poster #8: A biomimetic alveoli-on-a-chip system with relevant geometry and cyclical stretch.

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Rationale & Objectives: Alveoli are spherical, highly dynamic organs inside human lungs. In traditional in vitro studies, alveolar epithelial cells are cultured on flat (2D) surfaces, which lack physiological alveolar architecture. Thus, the need exists for a platform with similar alveolar architecture that allows recapitulation of both physiological and aberrant microenvironments that can evaluate physiological and pathological cellular changes in human alveoli. We herein propose a biomimetic alveoli-on-chip system composed of a controllable stretchable substrate.

Methods: The constructed alveoli-on-a-chip system uses a programmable pressure controller that connects with an opening of an Ibidi® µ-slide. A stainless-steel disc with holes 350µm in diameter is placed on top of the other opening, and then sealed with a thin Gel-Pak® PDMS diaphragm that is 38.1µm in thickness. To measure deflection of the PDMS diaphragm, the collagen coating was stained via immunofluorescence and subsequently imaged using confocal microscopy. A549 cells, which is a lung epithelial cell line, were seeded on the diaphragm with its viability confirmed with a Live/Dead Staining Kit. YAP and F-actin staining were performed to evaluate the morphological response of cells to static and dynamic stretch.

Results: We demonstrated that PDMS diaphragms in the system can withstand stretch between a physiologically relevant pressure range (-3.5 PSI to -4.5 PSI), which resulted in a maximum strain of 13.8%. Viability analysis showed that A549 cells grew well on PDMS diaphragms, with a higher cell density in the peripheral region of stretched dimples with higher tensile forces. F-actin staining showed that cells aligned perpendicular to the direction of radial stretch in cyclically stretched dimples, with significantly higher expression compared to that of cells under static stretch. YAP quantification showed the decrease in cytoplasmic YAP in statically and cyclically stretched cultures compared to those of flat cultures.

Conclusions & Significance: Results show that the alveoli-on-a-chip system is easy to fabricate, can provide physiologically relevant alveolar architecture, and can mimic physiological alveolar stretch. Our future plan will be evaluating lung epithelial cell performance under pathological cyclic stretch, as well as testing the effect of cyclic stretch on hiPSC-derived alveolar epithelial cells.

Poster #9: Automated microfluidic system with glucose output for point-of-need SARS-CoV-2 diagnostics.

Evan Amalfitano, Jennifer Doucet, Moiz Charania, Kebin Li, Matthew Shiu, Marc-Alexandre Chan, Aaron Bessof, Émilie Leblanc Gaudreau, Jarod Matwiy, Kaiyue Wu, Alexander A. Green, Tony Mazzulli, Claudia DosSantos, Keith Morton, Teodor Veres, Keith Pardee.

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Rationale & Objectives: Current gold standard diagnostics require sophisticated equipment including thermocyclers, fluorescence readers and specialized training, limiting wider distribution to hospital or lab settings. To create a distributed, automated diagnostic platform, we have developed a microfluidic device capable of performing a molecular diagnostic assay from sample to answer. Our system simplifies the workflow and reduces cost, relying on isothermal amplification coupled with a glucogenic reporter whose output can be measured with a standard glucose meter.

Methods: Our published glucogenic diagnostic system was modified to make it amenable to microfluidic automation. The isothermal amplification method was changed to speed up the reaction and simplify the workflow. A reagent for lysing viral particles and inactivating inhibitors in human saliva was used to eliminate the need for spin-column based RNA purification. For microfluidic operation, disposable chips were designed for the assay. A novel metering system was devised to measure small volumes, minimizing the required reagents and lowering cost. A novel microfluidic system was also built to apply the necessary pneumatic operations to carry out the various steps of the assay, which would normally be performed manually.

Results: The changes made to the molecular assay protocol resulted in a more streamlined molecular diagnostic. In combination with the microfluidic innovations in metering and mixing of the molecular reagents, we have created an effective automated diagnostic platform. The platform device is capable of reliably carrying out the assay, performing all steps that would normally be performed manually, such as RNA extraction, the metering and mixing of components, and incubation at the correct temperatures. Preliminary testing has demonstrated on-chip assay integration, with clear glucose generation in the presence of SARS-CoV-2 RNA using human saliva. Patient sample testing will be underway shortly.

Conclusions & Significance: We have created a novel diagnostic system with low cost per test. Our approach reduces the need for equipment and expertise, which often limit the accessibility of molecular diagnostics. This is a platform technology that can be adapted to address a range of unmet molecular diagnostics needs in low-resource settings.

Poster #10: Microfluidic platform with electrical sensors integrated onto a porous membrane substrate for biological barrier modeling

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Rationale & Objectives: Electrical cell-substrate impedance sensing (ECIS) provides a non-invasive alternative to traditional barrier assessment e.g. transendothelial electrical resistance (TEER), by measuring cells grown on electrodes. We integrated ECIS electrodes on porous membranes into microfluidic devices, enabling direct measurement of biological barrier integrity in organ-on-chip. This approach avoids the confounding effects of multiple cell types and biomaterials that prevent direct measurement of barrier function by TEER in advanced organ-on-chip platforms.

Methods: Gold electrodes were fabricated on porous membranes using hot embossing and UV lithography, and incorporated with acrylic and doubled-sided tape layers to form microfluidic devices. Top channels (area ~ 1.05 cm^2) were seeded with primary human umbilical vein endothelial cells (HUVECs) or human brain microvascular endothelial cells (HMVEC). Bovine collagen type I (2.7 mg/mL) containing DMEM was incorporated in bottom channels. HMVEC were co-cultured with primary human astrocytes in collagen hydrogel. Devices were connected to a syringe pump for application of fluid shear. Impedance was measured using a lock-in amplifier, and TEER with Millicell ERS volt-ohm meter electrodes placed in channel outlets.

Results: HUVECs cultured in devices showed increased ECIS resistance (4 kHz) vs. cell-free electrodes, indicating barrier formation. Importantly, presence of hydrogel in the bottom channel did not influence ECIS resistance ($p>0.4$), vs. TEER measurements in same devices, where hydrogels contributed 904 Ω·cm^2 ($p=0.006$) additional resistance over medium-only controls; endothelial barrier resistance is typically 50-500 Ω·cm^2. The platform supported co-culture over 5 days, as confirmed by confocal microscopy, immunostaining for GFAP (astrocytes) and ZO-1 (HMVEC). Measurements with cell-free electrodes in phosphate-buffered saline indicated ECIS resistance values were not affected by application of fluid shear (5 dyn/cm^2).

Conclusions & Significance: Building from previous work establishing porous membrane ECIS in co-culture inserts, we show the potential of this technology in organ-on-chip. In particular, this method can directly measure endothelial barriers in platforms containing multiple cell types and extracellular matrices, with broad applications to microphysiological models containing biological barriers.

Poster #11: Decellularized porcine skin reinforced by PCL-PEG frameworks as a 3D bioprinted skin substitute for burn healing applications.

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Rationale & Objectives: Tissue-engineered skin substitutes for wound healing have advanced dramatically in clinical trials during the past few years to improve wound healing and enhance functional and aesthetic outcomes. However, the management of burned skin remains challenging. The main objective of this study is to develop a 3D-bioprinted skin substitute to improve burn wound healing. In this study, a 3D-bioprinted skin substitute comprised of decellularized extracellular matrix (dECM) and biodegradable PCL-PEG was fabricated using 3D bioprinting technology.

Methods: For 3D printing of the framework, PCL and PEG were mixed in dichloromethane. Then the material was freeze-dried and pestled to obtain the powdery printing materials. Taguchi's design of experiments (DoE) method was used to optimize the 3D printing process. For dECM bioprinting, porcine skin was decellularized by chemical-enzymatic methods. The freeze-dried skin powder was dissolved in an acetic acid/ pepsin, and the pH was adjusted to 7.4 to develop a 3D bioprinting bioink. Engineering characterization, such as mechanical properties, SEM, FTIR, water contact angle, and water uptake tests, were conducted to investigate the mechanical, morphological, and Physico-chemical properties of the 3D-printed structure.

Results: The bioprinting parameters were optimized by L9 orthogonal array Taguchi DoE (i.e., Temperature 75˚C, pressure 30KPa, nozzle diameter 27G, and PEG concentration 30%Wt). An 8-layer bioprinted skin substitute (20×20×12 mm3) with a porosity of 56% and a mean diameter of 218μm was fabricated. The contact angle for the PCL structure is about 118°, which decreased to 33° for the PCL-PEG skin scaffold. The tensile strength of the optimized sample was recorded at 22 MPa, and the elongation at break was registered at 98%. Histological analyses verified the absence of cells and cell debris in dECM matrix. The dECM bioink was designated as a thermosensitive hydrogel for 3D bioprinting.

