A Novel 5mm NMR-Compatible Micro-Spindle Bioreactor for Steady-State and Dynamic in Cell NMR

K. R. Keshari[1], M. Van Criekinge[2], D. Vigneron[2], and J. Kurhanewicz[2]
[1]UCSF, San Francisco, CA, United States, [2]UCSF

INTRODUCTION: To present, NMR-compatible bioreactors have been utilized as a platform to explore the metabolism of cells non-invasively. These systems have utilized standard 10–25mm coils to exploit filling factor and ease of construction [1-3]. For immortalized cell lines, densities on the order of 10^8 cells are achievable, requiring extensive cell culture, but this is nearly impossible to achieve for stem cells as we all as other primary cells and tissues. Cultures of immortalized cells, the traditional platform for metabolic study, exhibit an unnaturally rapid mitotic rate, while primary cells and tissues more closely mimic *in vivo* metabolism [4]. Combing hyperpolarized MR with NMR-compatible 3D cell and tissue culture bioreactors allows for kinetic monitoring of metabolism on a second timescale without any background signals from the culture media under very controlled conditions. The use of primary cell and tissue cultures provides a more clinically relevant platform for testing new hyperpolarized MR probes and therapies. With this in mind, the goal of this study was to develop and implement a 5mm bioreactor system, which would provide comparable performance to 10 mm bioreactors while reducing the necessary cell/tissue and perfusate volume and thus facilitate the study of primary cells and tissues.

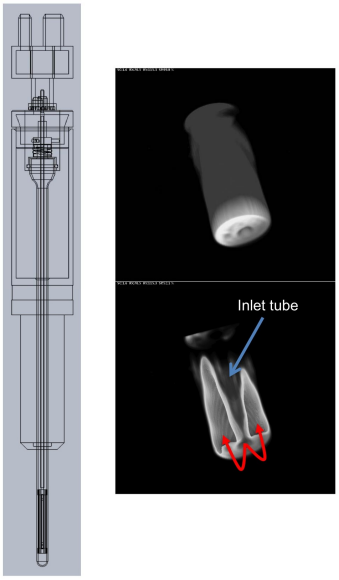

Figure 1. (left) A schematic of the 5mm design. (right) Representative

METHODS: *Cell Culture:* Human embryonic kidney (HEK) cells were cultured in T150 cm^2 culture flasks in DMEM medium (supplemented with 10% FBS and Penicillin/Streptomycin). *System design:* Cells were cultured in custom designed 5 and 10mm NMR-compatible bioreactor systems [6]. Both systems 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_2. *Bioreactor Studies:* 100 x 10^6 cells were trypsinized for study in the 10mm bioreactor. Cells were suspended in 2% alginate and cross-linked in 150mM $CaCl_2$ solution for encapsulation [6]. All NMR data was acquired on either a narrow-bore 11.7T Varian INOVA (125MHz ^{13}C, Varian Instruments) equipped with a 10mm broadband probe or 14.2T Varian INOVA (150MHz 13C) equipped with a 5mm broadband probe. Cell viability was assessed acquiring ^{31}P spectra (202MHz or 242MHz ^{31}P) with a 90° pulse and acquire sequence (nt=1024, at=1s, T_R=3s) to assess βNTP resonance. [1-^{13}C]pyruvate was hyperpolarized using the Hypersense^{TM} (Oxford Instruments) and 1mL of 4mM pyruvate was injected into the bioreactor where ^{13}C NMR spectra were acquired in intervals of 3 secs using 10° pulses for 300secs. Both prior to and after injection, the relative integrals of pyruvate and lactate were plotted as a function of time to determine maximum flux to lactate, which was then used for comparison between the 5 and 10mm design. To normalize the hyperpolarized ratio, the maximum pyruvate was assumed to be 4mM and the lactate was calculated accordingly. *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).

