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Biophysical Mechanisms of Microfluidic Devices: Future in Diagnostics and Microcellular Analyses

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ABSTRACT

Microfluidic devices (MFDs) have revolutionized fluid manipulation and analysis at the microscale. They sustain precise control over fluid flow through intricate networks of microchannels that allow for the integration of multiple laboratory practices onto a single chip, transforming scientific research and practical applications in biology, chemistry, and medical diagnostics. MFDs have fundamental characteristics that contribute to their functionality and versatility, such as materials like Polydimethylsiloxane (PDMS), providing composition flexibility, cost-effectiveness, and biocompatibility. In MFDs, fluid motion regulation involves employing various flows, microvalves, and capillary forces, stimulating fluid transport without external energy. The narrow width of the microchannels is advantageous in biomedical applications, as it assures efficient fluid separation and reduced sample volumes. This technology has exhibited practicality in protein characterization, DNA analysis, cell separation, and hormonal signal recognition, enabling sensitive detection and quantification of biomolecules. This provides researchers with valuable insight into complex biological processes and disease mechanisms. MFDs play a crucial role in simulating complex biological processes on a small scale, leading to the emergence of organ-on-a-chip and lab-on-a-chip technologies. MFDs hold remarkable promise in revolutionizing cancer diagnostics, serving as effective platforms for isolating and analyzing cancer-specific biomarkers, facilitating early detection, personalizing medicine, and monitoring tumor dynamics in real time. MFDs allow for the evaluation of therapeutic efficacy and investigation into cancer metastasis, enhancing intraoperative pathological evaluation. By leveraging their unique characteristics, MFDs have the potential to drive significant advancements in cancer diagnostics and improve our insight into complex biological processes, leading to enhanced and personalized therapeutics.

Introduction

MFDs consist of intricate networks of microchannels that offer meticulous control over fluid flow and have applications in various fields, exhibiting the potential to remodel experimental methods and practical applications in biomedicine. The history of MFDs traces back to the innovative work of Dr. George M. Whitesides and Dr. Andreas Manz, who initiated the field in the late 20th century(Convery et al., 2019). Noteworthy for introducing the vision of microfabricated devices, their research laid the foundation for MFDs, which integrate multiple laboratory functions onto a single chip. This field has quickly progressed, driven by advancements in assembly techniques, materials, and the use of novel functionalities. Due to this, MFDs are routinely used in laboratory, clinical, forensic, and agricultural domains. As shown in Fig. 1, MFDs have the capability to address questions in various biological fields that may require small diameters and precise cell manipulation. With around 39.5% of men and women being diagnosed with cancer at some point in their lifetime(National Cancer Institute, 2020), the need for advanced and effective cancer diagnostics has never been more critical. Further, the growing number of cancer survivors, projected to increase to 22.2 million by 2030(National Cancer Institute, 2020), stresses the need for improving medical diagnostics via MFDs to enhance patient quality of life. MFDs

hold enormous promise for revolutionizing cancer diagnostics. By leveraging its precise fluid manipulation capabilities, these devices can enable the isolation and investigation of cancer-specific biomarkers and the creation of microenvironments that emulate physiological states. Integrating MFDs into cancer diagnostics will improve early detection, personalized medicine practices, and patient prognosis. They allow for high-throughput screening, improved sensitivity, and real-time monitoring, addressing the current limitations of conventional diagnostic instruments. With ongoing advancements and innovations in microfluidic technology, MFDs can provide further avenues in research and diagnostics, promoting our understanding of diseases and improving patient care.