Conclusions & Significance: This study investigated the possibility of employing 3D bioprinting technology to fabricate a skin tissue-engineered using dECM and biodegradable PCL-PEG frameworks. The outcomes of this study demonstrated the promising potential of 3D bioprinting for developing a skin substitute with dECM and polymeric composites for skin tissue engineering applications.

Poster #12: Integrating ultrathin extracellular matrix-derived membranes for organ-on-a-chip applications.

Jeremy Newton, Edmond W. K. Young.

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Rationale & Objectives: Thin membranes are important structures in organ-on-a-chip (OOC) devices that enable precise control of the 3D arrangement of cells. In most devices, synthetic membranes with a biological coating are used, but recent trends have shown efforts to adapt hydrogel manufacturing methods to produce thin, purely biological membranes in OOC devices. Our project aims to develop a simple user-friendly method for fabricating thin extracellular matrix (ECM)-derived membranes in thermoplastic devices primarily for air-liquid interface coculture experiments.

Methods: A micro-milled thermoplastic device was designed to enable simple patterning of an ECM hydrogel using a micropipette, which was then air-dried to produce a thin ECM membrane in its place. Confocal reflective imaging was used to characterize the thickness and fibrous architecture of the membrane. Tunable control of the membrane thickness was enabled through variation in initial gel thickness and concentration. The device was designed to allow user-friendly cell culture on either side of the membrane without further manipulation after membrane fabrication.

Results: Device design optimization was conducted to improve control of membrane characteristics and enable robust adhesion of the membrane to the device. Characterisation of membrane thickness and solute permeability was conducted, and thickness was shown to be highly controllable. An arrayed well-plate version of the device was tested to demonstrate the potential for higher-throughput experiments and scaled fabrication. Cytoskeletal organization and focal adhesion expression of Calu-3 lung adenocarcinoma cells on fabricated membranes was characterised to further establish the OOC applications of the device.

Conclusions & Significance: The development and characterisation of a user-friendly culture platform utilizing a thin biological culture substrate has been completed. Proof-of-concept experiments establishing the capabilities and OOC applications of the device have been conducted. The developed device can be adapted to modelling a range of thin tissues through control of the membrane properties.

Poster #13: Developing a microfluidic platform to measure the elasticity of bioparticles by using acoustic forces inside a microchannel.

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Rationale & Objectives: Measuring cellular elasticity has an important role in both diagnosis and therapeutic applications. Atomic force microscopy (AFM) is currently being used as the gold standard in elasticity measurements. However, AFM has a few drawbacks, including low throughput in cell analysis, and results that depend on the probe used. Our aim is therefore to develop a technique with higher throughput, to enable elasticity measurements for a population of cells.

Methods: Our method employs acoustics to manipulate fluid in a microfluidic device. Acoustic actuation of adhered cells on a glass substrate inside a microchannel cause a rotational flow around the oscillating cells, termed microstreaming flow. As reported in the literature, microstreaming velocity is related to the mechanical properties of the cells. We aim to calibrate cell-induced microstreaming velocities with elasticity data measured from AFM. We have initiated this work, first by using polymers structures as a proxy for cells, to have predictable elasticity. We use stop flow lithography to polymerize specifically formulated array structures inside our microfluidic channel, for our initial calibration experiments.

Results: First, we are creating a robust protocol to measure microstreaming velocity around the synthesized polymer structures. Second, we are adjusting the parameters in polymer synthesis method to achieve homogeneous micropost structures with uniform elasticity. Finally, we are developing a standardized technique to measure the elasticity of microposts with AFM. We have achieved these three goals and measured velocity and elasticity for a group of microposts from one specific polymer composition, polyethylene glycol diacrylate (PEGDA250) with the microstreaming velocity of ~475.8 μm/s ±80 μm/s, and elasticity of 37.5 MPa ± 8 MPa.

Conclusions & Significance: After developing a robust method for creating the calibration graph, we need to synthesize polymers from different compositions and find both microstreaming velocity and the corresponding elasticity from AFM. Calibration graph can provide us with elasticity information for the unknown material by measuring the microstreaming velocity around them.

Poster #14: Additive manufacturing leveraged microfluidic setup for sample to answer nucleic acid-based detection of pathogens.

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Rationale & Objectives: The recent pandemic emphasized the necessity of rapid pathogen detection techniques. Loop-mediated amplification (LAMP) assay is an exciting technique owing to constant temperature and versatility with sensitive colorimetric readout methods. However, the assay is a multi-step process often requiring trained personnel. Moreover, colorimetric readouts are not sensitive to naked eye. In this work, we present a LAMP-on-chip microfluidic platform for multiplexed colorimetric detection of pathogen nucleic acid using additive manufacturing techniques.

Methods: To integrate all steps, from sample collection to endpoint detection onto a microfluidic cartridge, we leveraged, (1) tunable elastomeric chambers for fluid control, (2) additive manufacturing, and (3) open-source hardware and software. In brief, for fluidic operations, we employed a 3D printed contraption for angle-dependent actuation of pressure chamber for precise volumetric control, on our SLA 3D printed chip. For aiding in automation, we designed a portable setup with actuators, heaters, and an epi-illumination imaging setup for color change over our novel nanostructured platform. The whole setup is centrally concerted by microcontrollers operated via mobile phone.

Results: The automated setup was able to demonstrate the detection of SARS-CoV-2 wildtype nucleic acid in 18 minutes at a clinically relevant load of 8*10e5 RNA copies/µL. The mechanically actuated chambers demonstrated bidirectional pumping of fluid volumes down to 0.1 µl/per 30-degrees angular rotation. The imaging setup offered better illumination with the maximum relative intensity varying only 17% while achieving a resolution of 0.155µm/pixel.

Conclusions & Significance: This work demonstrates a novel 3D printed microfluidic chip for automation of amplification assays with minimal involvement of the user, further the microfluidic capabilities for rapid multiplexed pathogen detection. Further steps include validation with clinical samples.

Poster #15: Optimization of bioprinting parameters using deep learning for the high throughput fabrication of 3D organoids.

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Rationale & Objectives: To overcome the limitations of existing animal experiments and 2D cultured cell lines, organoids and 3D microtissues that imitate in vivo human organs have emerged. Organoids are multicellular components and can better represent complex in vivo cellular responses and interactions compared to conventional 2D cell cultures. However, with the current technology, the organoid fabrication process is time-consuming and expensive since they are produced by manual pipetting, requiring highly skilled personnel.

Methods: In this study, we develop an extrusion-based 3D bioprinting system that can be scaled up to fabricate organoids with high throughput. With deep learning technology, we optimize the bioprinting parameters (e.g., cell concentration, needle gauge, dispensing pressure, and dispensing time) required for predicting droplet volume to produce the various size of the organoids. Furthermore, we establish an automated system to print multiple droplets at once and measure their contact angle and volume simultaneously to collect many datasets, which are required for training the deep learning algorithms.

Results: As preliminary data, the droplet bioprinting and crosslinking were performed with bioink consisting of gelatin methacryloyl (GelMA) and 3T3 fibroblast cells, which contribute to the formation of connective tissue. After printing a droplet of 5 μL volume consisting of 5% GelMA hydrogel containing 13,300 cells on a transwell membrane, crosslinking was performed for 3 minutes using visible light to succeed in cell encapsulation. Cell viability was checked with live dead assay staining and healthy cell proliferation and elongation were continued until seven days.

Conclusions & Significance: Through this research, it will be possible to predict the optimized printing parameter condition to print desired droplet size. Finally, we expect to develop an automated extrusion-based 3D bioprinting system, which is cost-effective for organoid production for many biomedical applications.

Poster #16: Microfluidic spheroid-on-a-chip model for screening of liposome internalization in solid tumors.

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Rationale and objective. Nanomedicine has the potential to improve the biodistribution of encapsulated compounds by delivering them selectively to the pathological site and/or avoiding exposure of potentially endangered tissues (site-avoidance drug delivery). To improve the prediction of the performance of nanoparticles (NPs) within the human body, the development of preclinical *in vitro* tumor models to represent the complex processes of NPs transport under close-to-physiological flow is of great importance.

Methods. Fluorescently-labelled liposomes with different dimensions were generated by employing microfluidics (MF) and supplied to cell-free microgels and breast cancer spheroids in a MF spheroid-on-a-chip platform. Breast cancer spheroids were grown in tissue-mimetic hydrogels using breast carcinoma MCF-7 cells. Spheroids grew under near physiological flow conditions for 24 hours and were then subjected to liposome formulations with various size by continuous perfusion for 6 days. Uptake and retention kinetics of liposomes in individual cell-free microgels and spheroids were examined using wide-field fluorescence microscopy. The penetration of liposomes was assessed using confocal fluorescence microscopy after 48 h of incubation.

