Bioencapsulation of antigen secreting cells as a vaccination strategy

Hrynyk, M.T.[1], K. Rosenthal[2] , and R.J. Neufeld[1]
1. Department of Chemical Engineering, Queen's University, Kingston, Ontario, K7L3N6
2. Department of Pathology and Molecular Medicine, McMaster University, 1280 Main St. W., Hamilton, Ontario, L8S4L8
(mhrynyk@cogeco.ca)

Introduction

Vaccination is one of the most effective measures of preventing the spread of infectious diseases. For a vaccine to be effective, it must blend three important properties: 1) an antigen from which an adaptive immune response will be generated, 2) an adjuvant to stimulate the innate immune system, and 3) an effective delivery system (Ulmer, 2006).

Unfortunately, one of the pitfalls of conventional vaccine development is the reliance on cell or virus culture and immunogen fractionation to produce sufficient antigen for a vaccine (Gomez-Vargas, 2004). Although recombinant protein synthesis has been established to circumvent this problem, many recombinant proteins lack the innate post-translational modifications necessary to establish immunogenicity (Azizi, 2007). Furthermore, DNA vaccines often exhibit similar drawbacks and pose new challenges in eliciting the same level of immunity across species during development, leading to uncertainty in their efficacy (Gomez-Vargas, 2004; Jun-Ming, 2006).

Despite these difficulties, encapsulation of xeno- or allogeneic cells may hold the key toward providing all three necessary components of a vaccine in one package. Recently, Gomez-Vargas *et al*. (2004) demonstrated a continuous antigenic stimulation system (CASS) as a new immunization strategy. Using alginate-poly-L-lysine microcapsules, recombinant mouse C2C12 myoblasts secreting human factor IX (hFIX) elicited a strong humoral response and sustained immunity for up to 213 days after implantation (Gomez-Vargas, 2004). However, given this remarkable technology, little is known about the parameters required to maintain antigen production, nor the immunogenicity of alginates or coatings as adjuvants.

Although several strategies toward the use of immunoisolating alginate microcapsules have been identified (De Vos, 2006), the innate ability of alginate to attract antigen presenting cells such as macrophages and basophils (Ponce, 2006) may be a natural method of stimulating the immune system for vaccination. However, the question remains as to which properties of alginate microcapsules such as the type of polycation coating or quantity of low-, intermediate-, or high-guluronic acid content (G) alginate may lead to antigen presenting cell stimulation and antigen presentation. Table 1 lists the various combinations of polycation coatings and either intermediate- or high-G content alginate, as well as the proportions of cell types identified in the cellular overgrowth 30 days post implantation (adapted and modified from S. Ponce et al., 2006.) These data indicate that there are antigen presenting cells present in the fibrous capsule and may be capable of antigen processing and display to adjacent B and T cells.

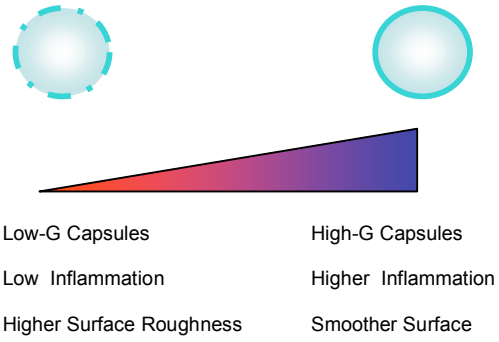
Table 1: Cell types determined in cellular outgrowth 30 days post implanation, with various combinations of particle core/coating (adapted and modified from Ponce, 2006).

Alginate/polycation	Macrophages	Lymphocytes	Granulocytes
Intermediate-G content			
PLL	7.75 ± 7.63	-	-
PDL	10.66 ± 3.78	3.33 ± 2.88	0.33 ± 0.57
PLO	63.5 ± 13.1	2.5 ± 7.63	2.5 ± 3.31
High-G content			
PLL	4.25 ± 1.5		
PDL	1.75 ± 2.21	3 ± 2.44	2 ± 2.44
PLO	32 ± 20	3 ± 1.825	3 ± 1.825

*Not all data shown; PLL, poly-l-lysine; PDL, poly-d-lysine; PLO, poly-l-ornithine.