Biophysical Mechanisms of MFDs

MFDs comprise several essential components that contribute to their functionality and versatility. These devices are fabricated using materials such as 3D-printed polymers and PDMS(Nielsen et al., 2019). 3D-printed materials offer advantages such as design flexibility and cost-effectiveness. PDMS is favored for its biocompatibility, the capacity to be integrated with live tissue, as well as its optical transparency(Wang et al., 2017). The fabrication process often involves photolithography, which utilizes light to produce microchannels and configurations on substrates. Photolithography enables precise and reproducible patterning of microfluidic features through steps such as photoresist application, exposure, development, and etching(Mukherjee et al., 2019). MFDs utilize different flows by which samples navigate through the channels, including continuous and droplet-based flows. Continuous flow refers to the steady movement of fluids within microchannels, facilitating processes such as mixing, reactions, and separation(Stanley et al., 2012). Droplet-based flows generate and manipulate discrete droplets within immiscible fluids, promoting high-throughput analyses and precise management of reagent volumes(Zagnoni et al., 2011). Different types of microvalves are used to regulate fluid flow within MFDs. Active microvalves depend on actuation using external stimuli, such as electrical or pneumatic signals, whereas passive microvalves leverage intrinsic fluidic properties or geometric elements to control flow(Zhang et al., 2007). Regarding fluid transport, passive microvalved MFDs rely on capillary forces, such as pressure, resistance, and surface tension, for fluid movement without the need for external power(Hassan et al., 2020). Furthermore, the small diameter of microfluidic channels, typically between 1-1000 µm, ensures efficient fluid separation, as shown in Fig. 2, and a reduction in sample volumes. Movement of a Newtonian fluid through a microfluidic channel is defined by Reynold's number ($\Re = \frac{\rho VD}{\mu}$), where ρ is the density of the fluid, V is the fluid velocity, D is the microchannel diameter, and μ is the dynamic viscosity of the fluid. The Navier-Stokes equation for an incompressible flow in the annular axisymmetric region around the droplet is given by $\frac{1}{r}\frac{\partial}{\partial r} = -\frac{1}{\mu c}\left(\frac{\partial p}{\partial z}\right)$, where p is the pressure of the fluid, u is the flow velocity, and r and z are radial and axial coordinates(Sajeesh et al., 2014). The droplet-based flow speed of microfluidics is dependent on several parameters, such as the volume of the droplets, geometric width of the channel, interfacial surface tension, viscosity μ_d, and viscosity μ_c, and rate of flow of the continuous phase(Jakiela et al., 2011). The size of the drops are inversely proportional to the capillary number (Ca) of the continuous phase (causes liquid to pinch, thus forming droplets) by $D(T) \propto Ca^{-1} = \left(\frac{\eta c(T) uc}{\gamma c(T)}\right)^{-1} = \frac{\gamma c(T)}{\eta c(T) uc}$, where D is the diameter of the drop, T is the temperature, η_c is the dynamic viscosity of the continuous phase, u_c is the characteristic speed of continuous phase in m/s, and γ_c is the interfacial surface tension between continuous and the disperse phase in mN/m(Stan et al., 2009). Flow rate can vary according to the geometrical structure of the microchannel. In cylindrical microchannels, the flow rate (volume/time) can be measured using the formula $Q = 2\pi \int_0^a u(r)\, r\, dr = \frac{\pi a^4}{8\mu}\left|\frac{dp}{dz}\right|$(Holmes, 2015). However, in rectangular microchannels, the approximate flow rate, can be measured using the

formula $Q = w\int_0^h u(y)dy = \frac{wh^3\Delta p}{12\mu L}$(Holmes, 2015). By combining these components, MFDs present a powerful medium for various applications, ranging from biomedical diagnostics to chemical analysis and lab-on-a-chip technologies.

