The kinetics of alginate-based microcapsules after adsorption of proteins

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INTRODUCTION

The efficacy of immunoisolation in alginate-PLL membranes in order to transplant pancreatic islets in the absence of immunosuppression has been demonstrated by several groups. Unfortunately, non of these groups have been able to show permanent survival of these grafts. Many factors have been shown to influence graft survival (de Vos 2006). Recent progress has allowed us to effectively eliminate the development of fibrosis surrounding the implanted microcapsules. Nevertheless, the long-term viability of encapsulated islets *in vivo* is still limited.
Triggered by the implantation surgery, inflammation at the transplantation site contributes to islet cell death and dysfunction. Macrophages in the vicinity secrete small molecules, such as cytokines and nitric oxide, that can penetrate the microcapsule membrane and harm the enclosed cells. This process can be enhanced by the capsule properties when which will further compromise the bioperformance of the cells in the microcapsules.
The adsorption of proteins in the immediate period after implantation is considered to be the initiator of all subsequent cellular responses to the implant, including inflammation (Ratner 2004 and Juste 2005). Immunoglobulin G and M (IgG and IgM) are opsonizing proteins that are well known to lead to complement activation (via the classical activation pathway) and inflammation.
In this study, we investigated the adsorption of immunoglobulin (Ig) from human serum and peritoneal fluid (PF) to the surface of alginate-based microcapsules *in vitro*. We show that Ig adsorption is influenced by the type of alginate applied and is strongly dependent on the presence of a polycationic membrane.

MATERIAL AND METHODS

Intermediate-G sodium alginate (~ 40% guluronic acid, Alginates UK Ltd) and high-G sodium alginate (▯ 50% guluronic acid, ISP Alginates UK Ltd) were used for microcapsule fabrication. Alginates were used as purchased (raw) or firstly purified using previously established methods (De Vos 1997). Poly-L-lysine (PLL) HCl (Mw 22 000, Sigma-Aldrich, Inc) was used for membrane formation. Human serum (HS) and peritoneal fluid (PF) was drawn from healthy volunteers.
Microcapsule fabrication. Microcapsules were fabricated using previously established methods (Wolters 1991). Briefly, sodium alginate powder was dissolved in a Ca^{2+}-free Krebs-Ringer-Hepes (KRH) solution, in concentrations ranging from 1.9% to 3.5% w/v to obtain optimal viscosity for capsule formation. Droplets of the alginate solution were extruded from a 25G needle using an air-driven droplet generator. Extruded droplets were allowed to gel for ▯5 minutes in a 100 mM $CaCl_2$ solution. The gel beads (CaB), measuring 650 ± 15 ▯m in diameter, were rinsed in a hepes solution then immersed in a PLL solution (0.1% w/v) for 10 minutes to form the microcapsule membrane. After rinsing 3 times with a Ca^{2+}-free KRH, the microcapsules were immersed in a 10x diluted solution of sodium alginate for 5 min before a final rinse with Ca^{2+}-free KRH. Microcapsules (APA) and alginate gel beads in their final form were stored in KRH at 4ºC.
Protein adsorption. Samples of 30 microcapsules were co-incubated with human serum and Peritoneal fluid (diluted 1:1:1 in KRH) in polypropylene test tubes at 37ºC with gentle agitation for

