Plasma protein adsorption on microcapsule surface: the first step of microencapsulated islet rejection

J. Dusseault, S.K. Tam, M. Ménard, J.P. Hallé*
5415 boul. L'Assomption, Maisonneuve-Rosemont Hospital Research Centre, Université de Montréal, Montréal, Canada (julie.dusseault@umontreal.ca)

Introduction

Since the publication of the Edmonton protocol in 2000(1), islet transplantation is considered one of the most promising treatment for type I diabetes. However, islet transplantation is only possible with the use of immunosuppressive drugs. These drugs decrease the host immune system efficiency and allow islet survival but are also known to have toxic effects. Immunosuppression can be avoided with the use of immunoisolating devices(2). This kind of device is conceived to protect the cells from the host immune system. The immunoisolation device that are used is this study is composed of an alginate gelled inner core surrounded by a semi-permeable membrane of poly-L-lysine, which is then incubated in alginate(2).

The semi-permeable membrane of microcapsules protects the islets from the immune cells and antibodies of the host immune system but allows nutriments, such as glucose, to pass trough this membrane to feed the encapsulated islets. When microcapsules are implanted *in vivo*, a host reaction against the microcapsule (HRM) surface occurs(3, 4). In this HRM, macrophages are known to play a key role(5, 6). Nevertheless, the mechanisms that are involved in macrophage recruitment are yet poorly understood.

Recent data suggest that the adsorption of specific host proteins onto biomaterial surface is the first step in the host immune cell reaction to a medical device(7). Inversely, other proteins (e.g. albumin) seem to possess the ability to protect the device by blocking the cell access to the surface or modulating the biofunctional properties (e.g. hydrophilicity) of the microcapsule surface(8).

Our hypothesis is that the identification of the proteins adsorbing onto microcapsule surface can lead to an improvement of microcapsule surface design in order to promote a favorable protein adsorption profile i.e. decreased/increased adsorption of proteins that correlates with enhanced/decreased immunogenicity respectively.

Materials and Methods

Alginate-poly-L-lysine-alginate microcapsules were made from purified(9) or non purified alginate (Pronova^{TM} LF10/60) using an electrostatic pulse generator (average diameter 280µM) and were mounted into chromatographic columns. Commercial mouse plasma or serum was incubated for 10 minutes with microcapsules. Then, non adsorbed proteins were eluted using 150mM NaCl. Adsorbed proteins were taken off from microcapsules surface using 1M NaCl eluting solution. At this point, 2M NaCl, 3M NaCl and 7M urea were also tested but were not effective to remove additional stuck proteins and were sometimes breaking the microcapsule column matrix.

Retrieved proteins were migrated on SDS-PAGE and stained with silver nitrate. After migration protein bands were cut and digested on Massprep robotik workstation and identified using mass spectrometry combined with a liquid chromatography (LC-MS\MS).

Results and Discussion

When mouse plasma or serum was incubated with microcapsules, most of proteins were easily washed out and did not adsorb onto microcapsule surface as shown into the typical elution profile in Figure 1 (peak A). However, some proteins were retrieved while increasing salt concentration of the elution solution from 150Mm to 1M showing that residual protein were stick to microcapsule (peak B).

Migration of these adsorbed proteins on SDS-PAGE showed that either proteins from mouse plasma (Figure 2) and mouse serum (Figure 3) were found to bind on microcapsule surface. Interestingly, migration patterns of adhering proteins were different between plasma and serum and also between microcapsule made from purified or non purified alginate (Figure 4). This result suggests the existence of pro-immunogenic proteins, particularly found on microcapsules made from non purified alginate, and anti-immunogenic proteins, particularly found on microcapsules made from purified alginate.