In addition to surface polycation coatings, surface morphology has been identified as another component leading to antigen presenting cell interaction and stimulation of local inflammatory responses (Ponce, 2006). Surface imperfections resulting from low-G content alginate microcapsules have been identified as increasing inflammatory responses *in vivo*, versus smoother morphologies produced by high-G content microcapsules (De Vos, 2006). In terms of antigen and adjuvant stimulation, it is difficult to define which combinations of G content and morphology would be better suited in vaccination. Figure 1 is an illustration indicating the relationship between surface roughness, immune response, as well as alginate G content.

Figure 1: Proposed relationship between alginate type and surface roughness on strength of immune response.



As such, alginate based microcapsules harboring recombinant cells for antigen production, immune stimulation and delivery can potentially be an efficient vaccination strategy. This study aims at achieving three main objectives important in understanding the relationship between the internal microcapsule environment and cellular survivability. The first objective is to test and determine the duration of encapsulated cell survivability in various low-, intermediate-, high-G alginate microcapsules coated with poly-l-lysine, poly-d-lysine and poly-l-ornithine. The second objective is to determine the internal and external physical morphologies of the prepared microcapsules and the third objective is to monitor antigen production during extended periods of implantation.

Experimental Model

Recombinant mouse C2C12 myoblasts excreting the human recombinant protein hFIX will be encapsulated using methods previously described by Gomez-Vargas et al. 2004. Briefly, a 3 x 3 combinatorial experimental setup formulating alginate microcapsules from low-, intermediate- and high-G alginate will be performed using an ionotropic gelation method in a calcium chloride solution. From each of the alginate formulations, three separate coatings of poly-l-lysine, poly-d-lysine and poly-l-ornithine will be applied.

Cell survivability will be determined using trypan blue staining to determine the relative ratio of living to dead cells per sample over time. hFIX secretion from alginate microcapsules will be monitored over time using an ELISA assay.

Analysis of the internal microcapsule environment will be determined using conventional imaging methods, and external morphology will be conducted using X-ray photoelectron spectroscopy as previously described by De Vos and colleagues (2006).

Conclusion

By determining cell survivability and antigen production under various alginate compositions and polycation coatings, a better understanding of which conditions are more favourable for encapsulated cell survivability and antigen production will be established. This will help advance this work toward *in vivo* studies, with the intent of identifying a formulation which can induce immunity.

References

J.B. Ulmer, Valley, U. & Rappuoli, R. (2006) *Vaccine Manufacturing : challenges and solutions*. Nature Biotechnology **24**: 1377-1383.

A. Gomez-Vargas, Rosenthal, K.L., McDermott M.R. & Hortelano, G. (2004) *Continuous antigenic stimulation system (CASS) as a new immunization strategy*. Vaccine **22**: 3902-3910.

A. Azizi & Diaz-Mitoma, F. (2007) *Viral peptide immunogens : current challenges and opportunities.* Journal of Peptide Science **13**: 776-786.

L. Jun-Ming & Dao-Yin, Z. (2006) *Therapeutic DNA vaccines against tuberculosis: a promising but arduous task*. Chinese Medical Journal **119**(13): 1103-1107.

P. De Vos, Faas, M.M., Strand, B. & Calafiore, R. (2006) *Alginate-based microcapsules for immunoisolation of pancreatic islets*. Biomaterials **27**: 5603-5617.

S. Ponce, Gorka, Orive, G., Hernandez, R., Gascon, A. R. et al. (2006) *Chemistry and the biological response against immunoisolating alginate-polycation capsules of different composition.* Biomaterials **27**: 4831-4839.

H. Uludag, De Vos, P. & Tresco, P.A. (2000) *Technology of mammalian cell encapsulation*. Advanced Drug Delivery Reviews **42**: 29-64.

P. De Vos, De Han, B.J., Kamps, J.A.A.M., Faas, M.M. & Kitano, T. (2006) *Zeta-potentials of alginate-PLL capsules: a predictive measure for biocompatibility?* Journal of Biomedical Materials Research **80**(4): 813-819.