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NMR STUDIES OF INSULIN SECRETING CELLS: BIOCHEMICAL CONSEQUENCES OF CELL ENCAPSULATION

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Introduction

Little is known about the biochemical consequences of alginate cell encapsulation. Recently, we demonstrated the effects of alginate composition on the growth characteristics of encapsulated murine insulinoma cells (1, 2). These effects were attributed to the strength of the “egg-box” configuration (3) which varies depending upon the number and frequency of consecutive guluronic acid residues and the cation concentration at the time of gellation. Although these studies illustrate the effect of alginate encapsulation on cell growth, they do not provide an insight on the effects of encapsulation on specific biochemical processes and their correlation to insulin secretion. Understanding these effects is critical in developing an optimum tissue engineered pancreatic substitute.

Experimental

βTC3 cells were obtained from the laboratory of Shimon Efrat, Albert Einstein College of Medicine, Bronx, NY. Cells were cultured in T-flasks as previously described (1). Alginate beads were generated using a 2% (w/v) alginate solution and an electrostatic bead generator crosslinking alginate with 0.1 M CaCl₂. Three bead sizes were tested in this study ranging in diameter as following: (i) 0.46±0.02 mm, (ii) 0.75±0.03 mm, and (iii) 1.15±0.16 mm. Perchloric acid extracts of monolayer and bead cultures were performed following exposure to uniformly labeled 13C-glucose to assess TCA cycle flux activity based on glutamate resonance isotopomer analysis. 13C NMR spectra were acquired at 11.1 T vertical bore Bruker Avance-spectrometer while NMR microimages acquired using a vertical 17.6-T 89-mm bore cryopumped magnet equipped with a Bruker Avance console and Micro2.5 gradients. These magnets are located at the AMRIS facility of UF.

Results and Discussion

1H decoupled 13C NMR spectra acquired from extract of βTC3 monolayer and alginate entrapped cultures show that the most prevalent metabolite produced by βTC3 cells in the consumption of glucose is lactate followed by glutamate, alanine, aspartate, and others. Alginate encapsulation has a dramatic effect in the appearance of the 13C NMR spectrum showing an increase in lactate and alanine resonances with encapsulation and a concomitant decrease in all TCA cycle related intermediates. It is important to note that there are no discernable differences in spectra profile with bead size. Isotopomer analysis of these spectra shows a statistically significant reduction in the relative flux through glycolysis and through Pyruvate Carboxylase, and an increase in a second anaplerotic entrance. These NMR based data are corroborated by insulin secretion measurements showing a significant reduction between monolayer and bead cultures but no difference among bead sizes.

Conclusions

Overall, our present data demonstrate that alginate encapsulation affects certain enzymatic processes related to glucose utilization as well as the insulin output of the encapsulated cells. Although neither of these effects was dependent on the diameter of the bead, the pattern of cell growth and some of the magnetic properties of the beads were size dependent.

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References