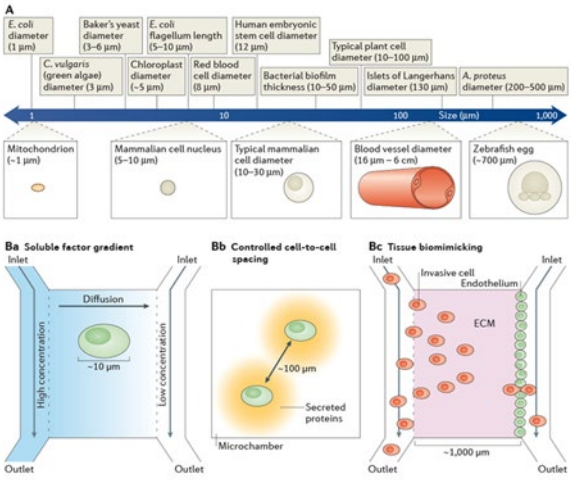

Figure 1. (A) Logarithmic ruler depicts the diameters of various biological tissue that MFDs can manipulate. (Ba) depicts specific intrinsic mechanics of MFDs that abide by properties of diffusion. (Bb) depicts MFDs ability to detect secreted biomarkers and hormones. (Bc) depicts MFDs capacity to mimic live tissue to simulate "organ-on-a-chip" functions(Duncombe et al., 2015).

Current Biological and Biomedical Applications of MFDs

MFDs have been used as practical instruments in various biological settings. Due to their specificity and practicality, microfluidics are widely used in laboratory experiments. One application is Microfluidic Modulation Spectroscopy (MMS), an innovative approach that incorporates microfluidics with infrared spectroscopic techniques, enabling precise characterization of the secondary structure of proteins that exhibit complex folding patterns and dynamics(Liu et al., 2020). This is essential for understanding protein behavior and for protein profiling. For instance, monoclonal antibodies (mAbs) have been analyzed using MMS, making it possible to analyze proteins with low formulation concentrations(Ivancic et al., 2022). Additionally, studies show that MMS provides higher sensitivity and reproducibility for low concentration samples over the conventional Fourier Transform InfraRed (FTIR) method(Liu et al., 2020). Current studies prove that MFDs are useful in forensic DNA analysis, reducing risk of contamination, yielding shorter analysis times, and allowing for direct application at the crime scene(Bruijns et al., 2016). Pathologists can obtain immediate and accurate diagnostic information by integrating microfluidic technologies with procedures such as rapid tissue sectioning, staining, and analysis(Schulte et al., 2002). As shown in Fig. 3, MFDs can also be utilized for rapid cytokeratin immunohistochemical staining in frozen sections(Brajkovic et al., 2017). Furthermore, fully integrated microfluidic systems allow for the completion of challenging processing steps and applications, such as microcapillary electrophoresis (µCE), microarrays, single-copy DNA sequencing, and single-cell gene expression profiling(Njoroge et al., 2011) Using intrinsic cell properties such as fluid dynamic forces, size, density, electrical and magnetic charges, microfluidics are able to separate heterogeneous cell populations(Nasiri et al., 2020). Biological, chemical, and hormonal signal recognition is another area in which MFDs are utilized, enabling the detection and quantification of signaling molecules with sensitivity and specificity. For example, microfluidics allow for islet hormone secretion detection in insulin resistance, as islet dysfunction leads to diabetes(Li et al.,

2023). In a study using MFDs to analyze Human Luteinizing Hormone (hLH), Human Chorionic Gonadotropin Hormone (hGH) and Immunoglobulin G (IgG) protein, an impressive regression coefficient and detection limit of 0.9985 and 0.61 IU/L were recorded(Ahi et al., 2022), highlighting the precision and specificity of microfluidics. This cultivates a deeper understanding of complex biological processes and disease mechanisms, offering insights into physiological responses and pathology. MFDs contribute to microscopy techniques, particularly in research involving *C. elegans*, a transparent nematode widely employed in biological studies due to their genetic homology to humans and ease in genetic manipulation[(Levine et al., 2020), (Aboobaker et al., 2000), (Kaletta et al., 2006)]. Integrating microfluidics with *C. elegans* allows for precise manipulation, immobilization, and real-time monitoring of individual worms(Levine et al., 2020). Microfluidics-based mammalian cell culture offers precision in spatio-temporal regulation of microenvironments, allowing for live imaging and the recapitulation of physiological conditions(Nocera et al., 2022). One disease commonly studied is Parkinson's Disease (PD), which emerges with the loss of dopaminergic neurons and the accumulation of α-synuclein (α-syn) protein(Youssef et al., 2019). *C. elegans* integrated microfluidics offers assay automation, giving advantages to PD neurobehavioral analysis based upon locomotion, speed, and reversal behaviors(Tong et al., 2013). MFDs are also employed in pathogen detection, offering sensitive identification and characterization of infectious agents. Although microfluidics replace traditional labor-intensive and slow-culture methods of detecting pathogens in food, capturing and purifying foodborne pathogens remain imperfect and inefficient(Zhao et al., 2019). This offers high-throughput analysis of genetic variation and biomolecular interactions, paving the way for innovative procedures in investigating cancer biology, drug screening, and personalized medicine. MFDs play a crucial role in simulating organ function (Organ-on-a-Chip) and complex biochemical processes on a small scale(Human Organs-on-Chips, 2014). Organ-on-a-chip, originally coined in 2007 by Donald Ingber MD, PhD, of the Wyss Institute, was inspired by the narrow channels of alveoli in a "lung-on-a-chip" system(Human Organs-on-Chips, 2014). These systems simulate various biological processes, designing realistic and dynamic platforms for therapeutics. Likewise, MFDs enable small-scale laboratory simulations (Lab-on-a-Chip), accelerating research, reducing costs, and offering novel insights into fundamental biological processes and diagnostic interventions(Franke et al., 2008).