1 hour. Microcapsules were then rinsed 5 times with KRH and permeability was determined immediately afterwards.
Animals. Male inbred Albino Oxford (AO/G), were obtained from Harlan (Harlan CPB, Zeist, The Netherlands) or the Central Animal Laboratory of Groningen. NHI-guidelines for the care and use of laboratory animals have been applied.
X-ray photoelectron spectroscopy (XPS). Intact capsules were washed tree times with ultrapure water and gradually lyophilized. The sample holder was inserted into the chamber of a X-ray photoelectron spectrometer (Surface Science Instruments, S-probe ,Mountain View, CA). An aluminum anode was used for generation of X-rays (10 kV,22 mA) at a spot size of 250 x 1000 µm. During measurements, the pressure in the spectrometer was around 10^{-7} Pa. Scans were recorded at a resolution (50 eV pass energy) C_{1s},N_{1s} and O_{1s} peaks over 20 eV binding energy range.
Permeability measurements. Microcapsules were incubated with a protein solution containing gamma globulins, albumin, insulin and glucose Samples were taken in time to determine the permeability of the capsules.
Data analysis. Results are expressed as mean SEM. Statistical comparisons were made with the Mann Whitney U test. .A p-value ▯ 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The recovery rates of capsules after implantation. Capsules were retrieved at one month after implantation into the peritoneal cavity of AO-rats. High G-content capsules had a recovery rate of 53.6 ± 7,5% with an inflammatory overgrowth percentage of 43.0 ± 14.0%. Intermediate G-content capsules had a higher recovery rate of 92.3 ± 5.3% with a lower overgrowth percentage of 3.0 ± 0.5%. Many has been efforts to understand the effect of alginate composition on this large difference in response.

	C (%)	N (%)	O (%)	Ca (%)	Na (%)	Cl (%)	others
High-G	58.6± 1.9	6.2 ± 0.5	31.4± 0.8	0	2.7 ± 0.4	0	1.1
Int-G	60.3± 0.5	7.6 ± 0.1	30.4 ± 0.5	0	1.3 ± 0.1	0	0.4
PLL	67.2	10.7	17.9	0	0	4.2	0

Table 1. The elemental surface composition of alginate-PLL capsules of different composition after prolonged periods of implantation in the peritoneal cavity of AO-rats

A significant difference between intermediate-G and high-G capsules is the PLL content of the membrane which has been reported as a potent stimulator of inflammatory reactions. As shown in table 1, the intermediate-G alginate capsules contains significantly ($P< 0.02$) less nitrogen (6.2±0.5 vs 7.6±0.5) than the high-G capsules. Since nitrogen is a minor component of no more than 10.7% of the total PLL molecule (Table 1) we calculated the actual difference between intermediate a high-G capsules by dividing the N/C ratio of PLL through the N/C ratio of the capsule membrane. After this calculation the high-G are composed for 79% of PLL while it is 66% for the intermediate-G capsules. Although significantly different there are also groups that claim that these small differences cannot fully account for the difference in response of the host. Therefore in a next study we the protein adsorption, eg the first step in an inflammatory response, on alginate capsules with PLL, i.e. on alginate beads of different composition. This was done by studying the adsorption by micro-FTIR after incubation in human peritoneal fluid and serum.

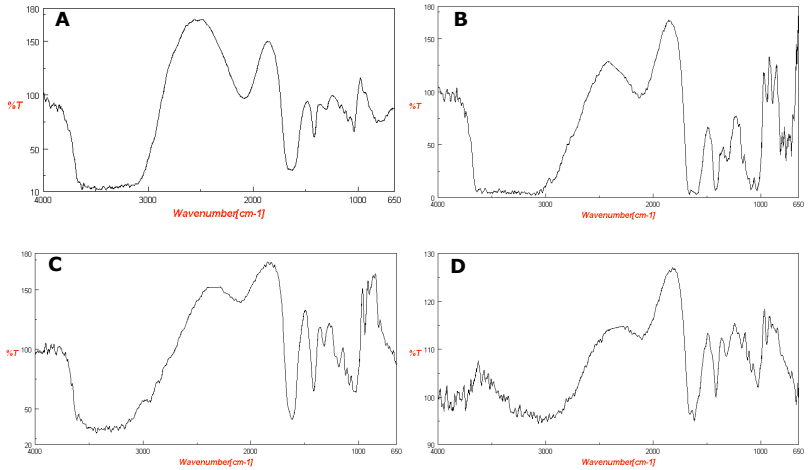

Figure 2. mFTIR spectrum of intermediate-G aAPA capsules (a), after incubation in peritoneaal fluid (c) mFTIR spectrum of high-G APA capsules (b) after incubation in peritoneaal fluid (d) .

