ON-CHIP DISAGGREGATION OF PRIMARY HUMAN CANCER CELLS FROM SOLID TUMOUR BIOPSIES FOR DOWNSTREAM CELLULAR ANALYSIS IN A MICROFLUIDIC DEVICE

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ABSTRACT

An integrated microfluidic device has been developed to disaggregate *ex vivo* tumour biopsies, liberating primary human cancer cells, which are then separated by asymmetric laminar flow and trapped downstream for subsequent analysis of intracellular and cell-surface markers. This methodology therefore offers the opportunity for fast patient-specific cellular investigations.

KEYWORDS: Cancer, Tissue, Disaggregation, Cell Separation.

INTRODUCTION

Cancer research currently utilises immortalised cell lines. Although useful, these robust models have nonetheless lost many original important biocharacteristics, due to long-term culture – often over 25 years [1]. Moreover, the complex molecular basis of cancer and metastasis varies between patients, which further detracts from the physiological relevance of cell line models that cannot reflect a multicellular 3D tissue architecture [2]. To address these problems a micro total analysis system has been developed which facilitates the disaggregation of the heterogeneous tumour tissue into different cell populations for subsequent analyses.

THEORY

The poly(dimethyl siloxane) microfluidic device is shown in Figure 1.

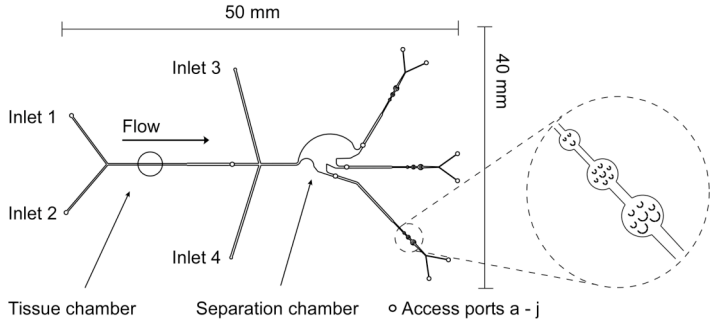

Figure 1. Schematic of integrated channels and chambers, with cell traps inset

978-0-9798064-1-4/μTAS2008/$20©2008CBMS

Disaggregated tumour cells from the tissue chamber enter the separation chamber along the short sidewall (bottom of Figure 2), together with laminar flow of medium via Inlet 3. Precise cell entry position is determined by cell diameter, as the centre of smaller cells is closer to the sidewall. Initial spatial differences become amplified as an asymmetric parabolic flow profile develops, with differently-sized cells following diverging flow paths. Separated cells are then trapped by a combination of surface modification and physical barriers (Figure 1 inset). Several cells can be accommodated within each trap, allowing intercellular signalling [3]. Variable trap size determines cell group size, enabling comparison of cell density effects in subsequent experiments.

EXPERIMENTAL

An enzyme mixture is flowed through the tissue chamber over the tumour biopsy at $2\mu l \cdot min^{-1}$ for 2 hours. Liberated cells are collected in a syringe for optimisation of the flow rate ratio of cells to medium for entry into the separation chamber. Cell separation is monitored in real time using an inverted microscope and CCD camera, and adjusted as required to achieve three-way sorting according to size by changing the flow rate ratio [4]. Cell traps are coated with collagen, fibronectin or laminin to provide different biomimetic surfaces for cellular adhesion, enabling investigation of integrin expression and signalling.

RESULTS AND DISCUSSION

The average cell count recovered from tissue disaggregation was $2.5x10^5 \cdot ml^{-1}$. Cancer cells were separated by size and density, as evidenced by video recordings of microfluidic sorting in progress. A video frame is shown in Figure 2.

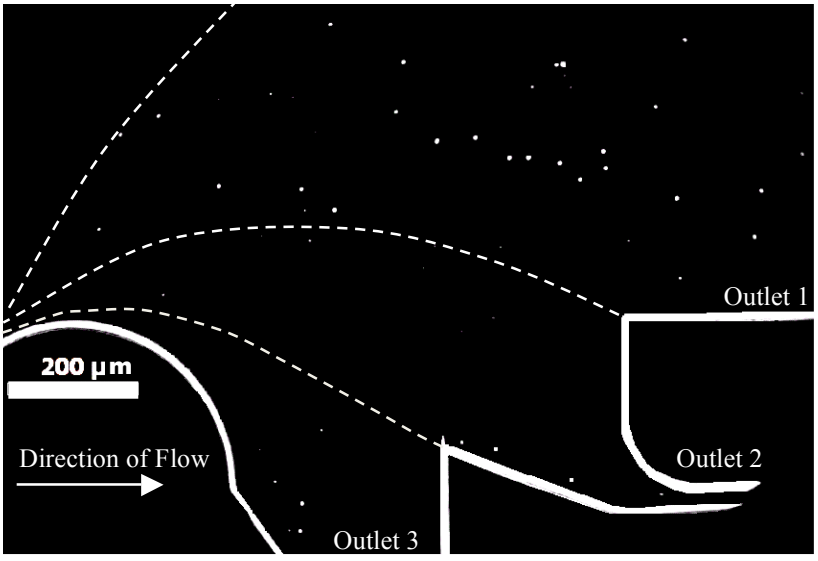

Figure 2. Photograph of cell sorting within separation chamber

Cells were in the range 3µm - 10µm and it can be seen that cells exiting via the upper Outlet 1 are generally larger than those exiting via the lower Outlet 3. In addition, cell debris was detected with the small cells collected from Outlet 3. The optimal flow rate ratio of cells to medium was found to be between 0.2:1.0 and 0.8:1.0, and optimum sorting speed 30-50 cells·sec^{-1}.

CONCLUSIONS

The multi-step process of tumour tissue disaggregation, cell separation and analysis is now possible on a single microfluidic chip, making patient cancer cells available for further investigations. These will include fluorescence-assisted detection of cellular adhesion molecules and intercellular signalling pathways to provide rapid information about the metastatic state and potential of an individual's cancer. Ultimately these on-chip investigations will lead to more effective diagnostic and prognostic decisions.

ACKNOWLEDGEMENTS

Grateful thanks to Dr. Zheng Shen and to Dr. Stephen Clark for PDMS and glass microfluidic chip prototypes, Joanna Butt and Sally Peyman for technical assistance, Professor Nicholas Stafford for providing tumour tissue, and the University of Hull for funding the research.

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