A Versatile NMR-compatible Bioreactor for Alginate-Encapsulated Liver Cells

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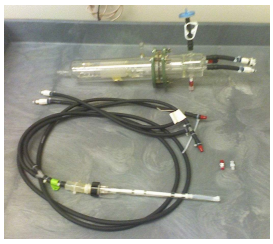
INTRODUCTION:

NMR studies with cell preparations permit the control of cell composition. Individual contributions from immune, endocrine, paracrine, and physico-chemical parameters can be explored that are not afforded in experiments with intact or perfused organ systems (1). Since the inception of NMR compatible bioreactors in 1973 (2), four categories of NMR-compatible mammalian cell perfusion systems have evolved: cell suspension, cell entrapment, cells on microcarrier, and cells sandwiched in membranes. Initially, NMR-compatible bioreactors utilizing isolated hepatocytes were cell suspensions. Viability and hepatocyte function lasted several hours before hypoxia-induced injury (3,4). In subsequent work, hepatocytes were encapsulated in collagen threads (5,6) and in membrane bioreactors (7). Our aim is to maintain for several days three-dimensional (3D) cultures of rat hepatoma cells (JM1) or primary rat hepatocytes in an autoclavable NMR-compatible bioreactor. Previous NMR-compatible bioreactor designs used 95% oxygen, a holdover from the Langendorf studies with perfused heart. With hepatocytes, oxygen is a differentiating agent, which spikes at birth and induces many phenotypic changes in the liver (8). Our initial study used 20% oxygen in a modified NMR-compatible bioreactor design (9) inoculated with JM1 cells to test the bioreactor's ability to support cell growth using ^{31}P NMR. Next, we used freshly isolated alginate encapsulated rat hepatocytes in the NMR-compatible bioreactor. Oxygen consumption and ^{31}P NMR were used to monitor cellular bioenergetics. The life support system of the NMR-compatible bioreactor is extremely versatile and relatively easy to use with any high-resolution NMR probe ranging from 5 to 10 mm.

MATERIALS AND METHODS:

The encapsulated cells were perfused in a 10-mm NMR tube with a milled Delrin™ fixture containing a 200µm porous polyethylene insert which retained the encapsulated cells (***Fig. 1***). The cap is connected to two water-jacketed (norprene) lines with internal ETFE tubing that is maintained at 37º C. ETFE tubing provides input of media, which extends to the bottom of the NMR tube. A second ETFE line is used to remove the medium above the end piece. This previously described (9) assembly of a bioreactor and gas exchange module (***Fig. 1***) provides oxygenated medium at 95% air, 5% CO_2 for the cells. Culture medium was recirculated through the encapsulated JM1 cells and primary hepatocytes with a peristaltic pump at a flow rate of 4ml/min and 8 ml/min, respectively. Cells were encapsulated (avg diameter, 500µm) with a 2% sodium alginate solution (1:1 v:v) using an electrostatic bead generation apparatus (10), resulting in a final concentration of 2.5x10^7 and 3.5x10^7 cells/ml for JM1 and primary hepatocytes, respectively. Confocal microscopy of the encapsulated primary hepatocytes maintained in a culture plate was used to optimize cell density of the encapsulates. The ^{31}P NMR spectra of JM1 cells were acquired on a narrow-bore 11.7T Varian INOVA equipped with a 10mm triple tune broadband probe. A time course of ^{31}P spectra (202MHz ^{31}P) was acquired with a 90^o pulse, nt=2048, and at=1s to assess the β-NTP resonance as a function of time and infer cell growth. The ^{31}P NMR spectra of the primary rat hepatocytes were acquired on a narrow-bore 9.4T Varian INOVA equipped with a 10mm broadband probe and using similar NMR parameters as described above. A second bioreactor was run in parallel that had two oxygen electrodes in-line with the input/output lines to the bioreactor in order to correlate oxygen uptake rates to β-NTP and intracellular pH obtained by ^{31}P NMR.



RESULTS:

Representative 1 hr ^{31}P NMR spectrum (nt=2048) of encapsulated JM1 and primary rat hepatocytes are shown in ***Figure 2*** and ***Figure 4,*** respectively. The plotted increase in β-NTP (***Fig. 3***) is characteristic of JM1 cell growth during the first 24 hours post trypsinization and is indicative of cell growth (12,13). Shown in ***Figure 5*** are confocal images of 10 µm thick slices through a 500 µm diameter alginate encapsulate containing 3.5 x10^7 rat hepatocytes/ml. The distribution of hepatocytes in red, show that they are tightly packed.

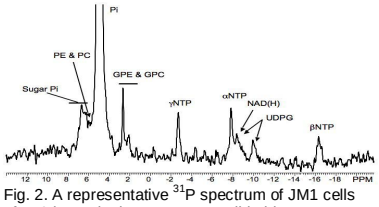


Fig. 2. A representative ^{31}P spectrum of JM1 cells after 9 hours in the NMR compatible bioreactor. Peaks corresponding to NTP, monophosphodiesters and diphosphodiesters are readily visible.

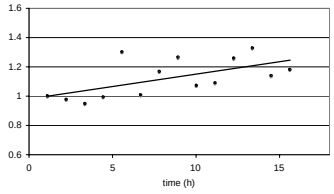


Fig. 3. The time course of β-NTP in JM1 cells.

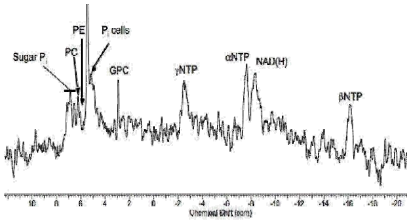


Fig. 4. ^{31}P spectrum of primary rat hepatocytes after 2 hours in the NMR compatible bioreactor.

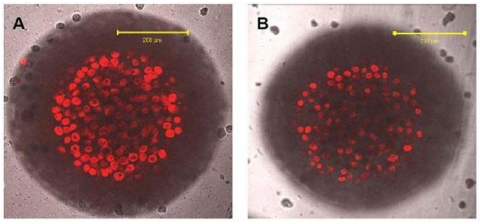


Fig. 5. Confocal images of rat hepatocytes stained with Mitotracker red.

DISCUSSION AND CONCLUSIONS:

This bioreactor design provides several advantages over previous designs such as longer period of viability than that of suspension, and superior mass transfer, while being economical and easier to use as compared to hollow fiber designs. In addition, the life support system is reusable with sufficient rinsing and autoclaveable. This economic and highly versatile (resized for 5mm, 8mm, 10mm) bioreactor can be utilized for models of various cell lines permitting tight control over physiological parameter, ultimately imitating the normal biochemistry. Future studies of human hepatocytes and drug-induced liver injury will be performed with this bioreactor model. Interestingly, comparison of the ^{31}P NMR spectra from the more undifferentiated JM1 cell line to the rat hepatocytes, there are diphosphodiesters present in the JM1 spectrum, which have been correlated to more undifferentiated cells discussed in previous NMR perfused cell studies (13)

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