Results. We generated arrays of biomimetic microgels and breast cancer spheroids with uniform size of 300 um using MF spheroid-on-a-chip platform. To study the size-dependent penetration of liposomes in the microgels and in the breast cancer spheroids, we selected 4 from 180 tested experimental conditions of liposome generation using MF hydrodynamic focusing method. We explored the effect of bidirectional flow on accumulation of liposomes with hydrodynamic diameters of 42, 92, 131, and 197 nm in cell-free biomimetic microgels. Using the generated library liposomes, we demonstrated the size-dependent accumulation, retention and penetration of liposomes in MCF-7 spheroids under near physiological flow.

Conclusions & Significance. We developed an innovative MF spheroid-on-a-chip platform utilizing a biomimetic hydrogel matrix, uniformly sized spheroids, and spheroid growth under near physiological flow. Given the versatility of the MF platform in growing various tissues, it can be used for the screening of nanomaterials for a wide range of applications, including cosmetics and vaccine development.

Poster #17: Printed electronics using metal nanoparticles and programmable fluorescent molecules.

Zlata Hadzifejzovic and Darius Rackus.

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Rationale & Objectives: There is an increasing trend for the fabrication of miniaturized objects for the computer and communication industries, which properties could be under spatial and temporal control, achieved by the ability of surface plasmons of metal nanoparticles to manipulate the behaviour of information-carrying molecules. We accomplished this by designing PDMS stamps with features dimensions of 100 μm that were used in the microcontact printing on glass surfaces to deposit a layer of silver nanoparticle ink, further utilized in metal-organic interactions.

Methods: The stamp models were designed in AutoCAD, etched to the silicon wafer in the clean room, and fabricated in PDMS with pre-design features. We then generated patterns of 3% APTES on glass slides through microcontact printing, followed by immersing glass slides in one of two nanoparticle solutions: silver nanoparticle ink (30 wt % dispersion in ethylene glycol made by MilliporeSigma) or custom-made triangular silver nanoplates. After 30 min immersion, the glass slides were rinsed with MilliQ, dried with nitrogen gas, and covered with a layer of fluorophores through spin-coating.

Results: We optimized a protocol to minimize background and non-specific manual deposition of silver nanoparticles on the glass substrate by adjusting the solution concentration while performing microcontact printing. We further characterized the patterns of silver nanoparticles by using scanning electron microscopy (SEM). Finally, we hope to present that a combination of stamp ink with spin-coated fluorophores demonstrates a spatial control of metal-enhanced fluorescence.

Conclusions & Significance: We used microcontact printing to generate patterns of AgNPs on glass substrates. Combining the AgNPs layer with a layer of fluorophores, we demonstrate spatial control of metal-enhanced fluorescence. This opens future opportunities for molecular data writing on the next-generation functional materials for informational technology and optical storage devices.

Poster #18: High throughput fabrication of fractal topographical substrates for high fidelity culture of kidney podocytes.

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Rationale & Objectives: Podocytes, with fractal branching morphology and wrapping around a dense cluster of capillaries in vivo, play a key role in the filtration function of the human kidney, which is enabled by their interdigitated foot processes and slit diaphragms. However, current 2D podocyte cultures do not exhibit hallmarks of mature podocytes such as interdigitations. This project was aimed at developing a high throughput method of fabricating fractal topographical (2.5D) cell culture substrates that could support high fidelity culture of podocytes in vitro.

Methods: A unit of fractal pattern was designed by tracing histology images of glomeruli (considered as a glomerulus slice) and packing each design unit at a high density to form a patterned area of desirable size (5cm x 5cm). The densely packed fractal pattern was directly laser-patterned onto AZ P4620 positive photoresist using a microwriter, followed by reflowing to smooth the edges, in order to fabricate the master mould. The structured TPEs were incorporated into well plate, sterilized, and tested for cell culture.

Results: The fractal dimension of the designed patterns was confirmed to approximate that of the native glomerulus. Human or mouse podocytes could be seeded and cultured on the patterned 2.5D substrates using standard cell culture techniques. Podocytes were able to form a confluent layer on TPE substrates. Interestingly, cell coverage was significantly higher on convex versus concave fractal patterns. Polydimethylsiloxane (PDMS) was used an alternative substrate material for proof-of-concept experiments to demonstrate the effects of fractal cues. Mouse podocytes cultured on fractal PDMS substrates showed higher expression of podocin and more glycocalyx-like apical protrusions compared to those grown on flat substrates.

Conclusions & Significance: Fractal topographical substrates made of biocompatible TPEs can be fabricated in a high throughput manner and allow podocytes to be cultured on topographical surfaces that resemble the shape complexity of the native glomerulus. They are a powerful tool that could be used for glomerular disease modelling, nephrotoxic drug screening, and testing of new drugs.

Poster #19: Engineering of architectural complexities of conical cardiac ventricle using polyesters.

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Rationale & Objectives: Currently, cardiovascular disease is responsible for highest number of deaths per year and hence we require transplantable engineered heart tissues. To construct these, the first step forward is to manufacture these tissues in a miniature, millimeter scaled with the physiological relevant features.

Methods: The focus of this design is to mimic the myofiber alignment of the native LV structure by topographically aligning cardiomyocytes on a thin scaffold sheet before wrapping the sheet around a mandrel. The architecture of the myocardium is comprised of multiple elongated myofibers with various cellular orientations (from +60° to -60°) from the endocardial to the epicardial surface. To model this, a planar scaffold was made from an unsaturated polyester, PICO polymer using previously described methodologies. Wrapping the planar scaffold around a central mandrel has resulted in a 3D ventricular model with the ability of incorporation of up to 3 different fiber orientation directionalities.

Results: Our results indicate that the left ventricle constructed from a planar scaffold drives cellular alignment in the direction of the microgrooves. Additionally, seeded scaffolds up to 3 layers showed no significant cell death using CFDA/PI staining. Furthermore, the wrapped scaffold was able to successfully contain fluid and had pressure volume changes over time detected by a Catheter inserted in the engineered cavity. Finally, the scaffold demonstrated the capability of detecting calcium transient traces and the engineered tissue was stimulable by electrical field stimulation.

Conclusions & Significance: We have constructed a a proof-of-concept for creating 3D ventricle models with physiological relevant fiber orientaton. In addition, our platform could allow better assessment of cardiac functionality and pharmacokinetics/pharmacodynamics predictability.

Poster #20: A flexible biosensing platform for high-throughput measurement of cardiomyocyte contractility.

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Rationale & Objectives: Emerging heart-on-a-chip platforms are promising approaches to establish cardiac cell/tissue models in vitro for research on cardiac physiology, disease modeling, and drug cardiotoxicity testing. However, challenges exist in integrating electrical biosensing components on heart-on-a-chip platforms to evaluate the contractile functions of in vitro cardiac cell/tissue models.

Methods: This project reports a novel carbon-based biosensing platform that has the capability of continuous measurement of contractility, beating rate, and beating rhythm in a monolayer of iPSC-cardiomyoytes. This device array contains 24 elements configured in the standard 24-well format, including ultrathin suspended membrane arrays integrated with carbon black-PDMS (CB-PDMS) strain sensors for contraction measurement and carbon fiber electrodes for electrical pacing of cardiomyocytes. This device array has been utilized to perform comprehensive measurement of cardiomyocyte contractile function with the increase of culture days and in response to different concentrations of cardiac drugs.

Results: Synchronous cell beating in a monolayer deflects each ultrathin suspended membrane and induces electrical resistance changes of the embedded strain sensor. Results show that the CB-PDMS strain sensor fabricated by the spray deposition method has a higher gauge factor value than that fabricated by the screen-printing method. The improvement of sensitivity is attributed to the more porous microstructures generated by direct spray deposition. Validation was conducted by monitoring the development of cardiomyocyte contractility with the increase of culture days and under drug administration.

Conclusions & Significance: This project reported a new flexible biosensing platform that enables in-situ and continuous measurement of contractile properties of iPSC-CM monolayers. The results confirmed that the biosensing array is capable of measuring changes in cardiomyocyte contractility for high-throughput cardiac drug testing.

Poster #21: Healing with sound: optimization of genetic and non-genetic cargo delivery with commercial ultrasound system and microbubbles.

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Rationale & Objectives: Targeted delivery of drugs, genes and siRNA using ultrasound and microbubbles (USMB) has emerged as a promising approach for treating diseases such as cancer and acute respiratory distress syndrome (ARDS). Currently, there are no systematic optimization studies investigating how different commercial ultrasound system settings and microbubble parameters affect the efficiency of cargo delivery. This project aims to optimize different commercial ultrasound and microbubble parameters for delivery of different genetic and non-genetic cargo in vitro.

