DEVELOPMENT OF MICROFLUIDIC BASED DEVICES FOR STUDYING TUMOUR BIOLOGY AND EVALUATING TREATMENT RESPONSE IN HEAD AND NECK CANCER BIOPSIES

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ABSTRACT

We report the novel use of a custom-made microfluidic based device to maintain biopsies of head and neck tumours in a viable state and quantify the effects of chemotherapy agents on these samples, with the aim of developing a personalised chemotherapy model. Using fresh and/or frozen samples, we measured cell viability via lactate dehydrogenase (LDH) release and WST-1 metabolism (cellular proliferation), evaluated preservation of tissue architecture by haematoxylin and eosin (H&E) stained cryostat sections and studied the effect of commonly used chemotherapy agents on cell death.

KEYWORDS

Microfluidic-based system, tumour viability, chemotherapy response

INTRODUCTION

The biological applications of microfluidic technology are expanding rapidly with recent uses including cell culture, DNA sequencing, and cytokine separation and detection [1]. Microfluidic-based technology is ideally suited to diagnostic applications with many studies describing the design and testing of antibody-based microwell arrays and electrochemical detection within microfluidic channels for various secreted and cell surface expressed tumour markers [2-4]. Analysis of chemotherapy resistance in cancerous cell lines has been assessed within a microchannel array that was reported to provide a more realistic representation of how cells would react in vivo than simply adding the drug to the cells in a static culture flask [5].

Worldwide, head and neck cancer is the sixth most frequent cancer with about 90% of these being squamous cell carcinoma (HNSCC) [6]. Despite advances in chemotherapy, radiotherapy and surgical techniques, 5-year survival rates remain largely unchanged. HNSCC encompasses a heterogeneous group of tumours with distinct epidemiology and clinical behaviour. Furthermore each sub-site differs greatly in its management and prognosis, however they are often grouped together in research due to insufficient tissue quantities and limited culture techniques. The relatively small biopsy-sizes available and the lack of advances in treatment make this malignancy ideally suited to a microfluidic-based analysis approach.

THEORY

The benefit of applying microfluidic technology to biological systems is the ability to replicate the in vivo environment, which is lost when using traditional culture flasks. Parameters such as laminar flow, mass transport driven by diffusion rather than turbulence, constant removal of waste products whilst maintaining cell-to-cell interactions via paracrine and autocrine signaling molecules can all be incorporated into microfluidic devices. A multicellular tumour spheroid culture has been described that allows assessment of how nutrient demands, shear stress and perfusion requirements affect tumour growth [7]. To date, the effectiveness of microfluidics for maintaining primary cancer biopsies has not been evaluated, however the viability of liver [8,9] and brain [10] has been studied.

Figure 1: Schematic diagram of the microfluidic chip used; two thermally-bonded glass layers of 1mm (base layer) and 3mm (top layer) thickness with 3 x 1.5mm inlets into which tubing is connected. Channel network of 190 µm width and 70 µm depth.

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EXPERIMENTAL

Fresh biopsies (5-10mg) of primary HNSCC or metastatic lymph node were placed in microfluidic devices (Fig.1) and subjected to continuous media perfusion for up to 8 days. The effluent was collected and analysed every 2 hours for the concentration of lactate dehydrogenase (LDH); a marker of cell death. Tumour viability was also assessed using the cell proliferation reagent WST-1 (both commercial assays, Roche Diagnostics). Architectural changes in the tissue following perfusion were studied by haematoxylin and eosin (H&E) staining of cryostat sections. Additional studies were carried out to compare the effects of snap-freezing, i.e. samples of the same biopsy were used fresh or following snap-freezing in liquid nitrogen and storage at -80°C for one week. Following optimisation of the approach using a cohort of patients (n=20) and >100 microfluidic devices, tissue was continuously subjected to the chemotherapy agents Cisplatin, 5-Fluorouracil (5FU) and Docetaxel, alone and in combination, to measure anti-tumour effects.

RESULTS AND DISCUSSION

LDH reproducibly showed a rapid decrease over the first 12 hours of incubation to minimal detectable levels (Fig.2). This relates to the large amount of initial cell death associated with the trauma of preparing, inserting and stabilising the tumour within the microfluidic device. The initial release and subsequent stabilisation has also been seen in liver biopsies treated in a similar manner [8,9]. Conversely, WST-1 levels (a metabolised product of mitochondrial succinate-tetrazolium reductase that is proportional to the number of viable cells) increased over four days (data not shown). The addition of cell lysis buffer at any point (seen in Fig.2 at 54 h incubation) resulted in an increase in LDH concentration (i.e. cell death) and reduction in WST-1 (cell metabolism). With regards to the frozen samples, although the levels of LDH released were initially slightly higher, they still demonstrated a similar pattern of response (Fig.2). Docetaxel, 5FU and Cisplatin increased cell death over control on day 1-6, with Docetaxel and 5FU peaking on day 1 and Cisplatin on day 4 (Fig.3). However, cell death was markedly increased over control and single dose chemotherapy from day 2 onwards, when a combination of Docetaxel, Cisplatin and 5FU were used.

Figure 2: Absorbance of LDH per mg of tissue for fresh (n=5) and frozen (n=7) from the same patient biopsys. Lysis buffer was added to both at 54 h incubation

Figure 3: Absorbance of LDH per µg of tissue following the addition of chemotherapy after 24 h of incubation

H&E stained cryostat sections demonstrated the preservation of tissue architecture after 4 days in the microfluidic device (Fig.4).
CONCLUSION
This study demonstrates a robust and reproducible microfluidic based system for the maintenance and ‘interrogation’ of tumour biopsies. The model provides a new platform for testing individual patient responses to chemotherapy, and is currently being tested in parallel with normal clinical management of a patient’s tumour, to investigate the correlation between in vitro and in vivo behaviour; ultimately paving the way for a ‘personalised’ treatment regimen.

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REFERENCES