The adsorption of immunoglobulin on alginate-based microcapsules

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Introduction

Important advances in the transplantation of islets of Langerhans have recently been achieved using alginate-based microcapsules (De Vos 2006). These microcapsules safely protect the transplanted cells from graft rejection in the absence of long-term immunosuppression. Recent progress has allowed us to effectively eliminate the development of fibrosis surrounding the implanted microcapsules (De Vos 2003). 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. Nearby macrophages secrete small molecules (≤ 51 kD), such as cytokines and nitric oxide, that can penetrate the microcapsule membrane and harm the enclosed cells. Simultaneously, the physico-chemical characteristics of the membrane can be influenced by the change in biological environment associated with inflammation (e.g. a drop in pH), which in turn may compromise the bioperformance of the microcapsule.

The immediate adsorption of proteins from surrounding biological fluids onto an implanted biomaterial is believed to govern all subsequent cellular responses to the implant, including inflammation (Ratner 2004). 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 upon their adsorption to foreign bodies.

In this study, we investigated the adsorption of immunoglobulin (Ig) from human serum to the surface of alginate-based microcapsules *in vitro*. We show that Ig adsorption is strongly dependent on the presence of a polycationic membrane and is also influenced by the type of alginate applied.

Material and Methods

***Materials*.** Int-G sodium alginate (~ 40% guluronic acid, Keltone LVCR, ISP Alginates UK Ltd) and hi-G sodium alginate (> 50% guluronic acid, Manugel, 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 (mol wt 22 000, Sigma-Aldrich Inc) was used for membrane formation. Human serum was obtained from healthy volunteers. For fluorescent staining, a FITC-conjugated anti-human polyvalent antibody to IgG, IgM and IgA developed in rabbit (Sigma-Aldrich Inc) was used.

***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 (depending on the alginate type) 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 μm in diameter,

were rinsed in a Hepes solution then immersed in a PLL solution (0.1% w/v) for 10 minutes to form the mcirocapsule 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 (diluted 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 fluorescently stained immediately afterwards.

Fluorescent staining. Microcapsules were incubated with a FITC-conjugated anti-human IgG/IgM/IgA antibody (diluted 1:600 in KRH) for 1 hour in the darkness. The samples were then rinsed 5 times with KRH before being transferred to a 96 well plate for fluorescence analysis.

Fluorescence measurements. The intensities of fluorescence emitted from the samples were quantified using a Bio-Tek FL600 fluorescence microplate reader. Readings were taken in triplicate to compensate for instrumental variation. Fluorescent emission from the stained microcapsule surface was also examined qualitatively using an inverted fluorescence microscope. Photos were taken through the microscope lens using a digital camera.

Data analysis. Because the capsules swelled and were occasionally aspired during sample preparation, the signal intensities measured using the fluorescence microplate reader were normalized by total surface area of the microcapsules for each sample. Fluorescent intensities between samples were compared using the Wilcoxon Signed Ranks Test with the help of statistical software (SPSS, Inc) and a difference was considered statistically significant for p-values of < 0.05.

Results and Discussion

Evidence of Ig adsorption to APA microcapsules in vitro

Figure 1 clearly shows the fluorescent glow emitted from FITC-labelled Ig that adsorbed to the surface of APA microcapsules (fabricated using purified int-G alginate) that were pre-incubated in serum. One can also see in Figure 1 that the fluorescence emitted from stained, non serum-incubated microcapsules is of negligeable intensity and comparable to the fluorescence emitted from unstained microcapsules, which is an indication that the fluorescent signal was not due to the non-specific adsorption of the FITC-labelled antibody to the microcapsule surface. As shown in Figure 2, the same trend is observed quantitatively in terms of the fluorescent intensity measured by the fluorescence microplate reader. The serum-incubated microcapsules showed a fluorescence intensity of 20.1 ± 1.4 arbitrary fluorescence units (AFU), compared to the non serum-incubated (12.8 ± 0.5 AFU) and unstained (11.0 ± 0.3 AFU) microcapsules ($p < 0.05$). These results provide direct evidence of Ig adsorption to APA microcapsules *in vitro*, as well as demonstrate the feasibility and effectiveness of measuring Ig adsorption to microcapsules using a fluorescence staining technique.

