INTRODUCTION: Development of MR-compatible bioreactor systems for the study of cell metabolism non-invasively has been limited by the sensitivity of low ν nuclei, such as 13C and 31P [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 10^6 cells, nearly impossible in primary cultures. The adaptation of this technology to smaller cultures (5 x 10^4 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 to extend these methods to primary renal tissue slice cultures (TSC), using dramatically reduced perfusate and tissue volumes.

METHODS: Cell Culture: Renal cell lines (HEK, UMRC6, 786-O) were cultured in T150 cm² flasks with DMEM medium (supplemented with 10% FBS and Penicillin/Streptomycin) [5]. Primary renal tissue slices were harvested from surgery, sectioned to 250μm, 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% CO₂ [6]. Bioreactor Studies: For cell studies, 10 million cells were suspended in 2% alginate and cross-linked in a 150mM CaCl₂ 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 1H) equipped with a 5mm broadband probe. Cell viability was assessed acquiring 31P spectra (242MHz 31P) with a 90° pulse and acquire sequence (nt=1024, at=1s, Ts=3s) to assess βNTP resonance. [1-13C]pyruvate was hyperpolarized using the Hypersense TM (Oxford Instruments) and 1mL of 4mM pyruvate was injected into the bioreactor where 13C 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 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 31P and HP 13C NMR experiments. Here we demonstrate the long-term perfusion (>48 hours) of 4 primary renal TSCs. 31P spectra demonstrate preserved bioenergetics in time, with no significant difference in βNTP over 48 hours (Figure 2a). Following injection of HP [1-13C]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 mmol/s/mg tissue, which is 60% lower than 786-O cells and 24% lower than HEK cells when normalized to βNTP. 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.

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).


ACKNOWLEDGEMENTS: NIH P41 EB013598 and DOD PC093725