Methods: For systematic optimization of different cargo delivery parameters, a standardized, 3D-printed, modular platform called ultrasound-microbubble-cell chamber (UMCC) is developed. UMCC is designed on the concept of modularity and consists different pre-designed blocks that snap-fit into each other to form the complete platform, analogous to LEGO® blocks. We use a commercial Phillips SONOS 5500 ultrasound system with Phillips S3 transducer, as well as commercial DEFINITY® microbubbles for optimization studies. As a model drug, we use different molecular weight dextrans, specifically, 4 kDa and 70 kDA fluorescent dextran. We quantify the delivery of these dextrans in HEK293 and CMT167 cells using flow cytometry.

Results: We studied the effects of different commercial ultrasound parameters such as mechanical index (MI), pulse interval (PI) and ultrasound exposure time as well as DEFINITY® microbubble concentration on the efficiency of dextran delivery. We found that proportion of dextran positive cells increases with increasing MI but decreases with increasing PI. Moreover, the proportion of dextran positive cells was found to increase with increasing ultrasound exposure time. However, beyond a certain exposure time, there is no significant increase in dextran positive cells. We also found that dextran delivery peaks in a narrow range of DEFINITY® microbubble concentration.

Conclusions & Significance: Our results show that different parameters such as MI, PI, exposure time and microbubble concentration affect USMB-delivery and these parameters can be optimized for maximizing the efficacy of cargo delivery. In future, we will be optimizing different parameters for siRNA delivery as well as utilize microfluidic micro- and nanobubbles.

Poster #22: Studies of cell interactions in clusters by a microfluidic device.

Kelsy Yuan, Mary Ma, Samantha McWhirter, Olga Tatsiy, Logan Zettle, and Gilbert Walker.

Department of Chemistry, University of Toronto.

Rationale & Objectives: Cell pairs or small cell clusters of the population can exhibit distinctive functional phenotypes characteristic of disease relapse. Our approach is to develop a combination of drug products that modulate the surface calreticulin (CRT) in a nano-formulation, which could enhance tumor cell recognition by macrophages from the immune system and increase therapeutic efficacy.

Methods: Based on a previously designed microfluidic chip,[3] modifications of the parameters (i.e. starting cell concentrations, microgel stiffness, and relative flow rates) were made to produce alginate microgels that were better suited for this work. ImageStream flow cytometry was then incorporated for measuring surface marker expressions and the extent of phagocytosis upon different enhancing treatments in a high-throughput manner.

Results: This poster provides updates on our work in developing such a microfluidic device to screen phagocytic cell recognition of AML cell lines by testing different enhancing agents. The statistical analysis of the produced microgel-encapsulated cell clusters was fully understood. The endocytosis and protein expression of lipid nanoparticle-encapsulated mRNA, the immune response to added drugs, the expression of surface markers such as CRT, and phagocytosis were measured and analyzed by image flow cytometry.

Conclusions & Significance: The microfluidic drug screening system was successfully tested and established to measure immune responses between AML cells and macrophages in a high throughput fashion in a 3D environment. This high-throughput system can also be easily replicated and applied to screen a variety of many other drugs or human diseases, with a high sensitivity and specificity.

Poster #23: Point of crime rapid DNA analysis using digital microfluidics.

Mohamed Elsayed, Leticia Bodo, Jonathan Millman, Aaron Wheeler.

Institute of Biomedical Engineering, University of Toronto; Department of Chemistry, University of Toronto; Biology Section, Centre of Forensic Sciences.

Rationale & Objectives: Rapid DNA instruments can perform forensic DNA analysis in less than 2 hours at the point of crime. However, sexual assault samples containing DNA from multiple sources, cannot be processed as-is in the field and must be sent to a lab for sample pre-processing known as differential extraction (DE). The objective of this project is to automate DE using digital microfluidics (DMF). This would enable quicker analysis of sexual assault samples collected at the point of care or point of crime.

Methods: The conventional DE method starts with lysing non-sperm cells, followed by a series of centrifugation steps to wash away non-sperm DNA prior to sperm cell lysis. A modified DE method, called differential digestion (DD), uses DNase to replace the need for centrifuge wash steps in removing non-sperm DNA. The multiple incubation steps in DD at varying temperatures are easy to automate using DMF. Using buccal swabs mixed with semen, the performance of these two methods were compared. DD was carried out using a DMF device. After DNA purification, qPCR was performed to quantify the total human DNA and male DNA. We also verified the effectiveness of the methods by processing a vaginal swab collected post-coitus.

Results: We assessed extraction efficiency, sperm fraction purity and DNA degradation. DNA extraction efficiency using DD was 30 to 35% in tubes and 13% using digital microfluidics, which were both higher than the DNA extraction efficiency using DE (9%). The retrieved sperm fraction contained 100% male DNA for the in-tube protocol and 86 to 100% male DNA for the DMF protocol. DNA degradation was assessed by quantifying different sized amplicons using qPCR and calculating their ratio. Larger amplicons are more susceptible to degradation, so a ratio of around 1 (less than 2) indicates no degradation. We found that the use of DNase did not degrade male DNA across all experiments.

Conclusions & Significance: We automated sample processing of artificial sexual assault samples using differential digestion on DMF. The sperm fraction contained > 86 % male DNA, and the DNA did not degrade. The next steps are to perform short tandem repeat typing to demonstrate that the DNA can be used for human identification.

Poster #24: Conductive PDMS electrodes for bioanalytical applications.

Kathy Ly, Darius G. Rackus.

Department of Chemistry and Biology, Toronto Metropolitan University; Institute for Biomedical Engineering, Science and Technology (iBEST).

Rationale & Objectives: Chemical measurements can be made within microfluidic devices using electrochemical sensors, which themselves can be miniaturized. However, many electrode integration methods rely on costly materials or processes. Instead, polydimethylsiloxane (PDMS) can be doped with conductive materials, such as carbon black, to produce electrodes that can be easily integrated with PDMS-based microchannels. Here, we report the characterization of carbon-doped PDMS (C-PDMS) electrodes for integration with a droplet microfluidic device.

Methods: C-PDMS paste was prepared by first dispersing carbon black in isopropyl alcohol followed by mixing with PDMS pre-polymer. The carbon black-pre-polymer was then mixed 10:1 with crosslinker with final carbon compositions of 12-20% (w/w). Microelectrodes were formed by applying C-PDMS paste to features formed from 40 µm deep trenches formed in a PDMS substrate. C-PDMS electrodes were characterized for their electrochemical performance using cyclic voltammetry of measure Ru(II)/(III) oxidation/reduction. Electrodes were integrated with a droplet generator through PDMS-PDMS bonding. Amperometry was used to measure electron transfer in droplets.

Results: Electrodes formed from C-PDMS with 20% (w/w) carbon black showed reproducible CVs with high faradaic currents. Further, the electrodes were shown to follow Randles-Sevcik behaviour, demonstrated by linearity of oxidation and reduction peak currents vs. (scan rate)_. Amperometry results from the electrodes integrated with the droplet generator demonstrate that we are able to measure analyte concentrations in single droplets.

Conclusions & Significance: We have established a protocol to form C-PDMS electrodes and integrated them with a droplet generator. In future, we will use this device to measure surface expressed alkaline phosphatase activity from single cells encapsulated in droplets, revealing cell heterogeneity in alkaline phosphatase expression.

Poster #25: An automation workflow for high-throughput manufacturing and analysis of scaffold-supported 3D tissue arrays.

Ruonan Cao, Nancy T Li, Jose L Cadavid, Simon Latour, Cassidy M Tan, and Alison P McGuigan.

Institute of Biomedical Engineering, University of Toronto; Department of Chemical Engineering and Applied Chemistry, University of Toronto.

Rationale & Objectives: Patient-derived organoids have emerged as a useful tool to model tumour heterogeneity. Scaling these complex culture models while enabling stratified analysis of different cellular sub-populations remains a challenge, however. We aim to develop an automated workflow to generate engineered tissues in scale.

Methods: One strategy to enable higher throughput organoid cultures that also enables easy image-based analysis is the Scaffold-supported Platform for Organoid-based Tissues (SPOT) platform. SPOT allows the generation of flat, thin and dimensionally-defined microtissues in both 96- and 384-well plate footprints that are compatible with image-based readouts. SPOT is currently manufactured manually, however, limiting scalability. In this study, we optimize an automation approach to generate tumour-mimetic 3D-engineered microtissues in SPOT using a liquid handler. Further, we develop a liquid handler-supported cell extraction protocol to support downstream analysis.

Results: Using the optimized liquid handler-supported seeding protocol, we have shown comparable within- and between-sample variation to standard manual manufacturing. As a proof-of-value demonstration, we generate 3D complex tissues containing different proportions of tumour and stromal cells and perform single-cell-based end-point analysis to probe the impact of co-culture on the tumour cell population and capture physiological relevant cell responses. We also demonstrate that we can incorporate primary patient-derived organoids into the pipeline to capture patient-level tumour heterogeneity.