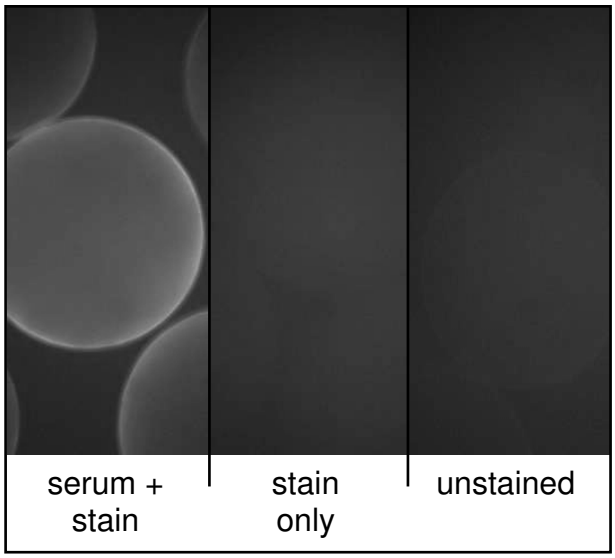

FIGURE 1. Fluorescent staining of human Ig adsorbed to APA microcapsules *in vitro*, as observed by fluorescence microscope. Microcapsules were fabricated using purified int-G alginate.

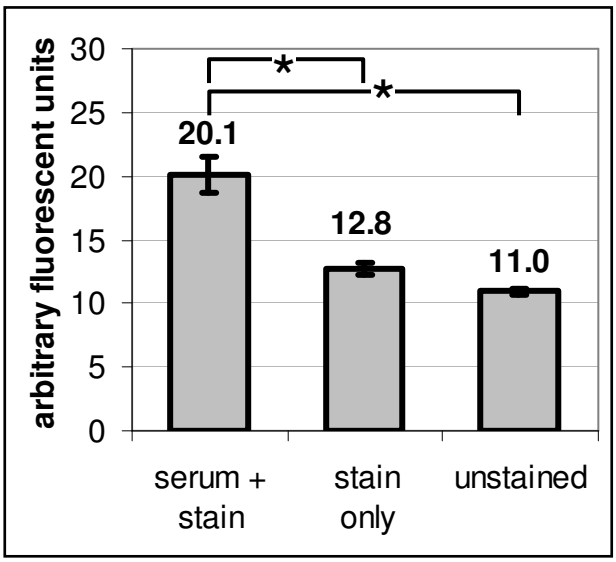

FIGURE 2. Fluorescent staining of human Ig adsorbed to APA microcapsules *in vitro*, as quantified by fluorescence microplate reader. Microcapsules were fabricated using purified int-G alginate. Bars represent the mean fluorescence intensity (normalized by total surface area of the sample) ± SEM (n=6). ⋆ indicates $p < 0.05$.

Effect of PLL membrane on the adsorption of Ig to APA microcapsules

Figure 3 compares the adsorption of Ig from human serum to microcapsules with a PLL membrane (labelled APA) and without a PLL membrane (labelled CaB). There is a significant drop in Ig adsorption in the absence of the PLL membrane ($p < 0.05$), represented by a 5-fold decrease in fluorescent intensity in the case of int-G alginate samples and a 15-fold decrease in the case of hi-G alginate samples. These results strongly suggest that Ig adsorption is mediated by the presence of the polycation or PLL-alginate complex that makes up the microcapsule membrane. This observation is very interesting considering that APA microcapsules are reputed to provoke inflammatory and immune responses *in vitro* and *in vivo*, in contrast to calcium alginate beads that do not have a polycationic membrane (Juste 2005).

