O4-1	**Structural surface changes and inflammatory responses against alginate-based micro-capsules after exposure to human peritoneal fluid**

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INTRODUCTION AND OBJECTIVES

During recent years, islet transplantation has been shown to be an efficacious method for the treatment of Diabetes Mellitus. Unfortunately, the technology of islet transplantation is only applied on a minor scale due to the necessity to apply lifelong immunosuppression. This obstacle can be overcome by microencapsulation of the islets.

Important advances have been made in encapsulation research during recent years. In spite of these advances survival of the graft is still limited to periods up to 6 months which is too short to merit clinical application. This limited graft survival is due to loss of up to 60% of the endocrine islet volume (De Vos et al. 1997) in the immediate period after transplantation. Recently it has been shown that this loss is caused by an up to now unrecognized inflammatory response in the immediate period after implantation. Due to the mandatory surgery, capsules are exposed to both blood and peritoneal fluid (PF) containing bioactive molecules. These bioactive molecules may adsorb on the capsule surface and induce deleterious immunological reactions or may change the chemistry of the capsule surface, in that way changing the functional properties of the capsules.

To investigate this aspecific adsorption of proteins we have performed two separate studies. First we studied, by applying microFTIR, the structural changes of capsules after exposure to human PF. Next we studied, the inflammatory response of PBMC after exposure to PF treated alginates capsules.

MATERIALS AND METHODS

The 3,3 % intermediate-G or 2% high-G alginate solution was converted into droplets using an air-driven droplet generator as previously described. A poly-L-lysine (PLL) membrane was formed by suspending the alginate beads in 0.1% PLL solution for 10 minutes. Finally, the capsules had a diameter of 750-800 µm.

For adsorption studies *in vitro*, human PF was obtained from male donors within 12 hours of decease. 100 microcapsules were transferred to a polypropylene test tube. PF was diluted 1:1 in KRH and 1 ml was added to each test tube. Samples were incubated in a warm water bath at 37°C with gentle agitation for 1 hour. Afterwards, the PF was removed and the microcapsules were rinsed 5 times with KRH before spectra were collected.

A Jasco MFT-2000 apparatus (Tokyo, Japan) was used for the microFTIR analysis. The spectra were collected at room temperature in the 4000-600 cm^{-1} wavenumber range in transmittance mode. Alginate beads with and without incubation in PF were placed on a hydraulic pressed KBr disk. The beads were analyzed on the top of a KBr disk at room temperature. Since the core of the beads were too concentrated for the mFTIR analysis in transmittance mode, the sample was analyzed by setting the microscope aperture on the marginal side of the bead. In all cases, the background was previously measured at a microscopic aperture size below the sample size.

Human Peripheral Blood Mononuclear Cell (PBMC) were isolated from peripheral blood by using Lymphoprep. The 100 capsules with or without previous PF treatment were cultured with 10 million PBMC in RPMI medium. Monensin (3 µM) was added to facilitate accumulation of TNFα and IL-6 in the Golgi complex by interrupting intracellular transport processes (Posma et al 2004). The calcium alginate-beads or microcapsules were incubated with the PBMC for 4 h at 37°C and 5% CO_2.

Intracellular cytokine production was measured as described by Veenstra et al. Cells were incubated with a saturating dilution of CD14 (PerCP/Cy5 anti human CD14 (Biolegend, San Diego, CA) for monocyt staining. The pellet was resuspended in 0,1 % saponin solution to permeabilise the PBMC for the intracellular staining with PE labeled mouse anti human TNFα and APC labeled mouse anti human Il-6 (both from Biolegend, San Diego, CA). Cells were analyzed with the Calibur (BD, USA). Ten thousand monocytes were acquired whilst gating on CD14 cells (i.e. monocytes). Using the unstimulated control sample, a linear gate was set in the histogram so that ± 99% of the unstimulated cells were negative for cytokine production. Analysis was performed using Win list 32 (Verity Software House, Inc., Topsham, ME).

RESULTS AND DISCUSSION

Both micro-FTIR spectra of alginate-beads prepared of intermediate-G alginate and high-G alginate show a broad absorption band in the 3370 cm^{-1} region, (–OH groups), and a small peak at about 2937 and 2965 cm^{-1}, (asymmetric -CH_2 stretching). It also shows two peaks at 1614 and 1419 cm^{-1}– COO^- groups), and an absorption band, similar to a fork with a shoulder and multiple peaks, between 1200 and 1000 cm^{-1} (vibration of C–O bonds).

After the beads prepared of intermediate-G alginate were incubated with PF the peak at 1317 cm^{-1} (C-H deformation vibration) shifts to 1311 cm^{-1}, and the peak at 1192 cm^{-1}(vibration of C-O bonds) shifts to 1174 cm^{-1}. This was different with beads prepared of high-G alginate. Here we observed a broadening of the shoulder in the

1200 cm^{-1} region (vibration of C–O bonds). Moreover a small peak was observed at 1261 cm^{-1} (C-H deformation vibration). Molecules in PF seems to have more influence on the vibrational modes of the C-H and COO^- groups, present in the alginate beads.

