In Vitro Development and Characterization of a Cortical Neural Stem Cell-Seeded Alginate Scaffold

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Statement of Purpose: The purpose of this study was to optimize alginate encapsulation techniques of cortical neural stem cells (NSCs) based on neurotrophic factor release from the cells and mechanical stability of the scaffold. Although alginate has been used to encapsulate many types of cells with the purpose of immunoisolation from the host, no available studies currently use this material to entrap NSCs. NSC lines have been shown previously to naturally secrete physiologically relevant quantities of neurotrophic factors [1], and this phenomenon was believed to be responsible for their therapeutic impact in rats with spinal cord injury [2]. Since alginate composition is known to affect the secretory characteristics of other types of entrapped cells [3], as well as the mechanical stability of the scaffold itself [4], the purpose of this work was to understand how alginate composition affects these variables for encapsulated neural stem cells. Future studies will apply the scaffold in vivo to repair cortical lesions in the adult rat brain.

Methods: E14 murine cortical neural stem cells were expanded with epidermal growth factor (EGF) and encapsulated by mixing a cell slurry with 1% alginate and dropping into a 100 mM calcium chloride cross-linking solution. Alginate beads were 1mm in diameter, where bead size was controlled by concentric parallel air flow around the alginate/cell stream. Four different conditions were employed: a high guluronic (G) alginate (68% G content, MW = 219,000 g/mol) or a high mannuronic (M) alginate (54% M content, MW = 222,000 g/mol), with or without a poly-L-lysine (PLL) coating layer. PLL coating was achieved as previously described [5]. At 1, 3, 7, and 10 days following encapsulation, media samples were collected for quantification of neurotrophic factor release with enzyme linked immunosorbent assay (ELISA). Specifically, brain derived neurotrophic factor (BDNF), glial cell line derived neurotrophic factor (GDNF), and nerve growth factor (NGF) were analyzed. Samples of beads were collected for analysis of viability with propidium iodide staining, quantification of proliferation with an tetrazolium salt (MTS) assay [6], and processing for staining with toluidine blue or various markers of differentiation. At 2 and 9 days after encapsulation, beads were tested for mechanical stability using a semi-quantitative osmotic pressure test [7]. All data were analyzed with ANOVA and an appropriate post-hoc test where significant differences were noted.

Results / Discussion: Both unencapsulated and encapsulated NSCs secreted significant quantities of NGF and GDNF as compared to media control at various time points (24 hours, 7 days, and 10 days for NGF and 10 days for GDNF, $p<0.05$). BDNF was not detected. Cells secreted surprisingly large quantities of NGF, in excess of 5 ng from one million initially encapsulated cells for the high G condition. Notably, greater quantities of both neurotrophic factors were detected in media collected from cells encapsulated in high G, non-PLL coated alginate compared to any other alginate condition at the 10 day time point, and this was statistically significant for NGF secretion ($p<0.05$). MTS data demonstrated that NSCs show a dramatic increase in proliferation following 7 days in alginate after an initial lag phase ($p<0.05$ for all conditions). This was corroborated by visual inspection of toluidine blue stained sections. The increase in cell number roughly corresponded with increased NGF and GDNF secretion as measured by ELISA. Propidium iodide staining revealed that cell death following encapsulation was primarily due to necrosis at the center of proliferating cell masses. Overall, the encapsulated cells tended to be approximately 60% viable throughout the study; cell viability was 86% prior to seeding. Encapsulated NSCs mainly express nestin, which is a marker of undifferentiated neural stem cells. In terms of stability as measured by an osmotic pressure test, G>M>G/PLL-coated>M/PLL-coated in the ability to remain intact following exposure to solutions of low osmolarity ($p<0.05$).

Conclusions: Alginate is a promising scaffold for neural stem cells, and the data presented here suggest that a high G alginate without PLL coating is optimal for cortical neural stem cells based on enhanced release of neurotrophic factors and improved mechanical stability. High G alginate is known to be more porous than high M alginate, and may possibly enhance the stability of growth factors [8], which may explain the detection of greater quantities of neurotrophic factors from high G-encapsulated cells. Since G is the cross-linking residue of the polymer chain, it is not surprising that alginate with this composition resulted in enhanced resistance to breakage due to osmotic swelling. It has been noted that osmotic swelling of the bead core against a less elastic polycation membrane may eventually cause capsules to burst, which may explain why PLL-coated capsules broke more frequently. However, it is important to consider that a variety of forces (osmotic, compressive, shear) are likely to contribute to alginate instability in vivo. While the recommendation to use a non-PLL coated, high G alginate to encapsulate neural stem cells is the conclusion of the present work, subsequent studies may further optimize the system by testing additional composition variables over a longer time period. Future studies will verify the bioactivity of secreted factors, and the scaffold will be employed in vivo to assess its ability to repair cortical lesions in a rat model.

References: [1] Lu P. Exp Neurol. 2003;181:115-29. [2] Teng YD. PNAS. 2002;99:3024-9. [3] Stabler C. Biomaterials. 2001;22:1301-10. [4] Smidsrod O. Trends Biotechnol. 1990;8:71-8. [5] Strand BL. J Microencapsul. 2002;19:615-30. [6] Bunger CM. Artif Org. 2002;111-6. [7] Van Raamsdonk JM. J Biomed Mat Res. 2001;54:264-71. [8] Peters MC. J Biomater Sci Polym Ed. 1998;9:1267-78.