

# Development of a Robust MR-compatible 5mm bioreactor for Primary Human Tissue Cultures and Hyperpolarized MR

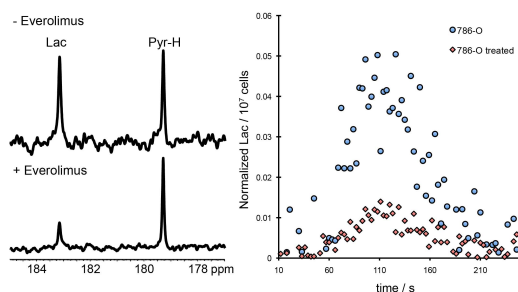
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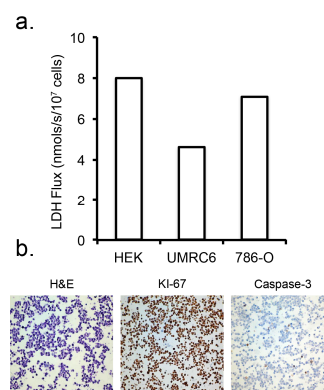
**INTRODUCTION:** Development of MR-compatible bioreactor systems for the study of cell metabolism non-invasively has been limited by the sensitivity of low  $\gamma$  nuclei, such as  $^{13}\text{C}$  and  $^{15}\text{N}$  [1-3]. With the development of dissolution dynamic nuclear polarization (DNP) technology, the requirements for cell density in MR-compatible bioreactors have been relaxed given the dramatic enhancement in SNR achieved. Typical studies in traditional 10mm MR-compatible culture systems require on the order of  $10^8$  cells, nearly impossible in primary cultures. The adaptation of this technology to smaller cultures ( $5 \times 10^6$  cells) is non-trivial and requires the optimization of both engineering parameters (flow rate, MR-compatible mechanical structure) and biochemical variables (dissolved oxygen, real time concentration of glucose). The engineering of more compact systems allows study of primary cultures of cells and tissues, which are more clinically relevant and cost efficient [4]. The goal of this study was to optimize a 5mm MR-compatible platform interfaced with hyperpolarized (HP) MR, small cultures of immortalized renal cells, and then extend these methods to primary renal tissue slice cultures (TSC), using dramatically reduced perfusate and tissue volumes.

**METHODS:** *Cell Culture:* Renal cell lines (HEK, UMR6, 786-O) were cultured in T150 cm<sup>2</sup> flasks with DMEM medium (supplemented with 10% FBS and Penicillin/Streptomycin) [5]. Primary renal tissue slices were harvested from surgery, sectioned to 250 $\mu\text{m}$ , and perfused using the same medium. *System design:* Cells and primary tissue were cultured in a custom-designed 5mm MR-compatible bioreactor system. The system utilized a completely enclosed perfusion system, providing a continuous flow of 37°C medium (analogous to the culture medium) dynamically oxygenated with 95% Air/5% CO<sub>2</sub> [6]. *Bioreactor Studies:* For cell studies, 10 million cells were suspended in 2% alginate and cross-linked in a 150mM CaCl<sub>2</sub> solution for encapsulation [6]. For renal TSCs, 4 slices were perfused in a custom-designed cartridge construct. All NMR data were acquired on a narrow-bore 14.2T Varian INOVA (150MHz  $^{13}\text{C}$ ) equipped with a 5mm broadband probe. Cell viability was assessed acquiring  $^{31}\text{P}$  spectra (242MHz  $^{31}\text{P}$ ) with a 90° pulse and acquire sequence (nt=1024, at=1s, T<sub>R</sub>=3s) to assess  $\beta\text{NTP}$  resonance. [ $^{13}\text{C}$ ]pyruvate was hyperpolarized using the Hypersense<sup>TM</sup> (Oxford Instruments) and 1mL of 4mM pyruvate was injected into the bioreactor where  $^{13}\text{C}$  NMR spectra were acquired in intervals of 3 secs using 10° pulses for 300secs. Peak integrals were calculated for each resonance and fluxes were calculated for label conversion to HP lactate [6]. *Histopathology:* After perfusion in the 5mm bioreactor, encapsulated cells were fixed in formalin and sectioned. These were stained for hematoxylin & eosin (for structure), KI-67 (for proliferation) and Caspase-3 (for apoptosis).