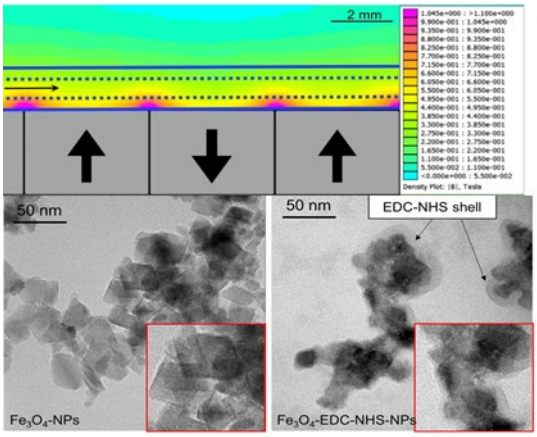

Figure 2. (top) Simulation of a magnetic gradient displaying locations with higher concentrations of nanoparticle (NP) accumulation in the microchannel of an MFD. (bottom) Transmission Electron Microscopy (TEM) images of NPs within the microchannels(María Sancho-Albero et al., 2020).

Future of Cancer Diagnostics Using MFDs

MFDs are poised to revolutionize cancer diagnostics, presenting innovative methods for early detection and personalized medicine. Liquid biopsy, which uses blood samples for the detection of biomarkers, has emerged as a favorable non-invasive cancer detection and monitoring procedure. MFDs provide an effective platform for the isolation and analysis of circulating tumor cells (CTCs), cell-free DNA (cfDNA), circulating tumor-derived DNA (ctDNA), exosomes, and other biomarkers present in liquid biopsy samples[(Nikanjam et al., 2022), (Khamenehfar et al., 2016), (Xu et al., 2019), (Heidrich et al., 2020)]. As shown in Fig. 4, MFDs facilitate imaging and quantitative analysis of cancer-specific biomarkers in tissue, such as tonsils. These devices allow for early cancer detection and real-time monitoring of tumor cell dynamics, improving personalized medicine strategies and enhancing patient outcomes(Heidrich et al., 2020). Characteristics of CTCs can be analyzed using MFDs, allowing for prostate-specific membrane antigen (PSMA)-directed diagnostics and therapies(Heidrich et al., 2020). Biomarker detection has an essential role in early cancer diagnosis and prognosis. MFDs can be utilized to design highly sensitive and precise assays for biomarker detection. By integrating capture probes, nanomaterials, and advanced detection processes, MFDs allow for the identification and quantification of cancer-specific biomarkers(Noor et al., 2023). Using MFDs with a disposable electrochemical biosensor, the breast cancer biomarker, HER2-extracellular domain (ECD), was detected in human blood with a limit of detection of 2.1 ng/mL(Noor et al., 2023). Tools such as these sensors embedded in MFDs allow doctors to detect cancer-specific biomarkers in patients even in the early stages of cancer. Evaluating the potency of therapeutics is paramount for effective and adequate cancer treatment. MFDs provide a unique opportunity to create assays that mimic tumor microenvironments and to evaluate the response of cancer cells to different drugs. By integrating microfluidic systems that imitate physiological conditions, researchers can experiment with multiple drug candidates and optimize treatment plans(Pérez-Rodríguez et al., 2021). By utilizing a multichannel microfluidic model, scientists were able to study hydrodynamic shear stress on epithelial-mesenchymal transition (EMT), which promotes cancer cell migration and invasion, and the cytotoxic drug responses to erlotinib and NSC-750212 in A549 lung cancer cells(Mani et al., 2019). Real-time monitoring of cancer cell proliferation is crucial for understanding cancer dynamics and tailoring treatment strategies. MFDs provide the ability to recreate the complex interactions within tumor microenvironments, facilitating studies on cancer progression and metastasis(Zhang et al., 2022).

