Characterisation of Tumour Cell Biomechanics using High-Throughput Microfluidic Aspiration Devices

Orlaith McSweeney[1], Raja Vadivelu[1], María-José Gómez-Benito[2], Eoin McEvoy[1]

[1]Discipline of Biomedical Engineering & IHDI, University of Galway
[2]Engineering Research Institute of Aragon, University of Zaragoza, Spain
Contact: **o.mcsweeney2@universityofgalway.ie**

Ollscoil na Gaillimhe
University of Galway

Background & Motivation

Breast Cancer Heterogeneity

Breast cancer tissue is highly heterogenous, both between and within tumours [1]. Personalised treatment requires a mechanistic understanding of heterogeneous cell behaviour within a patient's tumour material, to gain better insight into the complex mechanical determinants of cancer organisation and progression [2]. This project aims to characterise such heterogeneity by studying single cell biomechanical properties via high-throughput microfluidic aspiration.

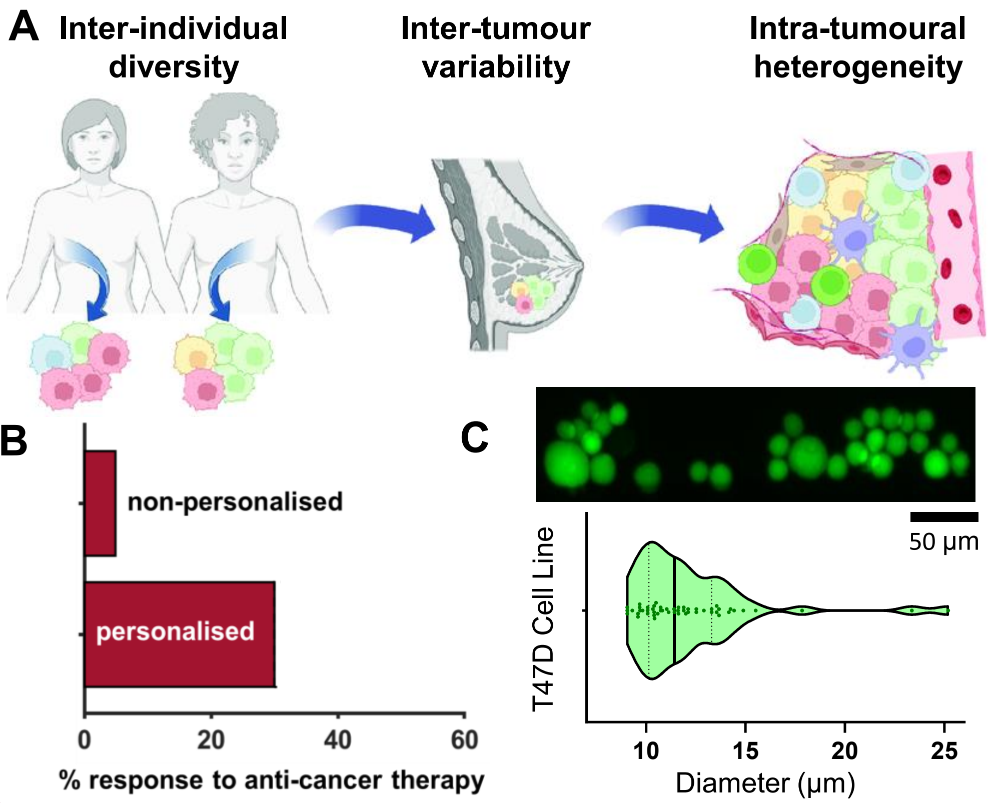


Figure 1: (A) Bio-mechanical hetero-geneity of breast cancer tissue, both between and within tumours [1]. **(B)** Personalised medicine elicits a much stronger response to anti-cancer therapy than non-personalised approaches [3]. **(C)** Sample distribution in cell diameter of calcein-stained T47D human breast cancer cell line.