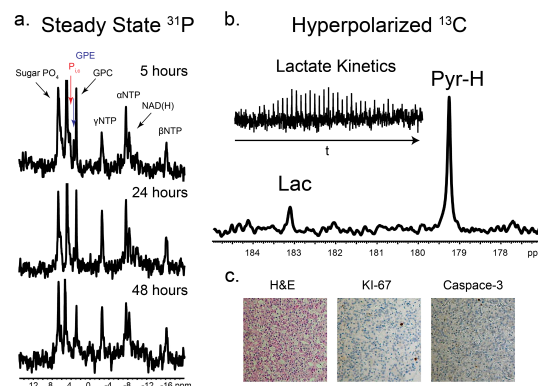
**RESULTS AND DISCUSSION:** When moving from to the 5mm design, the volume of encapsulated cells and primary tissue is decreased as well as the chamber size. This results in a reduced volumetric flow rate (0.8mL/min) necessary to turn over the chamber volume. With this flow, we were able to maintain a variety of renal cell lines and acquire hyperpolarized kinetics (Figure 1a). These rate constants describe the flux of pyruvate to lactate and vary with cell type. Maintained viability throughout the experiment is confirmed by preserved histopathology (Figure 1b). Subsequently, the platform was extended for use with primary renal tissue slices. In previous studies of tissue slices, 40-50 slices in a 10mm bioreactor were necessary to acquire sufficient SNR in both  $^{31}\text{P}$  and HP  $^{13}\text{C}$  NMR experiments. Here we demonstrate the long-term perfusion (>48 hours) of 4 primary renal TSCs.  $^{31}\text{P}$  spectra demonstrate preserved bioenergetics in time, with no significant difference in  $\beta\text{NTP}$  over 48 hours (Figure 2a). Following injection of HP [ $^{13}\text{C}$ ] pyruvate, dynamic conversion to lactate is observed (Figure 2b), similar to what has been observed in the first human studies. The flux in renal TSCs was 0.05 nmols/s/mg tissue, which is 60% lower than 786-O cells and 24% lower than HEK cells when normalized to  $\beta\text{NTP}$ . Histopathology following bioreactor studies verifies the preservation of tissue with minimal degradation (Figure 2c). These studies reaffirm not only the robust nature of the model, but also the potential for translating clinically relevant biomarkers.



**Figure 3.** (left) HP  $^{13}\text{C}$  spectra at 90 secs post injection of HP [ $^{13}\text{C}$ ]pyruvate demonstrating the effect of Everolimus (right) Normalized Lactate kinetics demonstrating the effect of the mTOR inhibitor on LDH flux observed on 10 million cells, resulting in a 2.8 fold drop in LDH flux.



**Figure 1.** (a) LDH flux measured in the 5mm bioreactor using HP pyruvate for immortalized renal cell lines. (b) Histopathology demonstrates the structural integrity of cells, proliferative status (>95%) and negligible apoptosis (<1%).



**Figure 2.** (a)  $^{31}\text{P}$  spectra from 4 living primary renal cancer TSCs demonstrating the preservation of bioenergetics over 48 hours. (b) HP Lactate kinetics after injection of HP pyruvate. (c) Representative histopathology of primary TSCs demonstrating cell viability and minimal apoptosis after 48 hours in the 5mm bioreactor.

**CONCLUSIONS:** This preliminary study demonstrates the feasibility of using a novel 5mm bioreactor design to robustly explore both cell and primary tissue metabolism at dramatically reduced cell and perfusate volumes. This is critical for studying small quantities of cells and tissues, for example stem cells, primary cell and tissue cultures. Here we demonstrate application of this new bioreactor platform to primary renal tissue slices removed from a patient and cultured for 48 hours. Ongoing studies are focused on the application of this design to the characterization of aggressiveness with primary tissue cultures using novel combinations of probes [4] and to explore hyperpolarized metabolism at baseline as well as in response to therapy (Figure 3).

**REFERENCES:** [1] Gillies RJ et al. NMR in Biomed 1993;6(1):95-104 [2] Macdonald JM et al. NMR in Biomed 1998;11(2):55-66 [3] Mancuso A et al. Biotech Bioeng 2004;87(7):835-48 [4] Zhao H et al. AJP 2010;177(1):229 [5] Grossman HB et al. J Surg Oncol 1985;28(3):237-44 [6] Keshari KR et al. Magn Res Med 2010;63(2):322-9

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