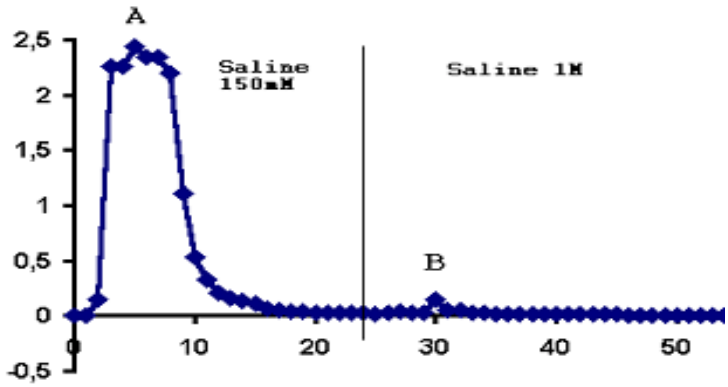
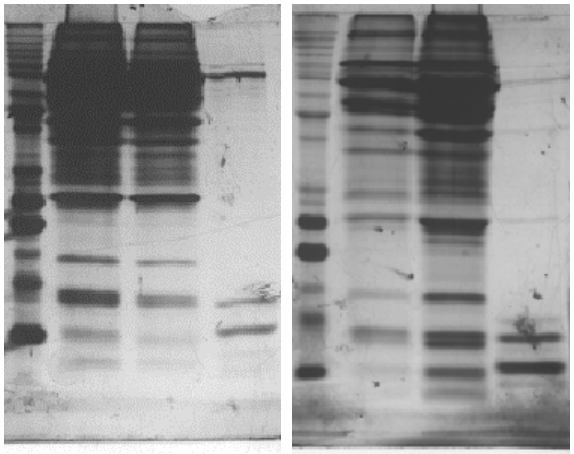
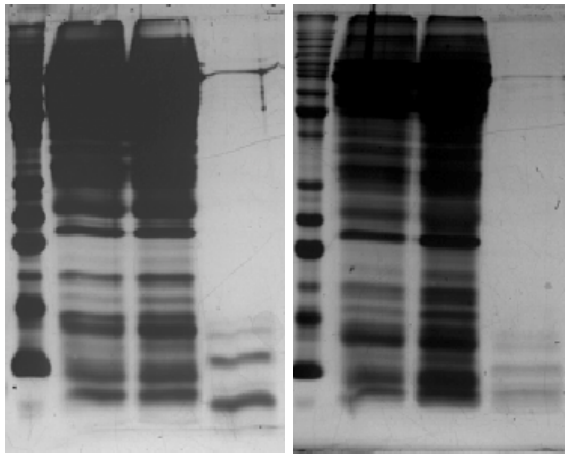


Figure 1: Typical protein elution profile of proteins.
A. Non adsorbed protein peak eluted with saline 150mM.
B. Adsorbed proteins peak eluted with saline 1M.



I

Figure 2: Mouse plasma proteins.
A. Non purified alginate microcapsules
B. Purified alginate microcapsules
Lane 1: Molecular weight ladder
Lane 2: Total plasma proteins
Lane 3: Non adsorbed plasma proteins
Lane 4: Adsorbed plasma proteins



A **B**

Figure 3: Mouse serum proteins.
A. Non purified alginate microcapsules
B. Purified alginate microcapsules
Lane 1: Molecular weight ladder
Lane 2: Total mouse serum proteins
Lane 3: Non adsorbed serum proteins
Lane 4: Adsorbed serum proteins

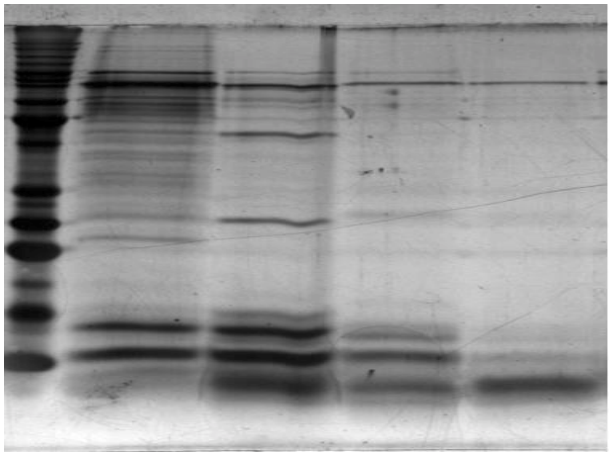


Figure 4: Plasma and serum adhered proteins
Lane 1: Molecular weight ladder
Lane 2: Plasma proteins-non purified alginate
Lane 3: Plasma proteins-purified alginate
Lane 4: Serum proteins-non purified alginate
Lane 5: Serum proteins-purified alginate

Table 1: Identification of adhered proteins.

