ALGINATE ASSESSMENT BY NMR MICROSCOPY

S.C. Grant (UF, Neuroscience), S. Celper (KTH, Sweden), I. Gauffin-Holmberg (KTH, Sweden), N.E. Simpson (UF, Medicine), S.J. Blackband (UF, Neuroscience), I. Constantinidis (UF, Medicine)

Introduction

In the development of a bioartificial pancreas, alginate has been used to encapsulate islets and transformed β-cells with considerable success. A layer of a polycation, such as poly-L-lysine followed by an additional layer of alginate is commonly used to coat the central alginate matrix, providing mechanical stability to the matrix and partial immunoprotection. Two issues of critical importance that have yet to be addressed about the long-term integrity of these beads are: (i) are there temporal changes in the alginate/PLL interaction, and (ii) are there temporal changes in the alginate gel structure. This report highlights our effort to visualize the PLL layer and assess the temporal stability of the alginate matrix.

Experimental

Alginate beads were generated using a 2% (w/v) alginate solution and an electrostatic bead generator crosslinking alginate with 0.1 M $CaCl_2$. Completion of the APA beads was based on the initial protocol developed by Lim and Sun (*1*). All MRI data were acquired using a vertical 17.6-T 89-mm bore cryopumped magnet equipped with a Bruker Avance console and Micro2.5 gradients. This magnet is located at the AMRIS facility of UF. Beads immersed in DMEM were loaded into a capillary and placed within a homebuilt solenoidal microcoil. Encapsulated cells were imaged in an unperfused state to avoid motion artifacts. Images were acquired using a gradient echo sequence with a TE=25 ms, TR=2 s, matrix of 512x128x128 and field of view of 6.4x1.6x1.6 mm as well as a spin echo sequence with a TE=15-120 ms, TR=2.5 s, matrix of 512x256 and field of view of 2.4x1.2 cm, slice thickness of 60 μm to measure T_2 value of the alginate as a function of time.

Results and Discussion

Figure 1 illustrates a NMR microimage of four alginate beads. The bottom two beads are coated with a PLL layer while the top two are not. The PLL layer is clearly demarcated by the dark circle surrounding the alginate matrix. This circle is attributed to the magnetic susceptibility between the PLL layer and the alginate matrix. T_2 measurements were obtained from such beads over a period of a month. The data show that T_2 of the alginate matrix decreases over time reaching a minimum within 10-14 days. Because a drop in T_2 correlates with a decrease in gel porosity, we can postulate that continuous culture of these beads has caused calcium ions to leak out of the bead, altering the alginate microstructure. Consequently, the pores of the alginate gel decreased since the egg box configuration (*2*) has collapsed with the decrease in available calcium.

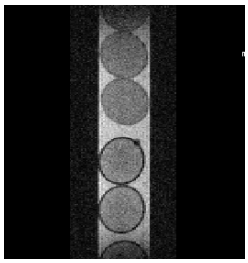

Figure 1: Spin echo images of non-coated and PLL coated alginate beads

Conclusions

NMR microscopy can visualize the alginate/PLL layer and monitor temporal changes in alginate structure. Thus, it has the potential to non-invasive assess biomaterials and tissue engineered constructs from both cellular and structural perspective.

Acknowledgements

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References

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