Conclusions & Significance: We envision that our work will offer new avenues for discovering novel personalized medicines as it offers the potential for investigating patient-level heterogeneity, with the possibility of combining with big-data analysis in the future.

Poster #26: Temporal multiplexed fluorescence imaging device for bead-based immunoassay detection.

Ziyin Wei, Xilong Yuan, Yali Gao, Jiahua Dou, Mianjun Wang, Nathan Ng, Stewart Aitchison.

Department of Electrical and Computer Engineering, University of Toronto.

Rationale & Objectives: Sepsis is a severe organ dysfunction that can progress rapidly and cause patient death within hours. Early detection is crucial to treating sepsis because it allows control at the initial stage and could significantly increase the survival rate of the patients. Sepsis detection involves multiple biomarkers, and this complexity is challenging when developing a point-of-care the diagnosis device. The development of a point-of-care device is desired to meet the needs for in-time, accurate, and multiplexed testing.

Methods: We propose a temporal multiplexed fluorescence imaging microfluidic device that is capable of detecting multiple biomarkers at the same time. Temporally multiplexed excitation encodes two wavelengths of light to illuminate the fluorophores in different time windows. Bead-based multiplex immunoassay can detect multiple biomarkers in a single assay. The biomarkers are captured by the antibodies on the fluorescent microbeads, and further react with a secondary fluorophore. The assayed sample is illuminated by red and blue LEDs and imaged by an epi-fluorescence optical system. In this way, the device can perform qualitative and quantitative detection in one run.

Results: The prototype device is able to distinguish three different fluorescence intensities of the microbeads, which allows for the classification of three biomarkers for sepsis detection: IL-5, IL-6, and IFN-a. Calibration curves of each individual biomarker are plotted, and their lower detection limits are 40 pg/ml, 40 pg/ml, and 10 pg/ml, respectively. Multiplexed tests are also recorded to test the performance of the device, and the comparison of these tests shows good independence of the fluorescence signals between the biomarkers and the capability of measuring concentration differences when multiplex biomarkers are present.

Conclusions & Significance: The results demonstrate that the proposed device can be used for sepsis diagnosis and potentially other complex disease detection where multiple biomarkers should be considered. With further optimization and miniaturized design, the device may achieve accurate, multiplexed, and low-cost diagnostic measurements and contribute to the point-of-care health service.

Poster #27: Label-free SERS liquid biopsy of single extracellular vesicles using a nanopatterned microchip for molecular profiling in glioblastoma.

Mahsa Jalali, Carolina del Real Mata, Olivia Jeane, Laura Montermini, Iman Hosseini, Cristiana Spinelli, Yao Lu, Walter Reisner, Janusz Rak, Sara Mahshid.

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Rationale & Objectives: Liquid biopsy represents an emerging paradigm in real-time monitoring of cancer progression with potential to assess cancer status rapidly with minimal invasiveness and in a clinically actionable manner. Extracellular vesicles (EVs) are heterogeneous structures shed into the biofluids by all cells thereby exteriorizing their unique fingerprints and therefore are a promising platform for cancer liquid biopsy applications. The main challenge with EVs liquid biopsy is their heterogeneity.

Methods: Here, by combining the working principles of plasmonic nanocavities, and the chemistry of semiconducting materials, we have developed a sensitive and easy-to-use micro-chip platform to perform SERS identification on single EVs derived from glioblastoma stem cells and blood samples in a minute amount of fluid (<10 µl). The micro-chip is benchmarked by identifying and distinguishing signals from single-EVs derived from liposomes, non-cancerous glial cells (NHA), glioma cells (U373, U87) and glioma stem cells (GSC83, GSC1005, GSC1123). SERS is an attractive alternative to immunoaffinity approaches, while providing biochemical information on a label-free basis for both EV surface chemistry and molecular contents.

Results: These cell lines were either wild-type or manipulated to express relevant molecular alterations such as oncogenic epidermal growth factor receptor variant III (EGFRvIII), and phosphatase and tensin homologue (PTEN) which is naturally lost during GBM progression. The capacity of the microchip in statistical differentiation of EVs from cancer cells and healthy ones demonstrated an accuracy of 91.37 ± 3%. This approach is effective in extra low amount of EVs which makes it suitable for non-invasive monitoring of blood plasma to differentiate between tumour EVs and platelet-derived particles.

Conclusions & Significance: The single EV SERS microchip successfully revealed the molecular diversity of Glioma cells by converting them into highly granular, actionable physical fingerprints, combining the complexities of individual EVs and their heterogeneous subpopulations.

Poster #28: Bioengineering human airway mimetic integrating airflow.

Kayshani R. Kanagarajah, Wuyang Gao, Christine Bear, Theo Moraes, Keith Morton, Teodor Veres, Axel Guenther, Amy P. Wong

Centre for Research and Applications in Fluidic Technologies (CRAFT); Department of Developmental and Stem Cell Biology, The Hospital for Sick Children; Department of Laboratory Medicine and Pathobiology, University of Toronto; Department of Mechanical and Industrial Engineering, University of Toronto; Medical Devices Research Centre, National Research Council Canada.

Rationale & Objectives: Current in-vitro models for respiratory diseases poorly reflect the native microenvironment of the human airways, questioning their use as representative models. Nonetheless, microfluidic chips have the ability to recapitulate key, dynamic components of the airway. Integrating this technology and extrusion based bioprinting, we develop a novel in-vitro model to enhance the study of human airway pathologies.

Methods: We developed an acrylic-based microfluidic chip prototype, capable of constant airflow perfusion through tubular collagen scaffolds to mimic the structural and dynamic components of the native small airways. The tube ends are cannulated on plastic needles in the media reservoir of the device, to maintain alignment with the microchannels during fluid and gas perfusion. After seeding cells, and formation of confluent monolayer, the devices are connected to a separate platform to perfuse warm, humidified air through the tube. The tubes were subjected to dynamic or static airflow conditions and characterized to capture changes in epithelium maturity and integrity with regards to airflow stresses.

Results: As proof-of-concept, 500,000 immortalized human bronchial epithelial cells were seeded in the collagen scaffold filled with media. The cells self-organize and form a uniform layer on the luminal surface within 5 days, demonstrating presence of viable cells, proliferation, strong attachment to collagen IV, and defined tight junctions using immunostaining. Our preliminary results with 50cm/s airflow condition 1 week indicated dense DNA content in the nuclei compared to no airflow and static air exposure conditions suggesting 50cm/s airflow velocity is not sustainable for cell viability long-term. Whereas 2.5cm/s has been identified as supporting cell viability for 2 weeks.

Conclusions & Significance: The integration of extrusion bioprinting and airway-on-a-chip technology, demonstrates the robust impact airflow stresses can have on airway epithelial cells, and which cannot be replicated or accounted for in current 2D ALI in vitro models.

Poster #29: Whole blood fractionation and cell isolation on a centrifugal microfluidic platform.

Byeong-Ui Moon, Liviu Clime, Daniel Brassard, Alex Boutin, Keith Morton, Teodor Veres.

Medical Devices Research Centre, National Research Council Canada.

Rationale & Objectives: Fractionation of whole blood components is an important process in many clinical applications. The existing blood fractionating methods are dependent on laboratory protocols. It uses serological pipettes requiring gently layering the blood sample on the density media followed by centrifugation; it is a time consuming and labor-intensive work. Here, we present an automating centrifugal microfluidic platform (CMP) of the entire blood fractionation and extraction steps using an aqueous two-phase system (ATPS).

Methods: We used a custom-made mixture of ATPS to create advanced separation layers. A mixture of stock solutions, polyethylene glycol (PEG) and dextran (DEX) are used to match the density of blood cells, which leads the densities of PEG and DEX to 1.071 and 1.086 g/mL, respectively. A microfluidic device was designed and fabricated in-house exclusively by CNC machining, injection molding and 3D printing. The main chip is composed of several fluidic reservoirs (ATPS, high density gradient media, waste, separation and collection chambers) as well as channels connecting these reservoirs mutually with the world-to-chip interface.

Results: We performed whole blood fractionation and isolation experiments on the CMP using ATPS. The entire process is as follows: 1) Blood transportation to the main chamber after ATPS separation; 2) Fractionation of four different blood layers; plasma, buffy coat, neutrophils and red blood cells; 3) Transportation of the plasma player to chamber A; 4) Moving the entire layers up using high density gradient media; 5) Transportation of the peripheral blood mononuclear cells to chamber B; 6) Transportation of the neutrophil cells to chamber C. The resulted cell counting analysis and cell viability studies showed equivalence to traditional manual methods.