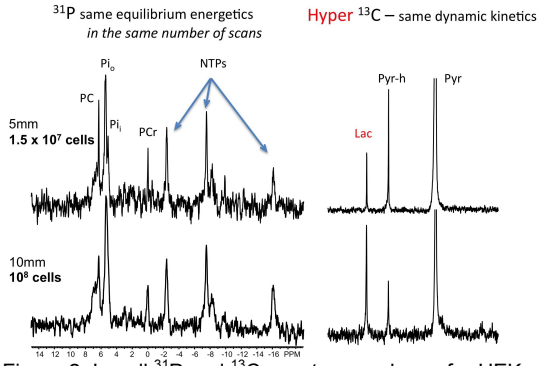

Figure 2. In cell ^{31}P and ^{13}C spectra are shown for HEK cells cultured in the (top) 5mm and (bottom) 10mm bioreactor.

RESULTS AND DISCUSSION: When moving from the 10mm to 5mm design, the volume of encapsulated cells is decreased 85%. Additionally, the volumetric flow rate must be reduced as a result of increased force on the cells with the smaller chamber volume, which resulted in an optimized flow rate of 0.5mL/min as opposed to 2.5mL/min at 10mm. The custom 5mm design and flow pattern is shown in *Figure 1*, where there is uniform media flow on both sides of the inlet tube. Representative ^{31}P spectra in *Figure 2* demonstrate the comparable signals observed for HEK cells in the 5mm and 10mm bioreactors. The average signal-to-noise ratio (SNR) of βNTP was 3.8 versus 5.6 for the 5mm and 10mm, respectively with a reduction in cell volume from 100 x 10^6 to 15 x 10^6 cells. As expected, this yielded an average NTP concentration measured in the coil that was not significantly different (0.39mM v. 0.36mM) for the 10mm relative to the 5mm system. Dynamic HP pyruvate metabolism was also tested in the 5mm setup and representative hyperpolarized ^{13}C spectra are shown post-infusion of 4mM hyperpolarized [1-^{13}C]pyruvate. The maximum lactate, when normalized to injected volume, for the 10mm and 5mm bioreactor, was not significantly different (0.23 v. 0.22 μmols/10^7 cells). Histopathology of encapsulated cells post-bioreactor (*Figure 3*) demonstrated intact, highly proliferative cells, reaffirming the viability of the cells in the 5mm design.

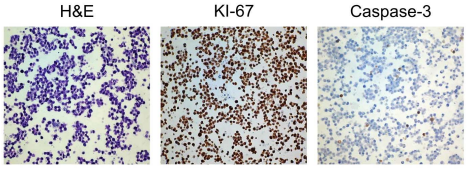

Figure 3. Histopathology demonstrates the structural integrity of cells, proliferative state (>99% stain positive for KI-67) and number of apoptotic cells (<1% stain positive for Caspase-3) after culture in the 5mm bioreactor.

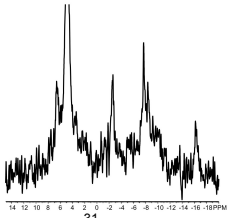

Figure 4. ^{31}P spectrum of 5 prostate tissue slices in the 5mm bioreactor

CONCLUSIONS: This preliminary study demonstrates the feasibility of using a novel 5mm bioreactor design to explore both in cell steady-state and dynamic metabolism using dramatically reduced cell and perfusate volumes. This is critical for the characterization of cells and tissues, which are otherwise unattainable, i.e. stem cells, primary cell and tissue cultures. Recent studies have shown that it can be applied to primary tissues. *Figure 4* demonstrates ^{31}P data acquired in this design on only 5 250μm human prostate tissue slices (i.e. 25mg). Ongoing studies are focused on the application of this design to primary tissue cultures from cancer patients to explore hyperpolarized metabolism at baseline as well as in response to therapy.

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):229-39 [5] Ardenkjaer-Larsen JH et al. PNAS 2003;100(18):10158-63 [6] Keshari KR et al. Magn Res Med 2010;63(2):322-9

ACKNOWLEDGEMENTS: NIH R21 EB007588 and R21 GM075941-01