Microfluidic Aspiration

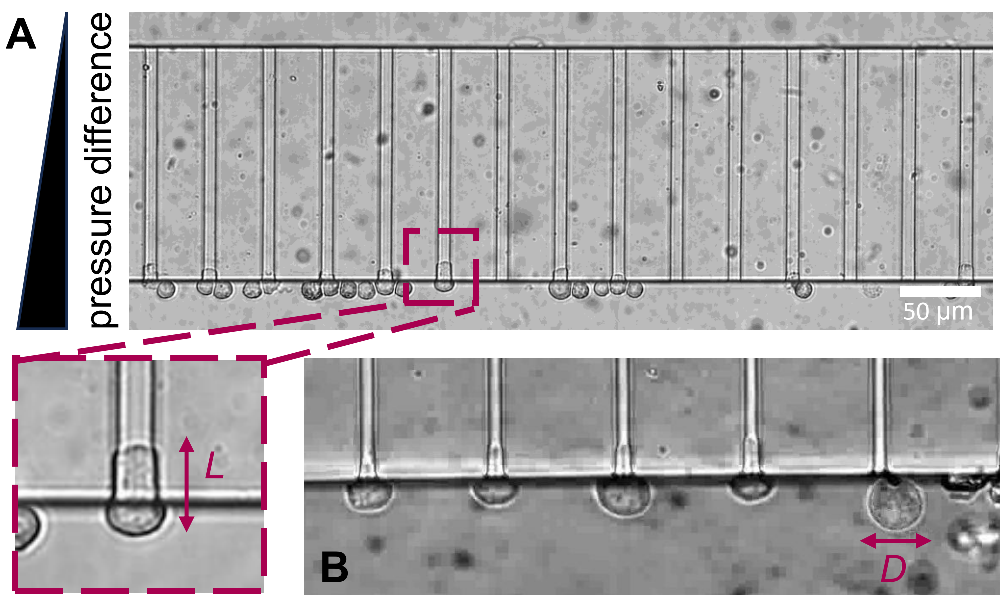


Figure 2: (A) Cells are trapped in chip microchannels, with subsequent aspiration tongue, development (*L*) upon pressure gradient establish-ment. **(B)** Aspiration lengths are coupled with reference cell diameter (*D*) pre-aspiration.

Microfluidic aspiration is an emerging technique which manipulates a pressure gradient, *ΔP*, to trap cells in chip microchannels. This leads to their deformation, which is characterized by the development of an "aspiration tongue", *L*, into the chip microchannel.

Initial cell diameter and protrusion length post aspiration are measured during experiments. From this, cell biomechanical properties can be inferred using inverse finite element (FE) models, such as the cell stiffness, cortical tension and actin contractility. This coupled platform parses complex non-linear cortical and nuclear biomechanics, to provide insight into biomechanisms of chemoresistance.

Experimental Methods

Device Design & CFD

Two device designs were employed for this study, with microchannel widths ranging from 3-14 µm. COMSOL models were developed to understand the influence of both chip design and experimental conditions in establishing a gradual pressure distribution across micro-channels. Simulations showed that cells at microchannel entrances will experience an average of the two inlet port pressures.