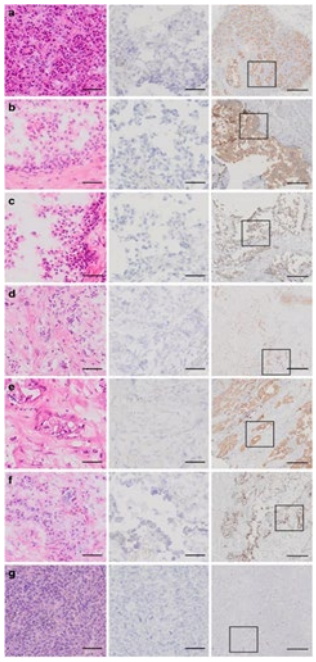

Figure 3. Results from optimized microfluidic tissue processor (MTP) staining protocol after development of a complete pan-cytokeratin chromogenic staining protocol on frozen sections. Figure depicts results of different tissue types[a=pancreas, b=ureter, c=prostate, d=breast carcinoma, e=pancreas adenocarcinoma, f=lung carcinoma (NSCLC), and g=Hodgkin lymphoma]. The first column is the conventional H&E staining, second is CTL staining for a negative control, then column 3 are cytokeratin stains at 200mm (column 3)(Brajkovic et al., 2017).

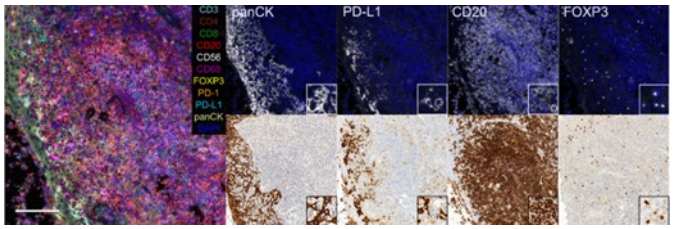

Figure 4. Fluorescent imaging of cancer biomarkers in tonsils. Images were obtained using microfluidic 10-plex immunofluorescence and bright-field imaging techniques of conventional single-plex immunohistochemistry on slides adjacent to one another. The images are on scales of 100 µm for the overviews and 15 µm for the insets(Migliozzi et al., 2019).