Conclusions & Significance: We demonstrated that the advanced layers of ATPS enables to fractionate whole blood and isolate blood cells on the CMP. This automating ATPS-CMP system can find in applications for cell isolation procedures and bioanalytical assays.

Poster #30: Patient stratification through COVID-19 patient biomarkers.

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Rationale & Objectives: Here we report the development of a quantitative isothermal assay for the clinical stratification of COVID-19 patients, using new and established biomarkers. The inspiration behind this project stems from the unmet need to rapidly identify patients with severe pathology presenting to emergency care to direct clinical resources to patients most in need of care. Therefore, the objective of this project is to build an analytical platform and method that can be used for patient stratification in emergency care settings.

Methods: Our proposed stratification method uses nucleic acid sequence-based amplification (NASBA), toehold-based DNA array circuit to power a multiplexed quantitative isothermal assay for target molecular biomarkers. In the reaction design, the NASBA component interacts with a toehold module to catalyse toehold-mediated strand displacement (TMSD). During TMSD, a displaced FAM-labelled strand is designed to interact with a DNA array component for quantitative purposes.

Results: To date, the TMSD mechanism and one-pot NASBA reactions specific to targets of the molecular signature have been established. We are now focused on the development of the quantification elements of the project and the overall system will then be evaluated using mock samples before patient sample testing, including deployment at the point-of-need at St. Michaels Hospital.

Conclusions & Significance: Currently, there are no point-of need tests to inform risk stratification patients. Therefore, the successful completion of the project will advance precision medicine for Covid-19 patients, providing the right drug at the right time to the right person.

Poster #31: A digital microfluidic-distance-based approach to portable sample processing and signal detection for nucleic acid amplification assays.

Man Ho, Sathishkumar N, and Aaron Wheeler.

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Rationale & Objectives: There is great enthusiasm for using loop-mediated isothermal amplification (LAMP) in point-of-care nucleic acid amplification tests (POC NAATs) as an alternative to PCR. While isothermal amplification techniques like LAMP eliminate the need for rapid temperature cycling in a portable format, these systems still require (1) a dedicated optical detection apparatus for analysis and (2) manual off-chip sample processing. Here, we developed an RT-LAMP-based microfluidic system to address these limitations.

Methods: The system comprised a Digital Microfluidic (DMF) device (fabricated at the CRAFT facility) for automatic liquid manipulation and a distance-based detector (DBD) (i.e., a slip of chromatography paper) for direct signal readout. Briefly, RNA from cultured, inactivated SARS-CoV-2 was extracted using QIAamp viral RNA kits (Qiagen, Germantown, USA) and amplified using a WarmStart® reverse transcribed LAMP (RT-LAMP) colourimetric assay kit with primers specific to SARSCoV-2 RNA. DBDs fabricated from Whatman™ Grade 1 and 4 paper were characterized by analyzing DNA standards; Two intercalating dyes, SYBR® Green I and SYBR® Safe were optimized to provide an adequate signal readout on DBD.

Results: The Whatman™ Grade 4 paper chosen over Grade 1 due to its higher flow rate, which speeds up analysis. Among the intercalating dyes, SYBR® Safe was less expensive, less toxic and exhibited superior performance for the DBDs. As the DBD method was quite sensitive to background signals found in LAMP products, we introduced a Capto™ adhere bead-based clean-up procedure to remove low-molecular-weight oligomers from the mixture before elution on DBDs. Finally, we automated all the steps (including amplification, clean-up of LAMP products, mixing with the reporter, and transferring to DBD) using DMF.

Conclusions & Significance: The DMF-DBD technique measured ~ 9000 copies/mL of the SARS-CoV-2 virus, which is in the clinically relevant concentration range (10^4 to 10^7 copies/mL). We propose that the combination of DMF with distance-based detection might be a powerful technique for performing portable NAATs for a wide range of applications in the future.

Poster #32: Micro-patterned BSA-based hydrogels for highly sensitive immunofluorescence assays.

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Rationale & Objectives: Having superior precision and accuracy in microscale, piezo-driven, non-contact dispensers have widely been used to print picoliter-sized droplets of bioinks for high throughput immunoassays in industries and research institutes. These printers, however, can typically create 2D microarrays. Generating 3D dots could greatly enhance the sensitivity of the assays due to the increased surface area. Here, we introduce a novel approach to print BSA-based micro hydrogels incorporating interleukin-6 (IL-6) capture antibodies.

Methods: Glass slides were first CO2 plasma treated followed by a 30 min fluorosilanization (FS) using chemical vapor deposition (CVD) method. While the quick FS treatment could increase the hydrophobicity of the glass surface which is required for the printing step, the remaining carboxy groups on the surface could be used for covalent attachment of hydrogels to the surface. Next, a bioink composed of BSA and 150 ug/ml IL-6 capture antibody diluted in PBS was printed on the slide. Further, 1-ethyl-3-(-3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) diluted in MES (pH:4.5) was printed on top to crosslink BSA proteins together and to the surface.

Results: The stability of the hydrogels as well as non-specific attachment of biomolecules to the hydrogels were assessed using different concentrations of BSA, EDC, and number of layers (achieved by repeating the printing cycle, and the optimum parameters were determined. SEM images showed surface topography and porosity of the hydrogels. We also evaluated the morphology and thickness the hydrogels using z-stack confocal microscopy. Compared to conventional bioinks printing, hydrogel microarrays could significantly increase the signal and sensitivity achieved after performing IL-6 immunofluorescence assay.

Conclusions & Significance: We could successfully print micro arrays of BSA-based hydrogels via a piezo-driven dispenser. The 3D shape of microdots and their associated porosities significantly increased the surface area thereby intensifying the signal and efficacy in IL-6 immunofluorescence assays.

Poster #33: Droplet-based microfluidic studies of the transport phenomena in the production of polymer microparticles by solvent extraction process.

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Rationale & Objectives: Encapsulating drugs in polymer microparticles using solvent extraction from polymer solution droplets is a popular method of preparing drugs for targeted drug delivery and controlled release treatments. The extraction rate of the solvent is a crucial parameter that impacts the morphology and the porosity of the microparticle, and this, in turn, affect the drug release kinetics. However, a fundamental mechanistic understanding of the solvent extraction dynamics as a function of shear is hitherto unexplored.

Methods: Our objective is to develop a model that can predict the extraction dynamics and describe the polymer microparticle morphology in the drop as functions of time and flow conditions. We use droplet-based microfluidics as a diagnostic platform to experimentally delineate the connection between shear and extraction dynamics at the level of a single drop. Our experiments, conducted in a microfluidic extensional flow device (MEFD), have enabled us to create a hydrodynamic trap in a diamond shaped channel, where we can trap polymer drops and observe their dissolution process under shear for an extended period of time.

Results: Our experiments were conducted with polymer solution drops of a biocompatible polymer, poly- lactic-co-glycolic acid (PLGA)with ethyl acetate (EtOAc) as the solvent, and solutions of polyvinyl-alcohol(PVA) as the suspending aqueous medium. We found a close agreement between our experimental results and our theoretical predictions of dissolution rates, and a surprising inference that the extraction dynamics is primarily controlled by the mass transport in the suspending phase and is seemingly independent of the viscosity of the drop phase fluid.We are also currently studying the extraction dynamics in drug encapsulated polymer drops, using piroxicam and rhodamine-B as model drugs for our preliminary experiments.

Conclusions & Significance: The results from our experiments will be used in developing a comprehensive model to predict the extraction rates and polymer microparticle composition, that can be used across various industrial drug manufacturing processes. It is also an important fundamental contribution to the existing literature on the mass transfer mechanisms in partially miscible emulsions.

Poster #34: 3D on-chip scaffold as a model to study galectin-1 in kidney transplant rejection.

Alex Boshart, Kieran Manion, Shravanthi Rajasekar, Chuan Liu, Sergi Clotet-Freixas, Milica Radisic, Boyang Zhang, Ana Konvalinka.

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Rationale & Objectives: Antibody-mediated rejection (AMR) accounts for >50% of premature kidney graft loss. AMR is caused by donor-specific antibodies (DSA) that bind human leukocyte antigens (HLA) on the graft endothelium, leading to pro-inflammatory cytokine production (e.g. TNF-**α**) by immune cells. AMR is not fully understood, and effective treatments are lacking, due in part to a lack of models recapitulating the kidney microvasculature. We have previously identified the immunomodulatory protein galectin-1 to be significantly increased in AMR glomeruli. Our goal is to study the role of galectin-1 in the renal endothelium and AMR using novel 3D models that recapitulate the renal microvasculature.