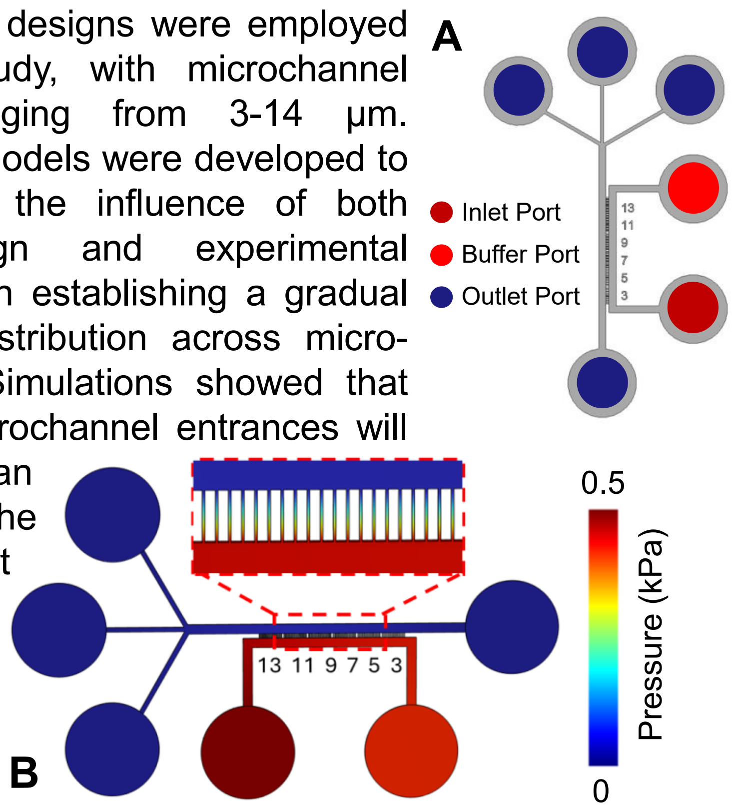


Figure 3: (A) Schematic of chip design A. **(B)** COMSOL model of chip design and experimental setup. Inlet port pressure: 0.5 kPa; Buffer port pressure: 0.4 kPa; Outlet port pressures: 0 kPa.

Chip Fabrication

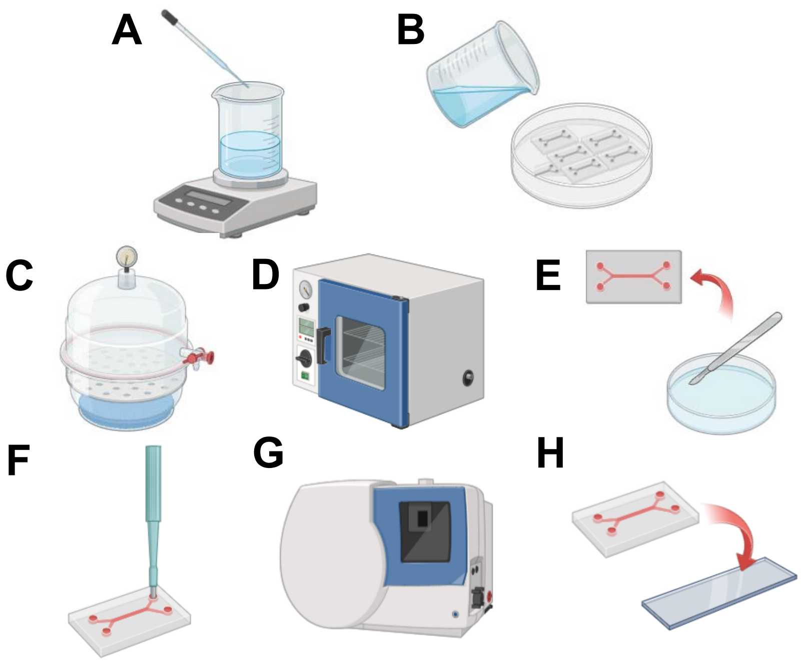
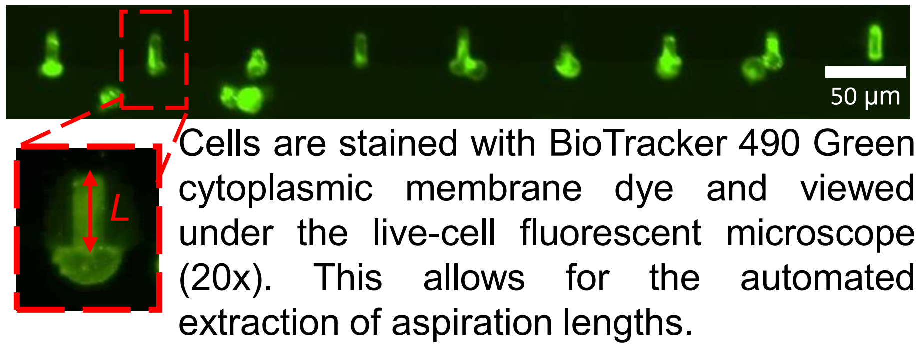


Figure 4: Steps for PDMS microfluidic device fabrication: (A) Make up PDMS. **(B)** Pour PDMS onto wafer. **(C)** Remove air bubbles via vacuum chamber. **(D)** Cure in oven overnight. **(E)** Cut PDMS chips from wafer using scalpel. **(F)** Punch out fluid ports using biopsy punch. **(G)** Treat chips and cover slips using plasma cleaning machine. **(H)** Seal chips to coverslips for use under microscope.