Integrating Artificial Intelligence and Imaging Technologies with MFDs

MFDs can be further integrated in fields such as artificial intelligence (AI), which bears tremendous prospects for ongoing microfluidics research. Automated data computation, pattern recognition, and decision-making can be accomplished by applying machine learning models to the data acquired from MFDs(Harofte et al., 2022). This integration can enhance the sensitivity and specificity of diagnostic assays, improve pathogen detection accuracy, and reveal correlations within datasets, leading to improved healthcare prognoses. As shown by Fig. 5, AI machine learning algorithms can help predict outcomes in scientific studies. By employing AI algorithms, the composition and structure of MFDs can be optimized, allowing for prompt prototyping and evaluation of new configurations. This integration can facilitate enhanced control and allow for adaptive optimization of fluid flow within MFDs, improving their functionality and performance. By regularly monitoring the fluid behavior within MFDs and leveraging AI algorithms, researchers can dynamically accommodate flow rates, pressure gradients, and other parameters to optimize the performance of MFDs in applications. This adaptive management enhances isolation efficiency and reaction kinetics, enabling more efficient and precise manipulation of fluids at the microscale. Combining the computational power of AI with the precision and versatility of MFDs opens prospects and drives further refinements in microfluidics. One domain in which AI integration can particularly influence MFDs is the field of imaging. Coupling MFDs with cutting-edge imaging techniques, such as fluorescence microscopy or single-cell imaging, facilitates the monitoring of cellular processes. Studies show that MFDs can be integrated with fluorescence microscopy and amperometric detection for real-time monitoring of exocytotic events, including the release of individual SH-SY5Y neuroblastoma cells(Shi et al., 2010), which originate from the SK-N-SH neuroblastoma cell line(SH-SY5Y: Human Neuroblastoma Cell Line (ATCC CRL-2266) | MSKCC, n.d.). Moreover, quantitative time-lapse fluorescence microscopy has been integrated with MFDs to control the nuclear localization of the budding yeast transcription factor Msn2(Hansen et al., 2015). This integration provides practical insights into cellular behavior, signaling pathways, and disease mechanisms. AI algorithms can examine the imaging data obtained from MFDs, allowing for automated cell tracking, image segmentation, and quantitative analysis. This cross between MFDs and AI imaging promotes new research in high-throughput screening studies, cellular diagnostics, and personalized medicine.

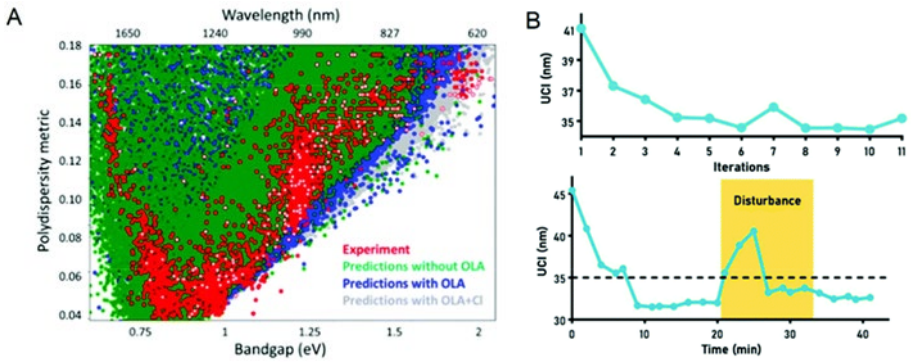

Figure 5. (A) Example of a machine learning model predicting the effects of oleylamine and chloride on Polybutylene Succinate (PbS) synthesis. (B) Microfluidic reactor model of self-optimization and self-interference for PbS nanoparticle synthesis(Liu et al., 2021).

Conclusion/Discussion

MFDs have already been used in a wide array of applications since their inception in the scientific community. They have been optimized for biomedical applications by the use of PDMS, making them biocompatible. MFDs hold tremendous promise in revolutionizing personalized medicine through their intrinsic separational properties and versatility in research. They have made significant impacts in domains such as diagnostics, therapeutics, immunology, microscopy, and imaging. MFDs have accelerated research timelines and discoveries, from basic biological research to translational and personalized medicine. Integrating MFDs with machine learning pattern recognition models could ensure sensitive quantification of samples, reducing human error, irreproducibility, and subjectivity in data analysis. One can imagine using MFDs to obtain non-invasive accurate results as opposed to highly-invasive techniques that may offer inaccurate results. Not only can MFDs enhance diagnostics, but also intraoperative pathological evaluations, where rapid frozen-section processing and histological interpretations occur to guide clinical decision-making during surgery. Besides intraoperative pathological evaluations, the role of MFDs in clinical settings have yet to be extensively explored. With further research, MFDs can help guide targeted therapeutics, advance our understanding of disease mechanisms, and expedite research in a wide array of sciences.

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