Parallel acquisition of hyperpolarized 13C1 pyruvate metabolism: multi-chamber MR compatible bioreactor

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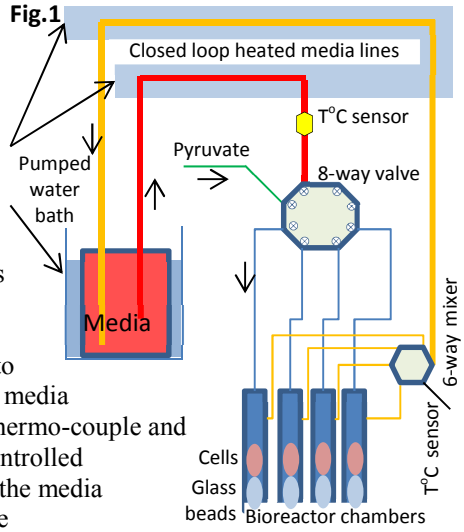
Introduction

In vivo solid tumour models are the most relevant for comparison with human disease models. However, animal studies are costly and time consuming. For hyperpolarised metabolite studies ethical considerations limit the injectable dose and number of permitted injections. Additionally, the requirements of hyperpolarised experiments mean that long waits are involved between injections. The use of bioreactors provides useful information of cellular metabolism and response to treatment[1, 2]. We have increased experimental throughput by developing an MR compatible bioreactor with multiple chambers to achieve simultaneous acquisition of multiple datasets with identical hyperpolarised metabolite concentration and polarisation level. The bioreactor permits the use of a surface coil or volume coil to simultaneously study 2 or 4 chambers respectively.

Methods

Bioreactor design (Fig.1). The bioreactor has been designed for use with up to four chambers (a further 2 chambers are possible), where each of the chambers can be sampled independently with a unique TR using a $^{1}H/^{13}C$ coil. The bioreactor uses a heated media approach in order to maintain the cells within a temperature range of 35-39°C. This was achieved by passing media containing tubes through heated water tubes, supplied from a TX-150 water bath (Grant Instruments Ltd) with temperature control. A stepper motor was used to pump media at 8ml/min from a stock bottle placed in the water bath. The media was distributed to each chamber via an Omnifit 8-way valve with individual control (Diba industries Inc). Also connected to the 8-way valve, via a 0.96 mm tube to an automated injection system[3], was the supply of hyperpolarised pyruvate. Heated media was returned to the stock solution (or waste vessel) via an Omnifit 6-way mixer (Fig.2). Temperature of the media was measured at the entrance to the 8-way valve and at the exit of the 6-way mixer with a thermo-couple and an optical temperature sensor (AMS Technologies AG), respectively. Flow direction was controlled using inline check valves (Cole Parmer). A gas permeable membrane (Liqui-Cel) placed in the media tube line before the temperature sensor was used for aeration. The bioreactor chambers were encapsulated in agar jelly within a plastic tray for additional mechanical support and further thermal insulation.

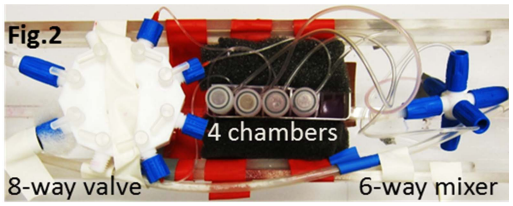
Fig.1



Cell preparation. Rat P22 sarcoma cells were cultured in DMEM media enriched with foetal bovine serum (FBS), glutamine (Gln) and antibiotics: penicillin and streptomycin (P/S); they were kept in an incubator at 37°C, in air with 5% CO_2. At confluence, media was removed, washed out with PBS and the cells were detached from the culture surface using trypsin, then brought to the magnet and carefully pipetted into the bioreactor chambers.

MRS/DNP experiments. An automated injection system[3] administered 1.5ml of HEPES buffered solution of $^{13}C_1$-pyruvate (PA) hyperpolarised by a HyperSense DNP polariser. Localisation was performed using a $^{1}H/^{13}C$ 90 mm volume coil and each chamber was sampled with 10mm slice selection in a coronal plane (the slices were positioned on a FLASH image as shown in Fig.3a). ^{13}C spectra were acquired with a Gaussian pulse (10deg flip angle, TR=1s) in a Bruker 7T animal MRI system. The PA signal and its metabolite lactate (LA), was processed using custom Matlab software to produce integrals for a temporal plot from phase-corrected spectra.

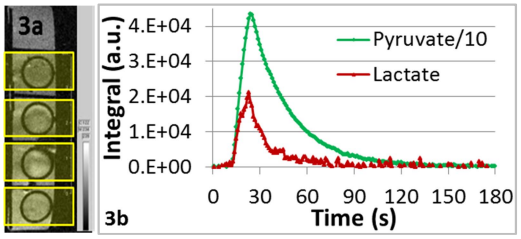
Fig.2



Results, discussions and conclusions

A typical time curve of ^{13}C PA and LA signals acquired from a chamber is shown in Fig.3b. The presence of lactate in spectra shows that the cells are metabolising even two hours after insertion into the chambers. The simultaneous use of the 4 chambers increases the throughput by four times and consequently reduces the corresponding experimental time, allowing the efficient use of hyperpolarised PA in MR/DNP studies on cells. The MR-compatible multiple chamber bioreactor permits comparison between untreated cells and up to three drug treatments or different doses at identical hyperpolarised metabolite concentration and polarisation levels. This could be an important step in investigation of drugs effects in animals.

Fig.3



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