Methods: We performed laser-capture microdissection to compare the tubulointerstitium of AMR to non-AMR biopsies. We examined secreted levels of galectin-1 in a dual vascular–tubular chip emulating the vascularized proximal tubule complexes in a kidney. To model rejection, the prototypical rejection cytokine TNF-**α** was perfused through the vasculature and supernatants from both the vascular and tubular compartments were collected and analyzed. The vasculature was also treated with anti-HLA Class I; adhesion molecule ICAM-1 was assessed with immunofluorescence microscopy.

Results: Galectin-1 protein was found to be significantly increased in the tubulointerstitium of AMR biopsies compared to no rejection.TNF-**α** treatment showed a numerical increase in galectin-1 secretion in the vascular compartment with no change in the tubular compartment. HLA Class I treatment showed increased ICAM-1 staining compared to vehicle.

Conclusions & Significance: Currently, there are no optimal animal models of AMR. Future experiments will use the novel in vitro model above to evaluate the effect of galectin-1 on immune and endothelial cells. Galectin-1 may represent a therapeutic target in AMR. This model of the peritubular capillary is important to AMR and other forms of kidney injury such as ischemia and fibrosis.

Poster #35: Assessing endothelial cell mechanosensory response to multi-directional wall shear stress.

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Rationale & Objectives: Atherosclerosis is a disease of the artery wall characterized by plaque formation at regions associated with flow disturbances. Blood flow through a vessel can become turbulent when passing through plaque-burdened regions within the vasculature. At sufficiently high wall shear stress (WSS) disturbances, endothelial cell dysfunction occurs, exacerbating the atherosclerotic characteristics of the vessel. Endothelial cell response to varying patterns of WSS has not been thoroughly investigated.

Methods: A novel microfluidic device was manufactured from optically clear PDMS, which can be used as a cell scaffold and is compatible with live-cell fluorescent microscopy. The device consists of a small channel for fluid to pass over endothelial cells, with two perpendicular jets to create varying levels of multi-directional flow, simulating physiological WSS in disturbed flow regions of the vasculature. Micro-PIV measurements and analysis were used to determine the time variation in the instantaneous WSS throughout the device. Human umbilical vein endothelial cells were seeded into the devices and loaded with FURA-2 AM, then the fluorescent emissions were captured to produce images of the cytosolic calcium dynamics.

Results: A transient spike in cytosolic-free calcium was observed in a fraction of the cells when exposed to changes in laminar flow. A muted but much more sustained response was seen in nearly all cells exposed to multi-directional disturbed flow, indicative of activation of a different mechanosensitive pathway. Endothelial cells did not respond to repeated pulses of laminar flow, leading us to believe that endothelial cells do not initiate signaling cascades due to changes in flow magnitude, rather strictly signaling changes in flow direction. With more data we will be able to begin correlating calcium profiles with specific flow parameters.

Conclusions & Significance: The results of this experiment will contribute to the understanding of the differential response of endothelial cells to WSS. The characterization of endothelial cell responses to varying flow patterns is essential in strengthening the link between endothelial cell response to flow and atherosclerotic development.

Poster #36: Incorporating elastomeric particles into bioinks to enhance 3D-printed tissues stability.

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Rationale & Objectives: 3D printing has emerged as a dominant fabrication tool in the field of tissue engineering. That said, the availability of bioink is capable of yielding reproducible results and is biocompatible remains one of the main challenges in the field. It is critical to fabricate a stable material that will maintain its printed shape while allowing tissue remodelling by cells in the environment and providing stiffness that is similar to native tissue.

Methods: To generate droplets, POMaC polymer was injected using a syringe pump into a glass beaker filled with 1%w/v PVA solution. The POMaC polymer solution forms particles upon being encapsulated by the PVA. Particles were then crosslinked under UV light. To create monodisperse POMaC particles, droplet microfluidic techniques were used. PDMS chips were made using conventional photolithography and soft lithography. The particles were collected and crosslinked under UV light. Following particle generation, the particles were mixed at a concentration of 1 M/ml in GelMA. Together, the GelMA and the particles made up our bioink for 3D-printed hearts. After the constructs were printed, they were seeded with cardiomyocytes.

Results: 72 hours after cell seeding, the 3D-printed hearts began beating. Mechanical testing performed of the GelMA-particles printed constructs reveals an increase in construct stiffness and plasticity when compared to particle-free constructs. Therefore, the addition of particles is shown to have mechanical stability, which is critical for shape maintenance. Cardiomyocyte seeding on GelMA-particles printed constructs resulted in higher cell coverage, increased tissue contractility, and enhanced cardiac functionality compared to constructs without particles. After approximately 7-10 days, we observed that the structures containing particles remained stable while those without were less likely to maintain their shape.

Conclusions & Significance: We show a novel combination between elastomeric POMaC particles and biological hydrogels. The results highlight how these particles are capable of mimicking an elasticity analogous to that found in native tissue as well as possess sufficient biocompatibility for cell growth and maintenance. This work will allow for the future development of engineered tissues.

Poster #37: Towards neural-network-on-a-chip: integrating microfluidics with DNA-based computers for powerful point-of-care diagnostics.

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Rationale & Objectives: Machine learning can diagnose diseases based on the amount of different RNA present in patient samples. However, this requires costly computers, equipment, and trained operators. DNA-based computers are a cheap and accessible alternative for reading and processing patient RNA. Currently, DNA computers are limited in processing power due to "leak", interference from two interacting signals. By designing DNA computers that integrate with microfluidic devices, we can overcome leak for powerful point-of-care diagnostics.

Methods: Here we introduce DNA-based biomolecular neurons for low-cost machine-learning-capable DNA computers. These neurons assemble together to form in vitro neural networks. First, we use enzymes to create highly pure DNA components to reduce leak. Second, we immobilize the DNA strands onto magnetic beads for modular assembly. DNA by-products from neuron assembly and computation can then be removed from solution using magnets, further reducing leak. This immobilization also allows us to synchronize computation across each layer of the neural network. Integration with microfluidics will replace manual magnetic separation with automated fluid exchange, allowing massively parallel computing for neural-network-on-a-chip.

Results: We demonstrate proof-of-concept for biomolecular neurons and enzymatic synthesis through modular primer mix-and-match and nucleic acid hybridization. We show synthesis of 6 unique species of neurons with input-specific actuation and high purity, requiring only 2 hours of preparation from start to finish. Our neurons actuate quickly, in less than 15 minutes, with 8-fold signal-to-background ratio, and are capable of making synaptic connections with up to 10 other neurons. Furthermore, we demonstrate self-assembly of these biomolecular neurons into computational layers, and show basic neural network features, such as neuron cascading, fan-in and fan-out motifs.

Conclusions & Significance: Our proposed design enables computer-free neural networks for complex decision making in portable diagnostics, autonomous lab-on-a-chips, compact data storage, and development of other in vitro neural network architectures. We plan for near future integration with microfluidic devices to bring further programmability and ease of operation to DNA-based computing.

Poster #38: Colorimetric detection of clinical biomarkers on paper-based analytical devices using digital microfluidics for sample manipulating.

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Rationale & Objectives: This study proposes the use of Digital microfluidics (DMF) coupled with a paper-based analytical device (PAD) for sample manipulation, image capture and analysis through a desktop scanner due to its efficient light control. As a proof-of-concept, the detection of creatinine (CR) was explored as a model. Creatinine is a useful biomarker for the assessment of renal function as it is the metabolite waste product of muscle contraction excreted by the kidneys.

Methods: The colorimetric detection of CR was performed using the Jaffé reaction, which consists of the reaction of alkaline picrate with creatinine to form an orange-red product. First, a polyester plate containing 96 square zones and a scanner were used to optimize the concentrations of picric acid (PA) and NaOH. The captured images with the scanner were analyzed with the Corel Photo-Paint software in the CMYK color system, exploring the yellow channel. Then, the mix type and mixing time on the DMF chip and the image acquisition time of the PADs after the reaction were optimized. Finally, an automated protocol was developed on the DMF chip for the construction of the CR analytical curve.

Results: The range of AP concentration analyzed was from 0.5 to 10 mmol L-1, keeping the NaOH concentration fixed at 250 mmol L-1, and the best result was achieved using 5 mmol L-1 of AP. The NaOH concentration was optimized ranging from 50 to 450 mmol L-1, and the highest color intensity was observed at 300 mmol L-1. These experiments were performed using CR solutions prepared at concentrations of 8 and 32 mg dL-1. For manipulation of the drops on the DMF chip, Tetronic 90R4 surfactant at 0.1% (v/v) was used. Linear and circular mixtures were analyzed on the DMF chip with time varying from 15 to 60 seconds, the highest intensity and lowest standard deviation were obtained with the circular mixture in 45 seconds.

Conclusions & Significance: Under optimized conditions, the integrated system involving DMF and PAD showed great potential for CR detection. Preliminary results revealed a linear behavior in the concentration range between 2 and 32 mg dL-1. Future efforts will be devoted to complete automation involving the entire analytical procedure and application to real samples.

