Assessment of Cellular Growth in a Double Microbead Alginate Construct Via MR Microscopy

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Introduction In recent years, considerable research has advanced the development of a bioartificial pancreas. Much of this effort has been directed toward the encapsulation of insulin-producing cells in a 3D structure that (a) provides the cells with a scaffold for development and (b) provides partial immunoprotection following *in vivo* implantation. For cells encapsulated in alginate/poly-L-lysine/alginate beads, immunoprotection is achieved by the poly-L-lysine (PLL) that also adds mechanical stability. In this study, alginates themselves are used to control cellular growth and provide structural stability by introducing a substantial outer layer of alginate beyond the cellular encapsulation layer. These double microbead constructs were analyzed with Magnetic Resonance Microscopy (MRM) to evaluate the growth and integrity of this construct during prolonged *in vitro* culture. To investigate the effects of the double microbead structure on the growth and metabolic activity of βTC3 cells, the rates of glucose consumption were measured over 30 days. At weekly intervals during this period, samples of the culture were taken for MR analysis. MRM was performed to assess the longitudinal distribution and growth pattern of βTC3 cells within the double microbead construct. Additionally, quantitative MR relaxation and diffusion data was acquired to assess cell viability and alginate integrity in both alginate layers.

Methods *Cell Culture & Encapsulation:* Mouse insulinoma βTC3 cells were cultured as monolayers in T-flasks and fed fresh medium consisting of complete Dulbecco's Modified Eagle's Medium (DMEM) every 2-3 days. The cells were harvested from monolayer cultures using trypsin-EDTA, and cell numbers were determined by a hematocytometer. Three types of alginate were used in this study: a high molecular weight, high guluronic acid content alginate (MVG); a lower molecular weight, high guluronic acid content alginate (LVG); and a lower molecular weight, high mannuronic acid content alginate (LVM). Harvested βTC3 cells were suspended in an alginate solution (inner bead always 2% LVM) at a density of 7 x 10^7 cells/mL. Alginate microbeads were made with the use of an electrostatic bead generator and gelled with a 1.1% $CaCl_2$ solution. These microbeads then were treated with $CaCl_2$, CHES, PLL and 0.2% alginate to create the inner APA beads (~500-μm diameter). To form the bead-in-bead (BIB) construct, the above procedure above was repeated with the appropriate alginate solution to create the outer bead. Blank and cell loaded BIB constructs (1-mm diameter) were generated for long-term culture.

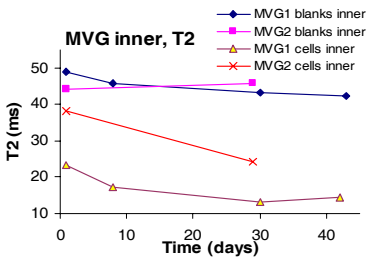
Glucose Consumption Rate (GCR): Glucose concentrations in medium samples (10 mL) were determined using an EKTACHEM DT60 bioanalyzer. Calculations of the rates of consumption were based on concentration changes and incubation time, and were normalized to a unit of 10^5 cells. The rate of glucose consumption was calculated from the change in the amount of glucose (amount = volume x concentration) within a 24-hour interval after feeding.

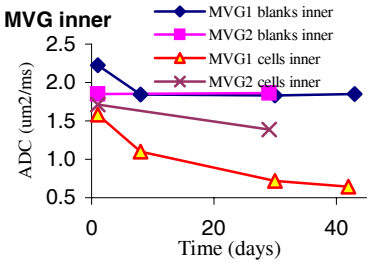
MRI: All MR data were acquired using a 17.6-T vertical magnet equipped with a Bruker Avance Console and Micro 2.5 gradient system. Three to six BIBs immersed in DMEM were loaded into a capillary, which was placed within a homebuilt solenoidal microcoil. Several beads were analyzed simultaneously to examine T2 relaxation and diffusion in both the cellular and alginate components of the BIB construct. For these quantitative experiments, a conventional spin-echo (SE) sequence was applied. For measurements of T2, separate images were acquired at seven echo times (TE=12.5-95.5 ms). For measurements of the ADC, separate images were acquired with increasing diffusion weighting (*b* value = 100-2500 s/mm2). All SE images were acquired at a resolution of 25x25x100 μm.

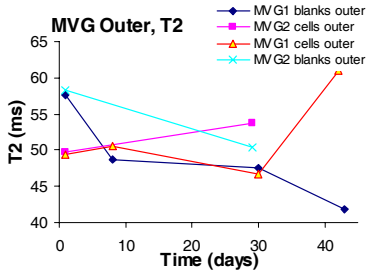
Results and Discussion Our data demonstrate that the T2 of the gelled beads were statistically different among the different alginates used in this study, similar to previous work [2]. T2 values of the guluronic-rich alginates (MVG and LVG) are significantly higher for the outer beads than T2 values of the mannuronic-rich alginate (LVM). Over the duration of culture, the MVG and LVG alginates displayed a consistent value of T2 and ADC, indicating a structural stability over that period in the absence of growing cells. The looser LVM alginates did display some increased in T2 over time in the absence of cells, which may indicate a further loosening of the alginate and/or an increased water content. For all samples, the inner bead that encapsulated the cells displayed decreases in T2 and ADC over the duration of the *in vitro* culture. These decreases are related to the growth of the cells, and correlate well with GCR curves. BIBs with an outer alginate consisting of LVG or MVG lasted much longer than BIBs generated with an outer LVM layer. In general, BIBs with an outer LVM layer ruptured within ~14 days. For an outer bead consisting of MVG or LVG alginate, the BIBs maintained their structure for nearly all of the culturing time; a significant number (~50%) of BIBs were intact after 42 days of culture. T2 and ADC measurements of the outer layer in the BIB constructs coated with MVG or LVG did not display significant changes over the duration of the cultures. T2 measurements of the outer layer in the BIBs with an LVM coating did show changes over the course of culture, but these changes reflect global changes seen in even cell-free blank preparations.

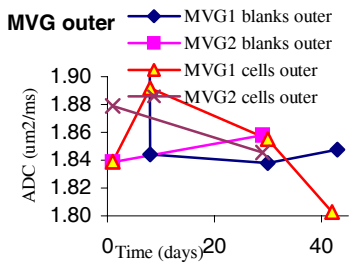
Microimages demonstrate that the encapsulated cells did not grow isotropically out of the inner bead, but rather along specific planes. These growth planes may represent fracture points in the inner bead that open as the pressure of cellular growth increases. However, the continued growth of these cells along the planes outside of the inner bead is not well understood and merits further study.

Acknowledgements and References Financial support provide by the NIH (R01 DK56890, R01 DK47858 and P41 RR16105) and the NSF through the National High Magnetic Field Laboratory. MRI data were obtained at the Advanced Magnetic Resonance Imaging and Spectroscopy (AMRIS) facility in the McKnight Brain Institute of the University of Florida.

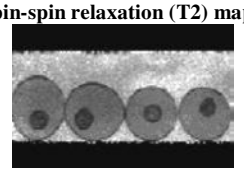




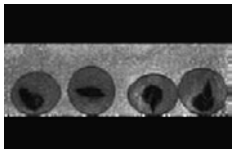




Spin-spin relaxation (T2) maps for the LVM-MVG culture:

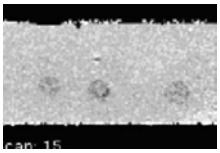


Day 1

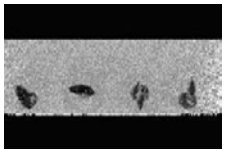


Day 30

Diffusion (ADC) maps for the LVM-MVG culture:



Day 1



Day 30