In the case of alginate-PLL capsules prepared of intermediate-G alginate the absorption band, corresponding to the NH_3^+ group of poly-L-lysine, shifted from 2020 to 2084 cm^{-1}. This shift should be explained by the interaction of NH_3^+ of poly-L-lysine with COO^- of sodium alginate. The peak related to the C=O stretching shifts to 1641 cm^{-1}. This band is reinforced and broadened by both the amidic C=O in poly-L-lysine and the carboxylic C=O in sodium alginate.

In the case of alginate-PLL capsules prepared of high-G alginate the absorption band, corresponding to the NH_3^+ group of poly-L-lysine, shifted from 2020 to 2065 cm^{-1}. The peak related to the C=O stretching shifts to 1619 cm^{-1}. Moreover, in both spectra, a slight broadening and indentation of the absorption band in the 3800 – 2800 cm^{-1} region was observed.

When alginate-PLL capsules were exposed to PF we found characteristic changes in the spectra which again was dependent on the type of alginate applied. With intermediate-G alginate PLL capsules exposed to PF we observed the shift of the band at 2138 and the peaks at 1671, 1425, 1313 and 1043 cm^{-1}. Moreover, the peak at about 1556 cm^{-1}, associated only to PF, shifts to 1587 cm^{-1} in presence of PLL. With high-G PLL capsules we found the shift of the band at 2107 and the peaks at 1658, 1178 and 804 cm^{-1}).

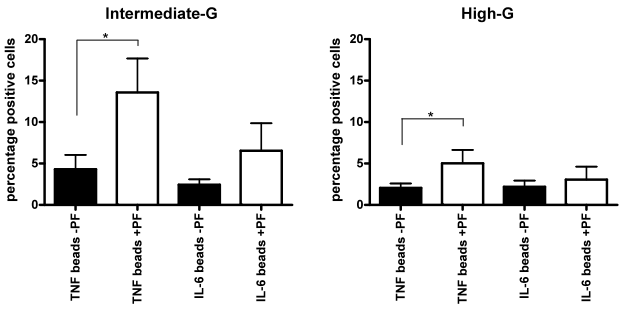

Figure 1. Number of TNFα and IL-6-positive monocytes after incubation with intermediate-G and high-G alginate beads in the presence and absence of a previous PF exposure.

Next, we determined the activation of monocytes. After exposure to intermediate-G alginate beads, monocytes showed an increase in TNF-α and IL-6 expression after PF exposure which reached statistical significant differences for TNF-α ($P < 0.05$) (figure 1). Also with high-G alginate beads, we observed an increase in TNF-α ($P < 0.05$) and IL-6 after PF exposure (figure 1). The increase was alginate dependent as exposure to PF showed less pronounced effect on high-G beads than on intermediate-G beads ($P < 0.05$, figure 1 left versus right).

Next we studied, the inflammatory response of monocytes after exposure to PF treated PLL capsules. A first observation was that PLL binding to the membrane reduced the body fluid induced activation of the monocytes for both types of alginates (see Figure 1 versus Figure 2). The PLL binding on the alginate matrix abandoned the increased inflammatory response of PBMC after exposure to PF. We found no statistical significant increases or decreases for TNF-α and IL-6 production from monocytes.

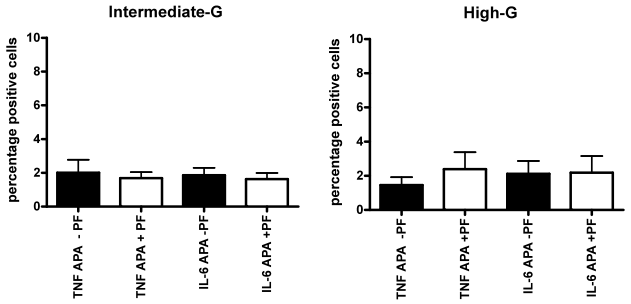

Figure 2. Number of TNFα and IL-6-positive monocytes after incubation with intermediate-G and high-G alginate PLL capsules in the presence and absence of a previous PF exposure.

CONCLUSIONS

A striking observation is that beads and capsules showed a unique change in intramolecular and intermolecular interactions at the surface of beads and capsules after exposure to human PF. This suggests that adsorption of PF always occurs.

This adsorption influences the responses of human monocytes. Monocytes did not respond with elevated TNFα and IL-6 production when exposed to untreated capsules. A minority of less than 4% of the cells (equal to medium control) expressed TNFα and IL-6. This changed when the alginate-beads were exposed to PF. Especially the number of TNFα expressing cells increased significantly. With both intermediate-G and high-G beads we found a two- to threefold increase in TNFα expressing cells in the monocytes. The PLL binding on the alginate matrix abandoned the increased inflammatory response of monocytes after exposure to PF.

REFERENCES

- Posma et al. (2004) *The Effect of Testosterone on Cytokine Production in the Specific and Non-specific Immune Response.* Am J reprod. Immunol. vol. 52 237-243.
- Veenstra van Nieuwenhoven et al.(2003) *Endotoxin-induced cytokine production of monocytes of third-trimester pregnant women compared with women in the follicular phase of the menstrual cycle.* Am J Obstet Gynecol. 188(4):1073-7).
- De Vos P et al. (1997) *Improved biocompatibility but limited graft survival after purification of alginate for microencapsulation of pancreatic islets*. Diabetologia 40 (3) 262-270.