Incubation of the intermediate-G APA with peritoneal fluid resulted in the appearance of four peaks in the mFTIR spectrum at 1664,1599,1369 and 1332 cm^{-1}(fig. 3c).
High-G APA capsules showed also a difference in spectrum due to incubation with peritoneal fluid. The incubation resulted in the appearance of four peaks in the mFTIR spectrum at 1664, 1619,1216 and 1174 cm^{-1}(fig. 3d). These results clearly show that under circumstances mimicking the in vivo situation (incubation in serum and peritoneal fluid) qualitative differences in spectra and thus in physical chemical properties can be found on capsules prepared from intermediate-G and high-G capsules. These differences in physical chemical properties can only be found after implantation and should be hold responsible for the differences in inflammatory responses against high and intermediate-G capsules.

Effect of protein adsorption on the permeability properties of APA microcapsules. In order to study the functional consequences of this protein adsorption we studied the permeability of the capsules for glucose, insulin, albumin, myoglobin, IgA, IgG, IgM after and before incubation with serum and peritoneal fluid. As shown in Figure 3, although protein adsorption is different on the two capsule types and probably associated with the higher response against capsules prepared of high-G alginate, we did not find any influence on the functional characteristics of the capsules. All tested solutes were limited or entered the capsules with the same kinetics irrespective of a previous incubation in peritoneal fluid or serum.

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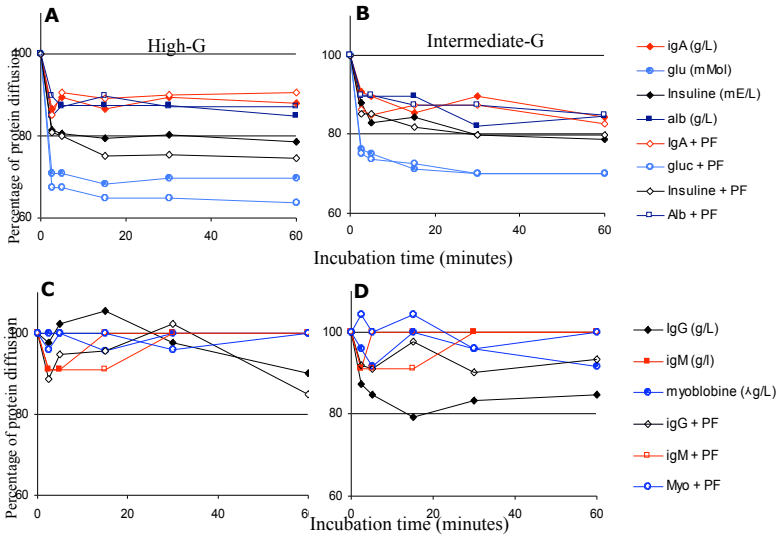

Figure 3. Percentage of penetration of proteins through capsules before (closed symbols) and after peritoneal fluid and serum incubation (closed symbols). The high-G APA capsules are presented in figure a and c and the intermediate-G in b and d.

CONCLUSIONS

High G capsules provoke a more severe response than capsules prepared of intermediate-G capsules. Incubation of the two capsule types in peritoneal fluid and/or serum showed different adsorption patterns as illustrated by differences in the mFTIR spectra. As this demonstrates differences in physico-chemical properties of the capsules it is tempting to speculate that this difference in adsoprtion is responsible for the large difference in the inflammatory reaction.
This change in spectrum due to protein adsorption did not change the permeability of the capsules for the tested solutes.

REFERENCES

De Vos P et al. (2006) *Advances and Barriers in Mammalian Cell Encapsulation for Treatment of Diabetes*. Immun., Endoc. & Metab. Agents in Med. Chem. 6 (2) 139-153.
Ratner BD et al. (2004) *BIOMATERIALS: Where We Have Been and Where We are Going* annurev.bioeng Vol. 6: 41-75
De Vos P et al. (1997) *Improved biocompatibility but limited graft survival after purification of alginate for microencapsulation of pancreatic islets*. Diabetologia 40 (3) 262-70.
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.
Wolters GH et al. (1991) A versatile alginate droplet generator applicable for microencapsulation of pancreatic islets. J Appl Biomater 3 (4) 281-6.