Effect of alginate composition and purity on Ig adsorption to APA microcapsules

Upon establishing that the PLL membrane plays a significant role in mediating the adsorption of Ig to APA microcapsules *in vitro*, we sought to investigate the effect of the alginate properties (which in turn influence the membrane properties) on Ig adsorption. Accordingly, we compared the extent of Ig adsorption to microcapsules composed of alginates differing in chemical composition (hi-G vs int-G) and purity (pure vs raw). The results are shown in Figure 4. The measured fluorescent intensities indicate that APA microcapsules composed of purified alginates adsorb slightly less Ig than samples composed of raw alginates ($p < 0.05$ in the case of int-G alginate). Microcapsules fabricated using purified int-G alginate were associated with the least amount of adsorbed Ig (8.3 ± 1.3 AFU) compared to the other samples (9.8 to 10.9 AFU). This observation was anticipated since our experience shows that microcapsules composed of purified int-G alginate display a better biocompatibility *in vivo* than other microcapsule types when implanted into rats (de Vos 2002).

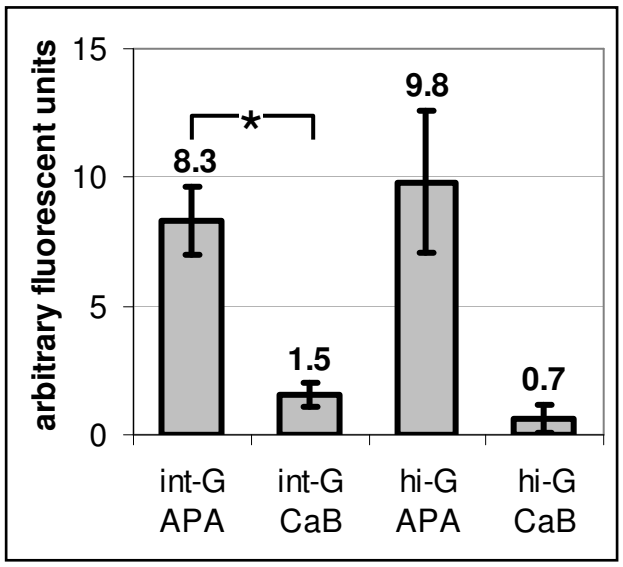

FIGURE 3. Fluorescent staining of human Ig adsorbed to APA microcapsules *in vitro*, as quantified by fluorescence microplate reader. Microcapsules with a PLL membrane (APA) and without a PLL membrane (CaB) were fabricated using purified alginates. Bars represent the increase in mean fluorescence intensity as compared to unstained samples (normalized by total surface area of the sample) ± SEM (n=6). ⋆ indicates $p < 0.05$.

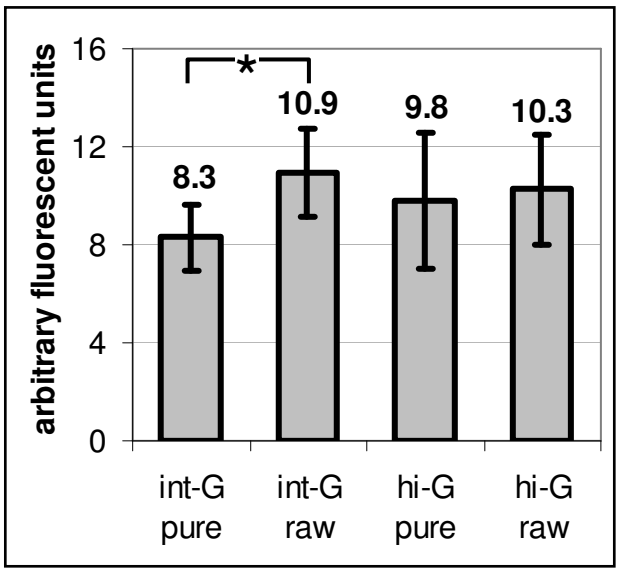

FIGURE 4. Fluorescent staining of human Ig adsorbed to APA microcapsules *in vitro*, as quantified by fluorescence microplate reader. Microcapsules were fabricated using alginates of different composition and purity. Bars represent the increase in mean fluorescence intensity as compared to unstained samples (normalized by total surface area of the sample) ± SEM (n=6). ⋆ indicates $p < 0.05$.

Conclusions

We provide clear evidence of the adsorption of human serum Ig onto APA microcapsules *in vitro*, as demonstrated using a fluorescent staining technique. Ig adsorption is strongly dependent on the presence of the PLL membrane. As well, Ig adsorption is slightly influenced by the composition and purity of the alginate used to fabricate the microcapsules.

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