Experimental Setup

Fluigent pressure controllers are used at inlet and buffer ports to create a precise *ΔP* optimal for cell trapping. T47D/4T1 cells are seeded into the device at a concentration of 1x10^6 cells/ml at 0.5 kPa, then drawn towards the buffer by slightly lowering its pressure (0.4 kPa). The stronger hydrostatic *ΔP* towards the outlet side governs the flow behaviour, drawing the cells into the microchannels where they become trapped and partially aspirated. Chip-to-chip and filling-to-filling variability was assessed in the interest of experimental sustainability. Results showed no significant difference in measured lengths.



Cells are stained with BioTracker 490 Green cytoplasmic membrane dye and viewed under the live-cell fluorescent microscope (20x). This allows for the automated extraction of aspiration lengths.

Figure 5: Fluorescent microscopy image (20x) of BioTracker 490-stained T47D cells post-aspiration.

Parsing Tumour Cell Biomechanics

Experimental Results

Heterogeneous 4T1 populations undergoing induced mesenchymal-to-amoeboid (MAT) transition were studied, with increasing anoikis resistance in amoeboid-like cells (F12). Experiments were conducted on control and chemotherapy-treated (DOX) groups. Cell aspiration lengths were extracted from experimental footage using ImageJ software, with the following trends observed:

- Aspiration length reduces with increasing anoikis resistance, indicative of stiffer cell populations.
- Aspiration length increases post-DOX treatment in mesenchymal cells, suggesting cell softening. However, no effect of chemotherapy on stiffness is observed as cells undergo MAT, indicating the development of chemo-resistant phenotypes.

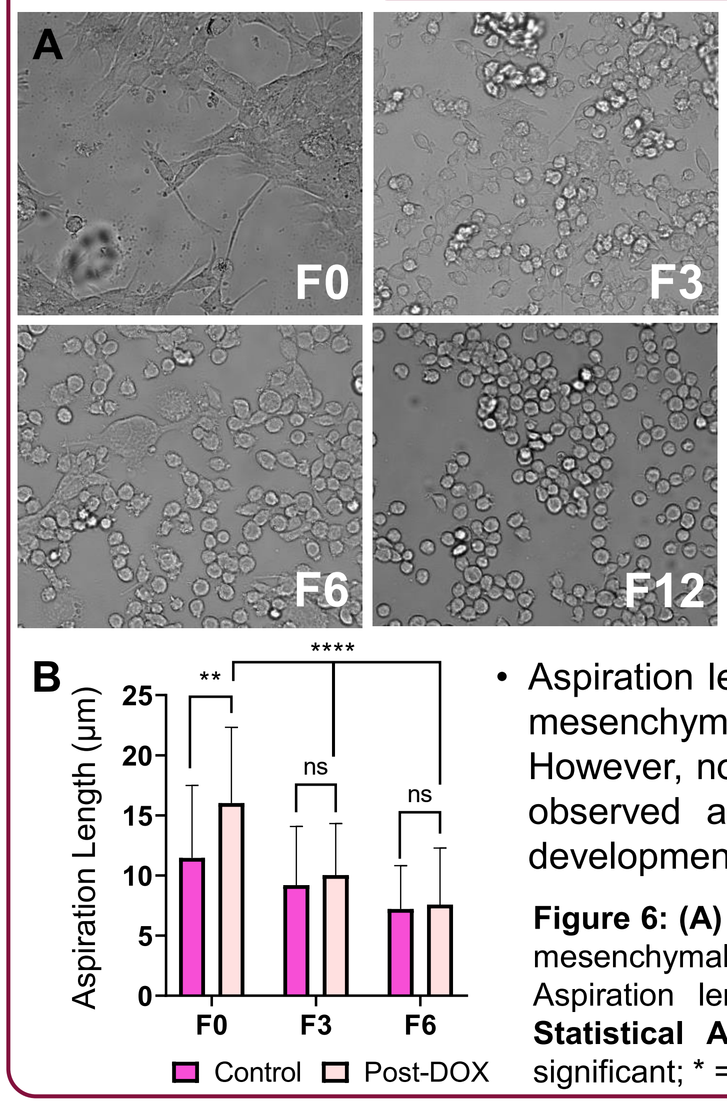


Figure 6: (A) Morphological changes as cells transition from mesenchymal (F0) to amoeboid (F12) phenotypes. **(B)** Aspiration lengths for control and DOX-treated groups. **Statistical Analysis:** Unpaired student t-test: ns = not significant; * = $p < 0.05$; ** = $p < 0.005$; **** = $p < 0.0001$.