CLASS	PROTEIN NAMES
Complement	Component 3
	B-factor, properdin factor B
	C1q subunit C
	C1q subunit B
	C1q subunit A
Coagulation factors	Plasminogen, heavy chain A
	Prothrombin, factor II
Immunoglobulins	Ig light chain
	Ig Gamma-2A chain C region
	Ig Lambda-2 chain C region
	Ig Kappa chain
	Ig Mu chain C
Others	Albumin
	Fibronectin precursor
	Fibronectin 1
	Thrombospondin
	Alpha1-macroblubulin
	Transferrin
	Apolipoprotein E

The most abundant adhering proteins are listed in Table 1 and gathered together by specific immune protein groups. A wide variety of proteins was found to bind to the microcapsule surface such as proteins from the complement system, the coagulation cascade and immunoglobulins. Albumin and fibronectin, proteins that are widely studied and generally known to bind to foreign surfaces(10-12), were also found on microcapsule interface. Fibronectin is particularly correlated with cell attachment because of his RGD peptide sequence(13). Complement is also suspected to play an important role in host immune response to biomaterials(14, 15). Complement components, such as C3, C1q and B factor were detected in proteins that adhered onto microcapsules. In fact, it was already observed on polystyrene surface(14). It is noteworthy that proteins that would be covalently linked to microcapsule surface would not be detached from microcapsule using saline elution, and therefore cannot be identified in these experiments.

Conclusions

Using the methodology that is described in the present paper, we were able to show that adsorbed plasma/serum proteins onto alginate-poly-L-lysine-alginate microcapsules can be retrieved and analysed on gels.

Our preliminary results from mass spectrometry analysis showed that we were able to identify proteins adsorbed to microcapsule surface.

Different protein migration patterns were seen between microcapsules made with purified or non-purified alginates, suggesting that specific proteins may increase or, inversely, decrease the immunogenicity of microcapsules.

In perspective, we foresee that making a differential analysis between adhered proteins (purified vs non purified alginate) will lead to the identification of proteins playing a key-role into the rapid initiation of an immune response against microcapsule surface or in the prevention of cell adhesion.

Furthermore, microcapsule surface design will eventually be modified to promote adhesion of anti-immunogenic proteins and prevent adhesion of pro-immunogenic proteins.

References

1. Shapiro AM et.al (2000) *Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen*. N Engl J Med 343(4):230-8.
2. Lim F et al. (1980) *Microencapsulated islets as bioartificial endocrine pancreas.* Science 210(4472):908-10.
3. Vandenbossche GM et al. (1993) *Host reaction against empty alginate-polylysine microcapsules. Influence of preparation procedure*. J Pharm Pharmacol 45(2):115-20.
4. Robitaille R et al. (2005) *Inflammatory response to peritoneal implantation of alginate-poly-L-lysine microcapsules*. Biomaterials 26(19):4119-27.
5. Pueyo ME et al. (1993) *In vitro activation of human macrophages by alginate-polylysine microcapsules*. J Biomater Sci Polym Ed 5(3):197-203.
6. Juste S et al. (2005) *Effect of poly-L-lysine coating on macrophage activation by alginate-based microcapsules: assessment using a new in vitro method*. J Biomed Mater Res A 72(4):389-98.
7. Allen LT et al. (2006) *Surface-induced changes in protein adsorption and implications for cellular phenotypic responses to surface interaction*. Biomaterials 27(16):3096-108.
8. Rouxhet L et al. (1998) *Adsorption of albumin, collagen, and fibronectin on the surface of poly(hydroxybutyrate-hydroxyvalerate) (PHB/HV) and of poly (epsilon-caprolactone) (PCL) films modified by an alkaline hydrolysis and of poly(ethylene terephtalate) (PET) track-etched membranes*. J Biomater Sci Polym Ed 9(12):1279-304.
9. Klock G et al. (1994) *Production of purified alginates suitable for use in immunoisolated transplantation*. Appl Microbiol Biotechnol 1994;40(5):638-43.
10. van Raamsdonk JM et al. (2002) *Deterioration of polyamino acid-coated alginate microcapsules in vivo*. J Biomater Sci Polym Ed 13(8):863-84.
11. Renner L et al. (2005) *Fibronectin displacement at polymer surfaces*. Langmuir 21(10):4571-7.
12. Bumgardner JD et al. (2003) *Contact angle, protein adsorption and osteoblast precursor cell attachment to chitosan coatings bonded to titanium*. J Biomater Sci Polym Ed 14(12):1401-9.
13. Takahashi S et al. (2007) *The RGD motif in fibronectin is essential for development but dispensable for fibril assembly*. J Cell Biol 178(1):167-78.
14. Andersson J et al. (2005) *Binding of C3 fragments on top of adsorbed plasma proteins during complement activation on a model biomaterial surface*. Biomaterials 26(13):1477-85.
15. Wettero J et al. (2002) *On the binding of complement to solid artificial surfaces in vitro*. Biomaterials 23(4):981-91.