Poster #39: Heart-on-a-chip with mechanically matched 3D microelectrodes and force sensors enables multiparametric functional recordings.

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Rationale & Objectives: The development of soft bioelectronics and force sensors with tissue-like mechanical properties for in situ recording in organ-on-a-chip devices remains a challenge. Most of the available microelectrode arrays are built as planar sheets, preventing recording from 3D tissues without interfering with tissue contraction. Here, we developed a heart-on-a-chip platform that integrates soft 3D micropillar electrodes and highly flexible force sensors into a single device.

Methods: The high-aspect-ratio microelectrodes were 3D-printed into the device using a conductive hydrogel, poly(3,4-ethylenedioxythiophene): poly(styrene sulfonate). Force sensors were constructed from flexible nanocomposite 3D printed into a pair of parallel wires that also acted as tissue anchor points.

Results: Highly flexible potential and force sensors enabled unobstructed human iPSC-based cardiac tissue formation and contraction, suspended above the device surface, under both spontaneous beating and upon pacing with a separate set of integrated carbon electrodes. Recording of extracellular field potentials was demonstrated with and without epinephrine as a model drug non-invasively and at a high signal-to-noise level, along with in situ monitoring of tissue contractile properties and calcium transients.

Conclusions & Significance: Together, the integrated smart sensors, with tunable structural and mechanical properties supported efficient, stable, and non-invasive real-time recordings of electrophysiology and contractility of engineered heart tissue.

Poster #40: Biomimic assay based on molecularly-imprinted-polymers for the impedimetric detection of SARS-CoV-2/Influenza A spike proteins.

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Rationale & Objectives: With the continuously fluctuating incidence of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), critical gaps in the field of rapid diagnostic testing have been exposed, particularly in the diagnosis of viral respiratory infections. Current gold standard methods rely on real time quantitative polymerase chain reaction (RT-qPCR) for the detection of viral nucleic acids, but these tests are challenged with long turnaround times, costly centralized laboratory equipment and the need for trained personnel to execute the protocols.

Methods: Here, we propose the design of a novel assay based on our previously studied gold nano/micro islands (NMIs) as a core for the fabrication of an ultrathin molecularly imprinted polymer (MIP) for the impedimetric detection of SARS-CoV-2 and Influenza A spike proteins (SPs) in untreated saliva within 10 minutes. The proposed electrofabrication protocol is rapidly adaptable to a diverse repertoire of protein biomarkers.

Results: In this work, we demonstrate the impedimetric detection of the SARS-CoV-2 original strain, Alpha B.1.1.7, Delta B.1.617.2 and Omicron B.1.1.529 variant SP, as well as the Influenza A SP within physiologically relevant ranges and at a low limit of detection to enable the diagnosis of acute infections. Validation was performed at two unique test sites with 51 SARS-CoV-2 patient samples to demonstrate an overall 100% sensitivity and 100% specificity of the NMIs/MIPs assay. Robust quantification of the electrochemical assay was confirmed against RT-qPCR, which effectively enabled statistically significant ($p < .0005$) viral load quantification on a rapid, miniaturized, and ultrasensitive platform.

Conclusions & Significance: This novel technology presents the development of a quantitative and versatile electrochemical assay with the potential for the rapid detection of current and future viral respiratory infections, which can guide future electrochemical clinical and commercial point-of-care testing platforms.

Poster #41: Towards microplastics separation by microfluidics and analysis with laser-directed infrared spectroscopy.

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Rationale & Objectives: Conventional methods of microplastic sampling rely on filters and infrared spectroscopy for composition, both of which can be inefficient and tedious. Microfluidics has emerged as a technique for microparticle sorting and sample pre-processing for spectroscopic analysis. Literature reports do not characterize device performance with realistic samples and tend to use slow analytical methods. We seek to better characterize microfluidic particle sorters with more realistic standards and couple them to laser-directed infrared (LDIR) spectroscopy.

Methods: We designed two microfluidic particle separators – one based on differential lateral displacement (DLD) and one based on pinch flow fractionation (PFF) – based off designs previously reported for microplastics separation. The devices were first tested using commercial standards of 8 µm and 50 µm diameter PMMA and PS microparticles, respectively. Concurrently, we developed microplastics standards ranging in 1-100 µm from polymer pellets. Reference spectra of the microplastics were recorded using LDIR spectroscopy. Microplastics were sorted using the two devices. Sorted samples were collected and then identified using LDIR spectroscopy.

Results: We have been able to fabricate two microfluidic particle separators and evaluate one sorter. Our device using differential lateral displacement has successfully been able to separate PMMA particles of 8 µm with high efficiency, and we are currently working on the separation of 50 µm PMMA particles. Development of complex microplastic standards have been formed and referenced into a spectra library using LDIR spectroscopy. Evaluating our second microfluidic sorter based on pinched flow fractionation is also still in progress.

Conclusions & Significance: Microplastics affect human and aquatic species, the need for rapid analytical methods to determine microplastic properties (eg., size and composition). This will help determine their transport fate and reduce contamination. We have been able to design a microfluidic chip that is capable of sorting microplastics and providing fast characterization of these plastics.

Poster #42: Characterization of biosludge flocs from pulp and paper mills.

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Rationale & Objective: The activated sludge process is a common wastewater treatment technology in pulp and paper mills that generates a large amount of biosludge. Biosludge management expenses are considerable accounting for approximately 60 percent of overall treatment system operating expenses. Dewatering is the process of removing water from sludge to reduce its volume and make it more manageable. Due to the colloidal nature of the particles and the gel-like structure of the flocs (biosludge's building blocks), it is difficult to dewater biosludge, which contains up to 98% water. Biosludge is typically regarded as a non-Newtonian fluid with viscoelastic characteristics. However, the flocs' structures (physical and biological) and their effect on dewaterability remain poorly understood.

In preliminary microscopic images, some flocs of biosludge appeared to be darker than others. We became interested in exploring whether there were differences in the characteristics and structures of light and dark flocs, and whether these differences could impact their dewaterability.

Methods: By examining individual flocs at the microscale, my research project attempts to get a deeper and more mechanistic knowledge of the biosludge dewatering process starting with microfluidic channel measurements of the water permeability of single flocs by measuring the flow rate through single flocs. Furthermore, my research employs a mechanistic strategy to manipulate the dewatering process by investigating what happens in rheology experiments as the flocs are compressed and measuring the viscoelastic properties (storage and loss modulus). This, I believe, will eventually aid in the development of a comprehensive understanding of bulk-scale processes such as mass transfer through sludge flocs and sludge dewatering.

Results: The results at the single floc level indicated that light flocs have a higher water flow rate at a given pressure drop (i.e. are more permeable) in comparison to darker flocs. In addition, to completely compress darker flocs, roughly ten times higher pressure difference is required. Both light and dark flocs exhibit solid-like behaviour (G'>G") at the multiple floc level, although dark flocs are more structured than light flocs.

Conclusions & Significance: These results suggest that biosludge flocs from pulp and paper mills can be separated into at least two major classes with distinct properties: light and dark flocs. Understanding the various types of flocs and developing a model to better comprehend the dewatering mechanism may contribute to the optimization of the dewatering procedure.

Poster #43: Design of multi-model biodegradable brain stimulation electrodes towards activating endogenous neural precursor cells (NPCs).

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Rationale & Objectives: Harnessing the potential of endogenous neural precursor cells (NPCs) to enhance neural repair is a promising strategy with demonstrated success. The use of electrical stimulation and delivery of neurotrophic factors has shown great potential for activating NPCs. However, traditional implantable electrodes of such purposes involve interfaces that can cause inflammatory response and neuronal cell death around the implant site, which impose risks and costs due to requirements for surgical extraction after treatment period.

Methods: Here, we introduce a biodegradable, multi-model penetrating electrode that enables activation and promotes survival of NPCs. The design consists of microfabricated stimulation electrodes integrated with microfluidic pharmaceutical delivery channel made of bioresorbable materials.

Results: Optimized stimulation electrodes demonstrate superior electrochemical performance compared with conventional electrodes, showing the efficacy of delivering required current and electric field to the brain. Bioresorbable delivery system also showed its ability to inject neurotrophic factors into the cortex of live mice without leakage, backflow, or backtracking. The entire design undergoes harmless degradation over time and disappear in physiological conditions. The development of such platform would provide combinatorial approaches to enhance neuroplasticity of activated NPCs, while supporting host cell survival for neural repair through the delivery of therapeutic molecules to the central nervous system.

Conclusions & Significance: The biodegradable electrode can also be used in applications involving temporary neuromodulation such as neuroscience research as well as stem cell therapy for less-invasive treatment of neurodegenerative diseases.