Modelling Experimental Data

An axisymmetric inverse finite element (FE) model of cell aspiration was developed using Abaqus (Simulia), implementing Neo-Hookean hyperelastic and Prony series viscoelastic material properties. The cell was sectioned into cortical, cytoplasmic and nucleic regions, with properties varied until aspiration lengths similar to those visualised in experiments were achieved. Parametric studies of cortical stiffness conveyed the non-linear behaviour of tumour cells. The model showed that stiff cells are more readily aspirated at smaller sizes, whilst softer cells exhibit greater aspiration lengths when larger.

Next steps will involve inverse FE techniques to characterise the biomechanical properties associated with the anoikis-resistant populations, to identify if biomarkers of chemoresistance may exist in these subgroups.

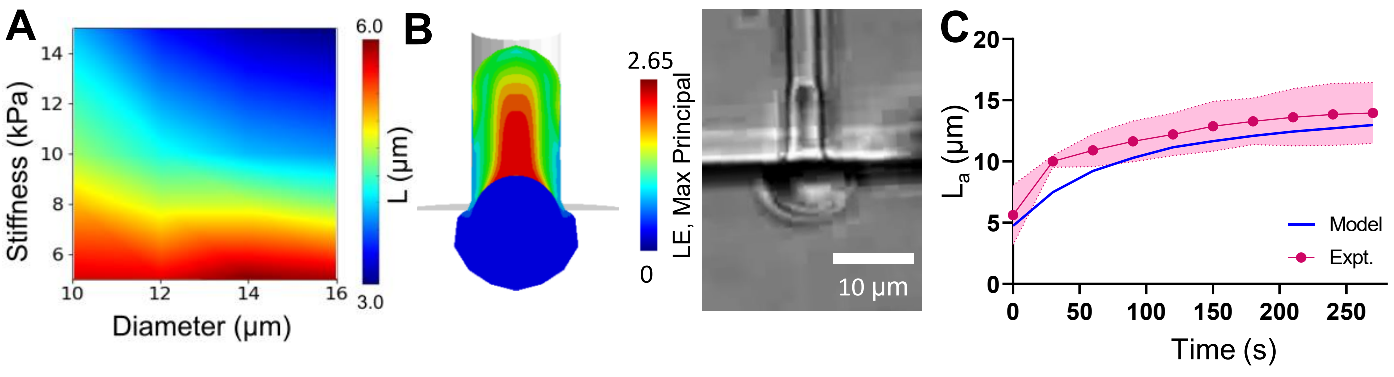


Figure 7: (A) Parametric study on influence of cortical stiffness and cell diameter on aspiration length. **(B)** Inverse FE model of cell aspiration compared to experimental data. **(C)** Hyper-viscoelastic cell model's predicted aspiration length fitted to experimental data.

Key Points

1. Computational modelling provided insight into the device design parameters and experimental conditions required to achieve pressure gradients optimal for cell trapping.
2. Experimental results revealed the non-linear response of tumour cells undergoing MAT to chemotherapy.
3. A calibrated computational model has been developed to parse the time-dependent behaviour of cell cortex and cytoplasm, with next steps being to quantify stiffness associated with induced heterogeneous populations.

→ A high-throughput platform has been established to characterise tumour biomechanics and heterogeneity, using coupled experimental and computational techniques.

References

1. Azam et al. (2022). *MDPI Cancers*
2. Massey et al. (2024). *Nature Reviews Physics*
3. *JAMA Oncology* (2016)

Acknowledgments

This work is kindly supported by the European Research Council (Grant No. 101116234).