Digital Microfluidic Lab-on-a-chip for multiplex detection of biomarkers in Exhaled Breath Condensate

Daisy Ashton

Supervisors: Loic Coudron, Laura Urbano and Ian Johnston

Background

- Exhaled breath (EB) carries diagnostic biomarkers, which are biological indicators of infection and disease.
- Microfluidics is the science of miniscule volumes of fluid and its manipulation and the study of its behaviour.
- Digital Microfluidics (DMF) technology involves the manipulation of an ultra-small droplet on an array of microelectrodes.
- A lab-on-a-chip (LOC) device combines laboratory tests, such as blood analysis, ELISA assays and DNA amplification, all on a single miniature chip.
- Digital microfluidic multiplex LOC detection of lung disease biomarkers from EB can be carried out noninvasively and painlessly at point-of-care by the use of EB collection devices.

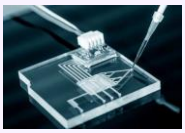

Figure 1: A digital microfluidic system (Berthier, 2018). Figure 2: A multiplex lab-on-a-chip device (Maxwell, 2016).

Motivation and Aim

- British Lung Foundation/Asthma UK states that 'lung diseases are responsible for more than 700,000 hospital admissions and over 6 million inpatient bed-days in the UK each year' and that 'somebody dies from lung disease in the UK every 5 minutes' (British Lung Foundation, 2017).
- 'It is thought that approximately 10% of the population have a needle phobia' (NHS Foundation Trust University Hospital Southampton, 2018). Therefore more non-invasive testing and diagnostic devices are necessary.
- At the end of this project, the goal is to have developed a fully automated multiplexed DMF system with bioprinted detection sites that can detect lung disease biomarkers at a low cost and at point-of-care. Beyond contributing to the progress of DMF technology in diagnostics, the project's results hold the potential for broader applications in fields such as agriculture and air quality monitoring.

Objectives

1. Biomarker selection
2. Selecting the most appropriate ink composition
3. Finding suitable geometric structures for separation sites on employing total extraction DMF approach
4. Selecting appropriate immunoassays for separation and detection
5. Creating artificial exhaled breath condensate

1. Biomarker selection

Table 1- Expected concentrations of chosen disease biomarkers

	8-isoprostane	IL-6	LB4
Control	7-64.23 pg/ml	1.5-5.1 pg/ml	7.9-53.6 pg/ml
Asthma	30.9-54.1 pg/ml	7.1 ± 1.1 pg/ml	88.9 ± 10.9 pg/ml
Chronic obstructive pulmonary disease	40 ± 3.1 pg/ml	8.0 ± 0.1 pg/ml	73.5-170.5 pg/ml
Cystic fibrosis	42.7 pg/ml	8.7 ± 0.4 pg/ml	N/A
Non-small cell lung cancer	N/A	9.3-11.4 pg/ml	24.2-61.5 pg/ml

2. Selecting the most appropriate ink

- To create the individual biosensing structures, a combination of printing methods including inkjet printing and extrusion 3D-bioprinting will be investigated.
- Inks will be initially selected based on their mechanical and rheological properties, wettability, printability, and of course their known compatibility with antibodies.
- The investigation will then consider two different avenues for functionalisation of the printed structure: (a) embedding antibodies within the ink itself or (b) using a post-functionalisation step of the pre-printed structure.
- Inks currently being investigated include: SU8, Mebiol and Gelatin Photogel.

3. Finding suitable geometric structures

- Inks can be printed in many different shapes and designs such as a pillar, a scaffold, a droplet shape, or simply a standard 2D spot.
- The geometry of the structure will affect its functionality, trapping and cleaning efficiencies.
- Fundamentally, the droplet must be able to detach from the structure. It is anticipated that droplet detachment will be correlated with the structure-to-electrode size ratio (area occupied by the structure footprint compared to the area of the electrode on the EWOD plate.
- Geometries will be coded using G-Code.

Figure 3: Geometries made using Tinkercad: (a) scaffold, (b) pillar, (c) droplet.

5. Selecting appropriate immunoassays

Table 2 - Standard assays for chosen biomarkers, their detection assays and specificities.

Biomarker	Standard Assay	Detection method	Sensitivity	Range
8-isoprostane	ELISA	Colorimetric	1 pg/ml	0.005 ng/ml – 5 ng/ml
IL-6	ELISA	Colorimetric	< 2 pg/ml	6.25 pg/ml – 200pg/ml
LB4	ELISA	Colorimetric	5.63 pg/ml	11.7 pg/ml – 3000 pg/ml

4. Creating artificial exhaled breath condensate

- Exhaled breath is composed of approximately 78% nitrogen, 16% oxygen, 4% carbon dioxide and 0.09% noble gases such as Argon, while the rest is made up of water vapour and over 3500 volatile organic compounds (Johnson, 2018).
- Would comprise of realistic ratios of the main components of exhaled breath in liquid form, salts, a buffer to ensure the stability of pH alongside, reported contaminants that are found in EBC samples and the chosen biomarkers.
- The components of the artificial exhaled breath will be mixed manuallv.

References

Berthier, Jean. "Digital Microfluidics - an Overview | ScienceDirect Topics." *Www.sciencedirect.com*, 2018, www.sciencedirect.com/topics/chemistry/digital-microfluidics. Accessed 14 May 2023.

British Lung Foundation. "Lung Disease in the UK | British Lung Foundation." *Blf.org.uk*, 2017, statistics.blf.org.uk/. Accessed 31 May 2023.4

Johnson, D. (2018). *The Chemical Composition of Exhaled Air From Human Lungs*. [online] Sciencing. Available at: https://sciencing.com/chemical-composition-exhaled-air-human-lungs-11795.html.

Maxwell, Amanda. "Microfluidics and Mass Spectrometry-Based Proteomics." *Accelerating Proteomics*, 10 Aug. 2016, www.thermofisher.com/blog/proteomics/microfluidics-and-mass-spectrometry-based-proteomics/. Accessed 14 May 2023.

NHS Foundation Trust University Hospital Southampton. "Blood, Injury and Needle Phobias and Procedural Anxiety." *NHS*, 2018, www.uhs.nhs.uk/Media/UHS-website-2019/Patientinformation/Tests/Blood-injury-and-needle-phobias-and-procedural anxiety-patient-information.pdf. Accessed 31 May 2023.