O7-1 **Characterization of surface-modified alginate-based microcapsules**

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INTRODUCTION AND OBJECTIVES

Diabetes represents a major public health problem in industrialized countries (Kleinman J.C. 1988). Transplantation of microencapsulated pancreatic islets is a promising approach for the cure of this disease. Long term survival of microencapsulated grafts has been demonstrated, but the reproducibility of the procedures is low due to the complexity of the technology. In many cases researchers end up with bioincompatible capsules with graft failure as a consequence. In order to overcome these issues we propose to design less complex and reproducible procedures with a high degree of biocompatibility. We propose to accomplish these goals by applying stable polymer brushes on the capsule surface. Obviously the creation of such a brush is complex but its final applicability will be simpler than the present procedures.

The polymer brushes consist of two blocks: one block (polylysine HCl-salt) forms the polyelectrolyte complex with alginate, the other block is neutral, incompatible with alginate and forms the polymer brush. The length of both blocks must be optimized in order to maximize repulsion of the proteins and minimize adhesion of inflammatory cells on one side and achieve optimal permeability of capsules on the other side.

MATERIALS AND METHODS

Intermediate-G (Keltone LV) sodium alginate was obtained from ISP Alginates Ltd UK. It was purified and dissolved at 4^0C in KRH (3%) (De Vos. P. 1997). Capsules were produced by collecting Na-alginate droplets in a Ca-rich solution. Rigid Ca-beads were then collected in the polymer solution (block copolymers PEG_x-PLL_y, x=22, 113 and 454; y=10, 50 , 100 and 200). Influence of different accumulation time in the polymer solution was observed. All solutions used in this procedure were made in D_20.

Surface characterization of the capsules was done with horizontal FT-IR-ATR (hATR) and by optical microscopy using fluorescent labelling of PEG.

RESULTS AND DISCUSSION

The capsules, with a diameter of 600-700 µm, were successfully prepared. Figure 1 shows all possible positions of the block copolymer at the capsules' surface. The presence of the block copolymer at the surface of the capsules was demonstrated with hATR and fluorescence microscopy. In order to prevent large spectral interferences with water absorptions, FT-IR spectra were recorded in D_2O.

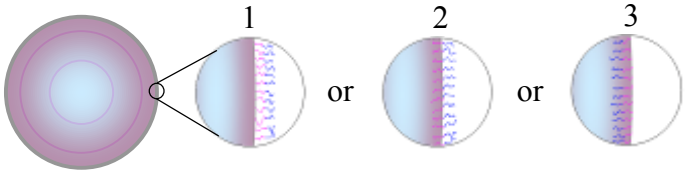


Figure 1: Possible positions of the block copolymer at the surface of the capsules

Horizontal ATR

hATR measurements of the capsules with different PEG block and higher PLL blocks (y=50, 100, 200) showed the presence of polylysine but visible signs of PEG were not identified.

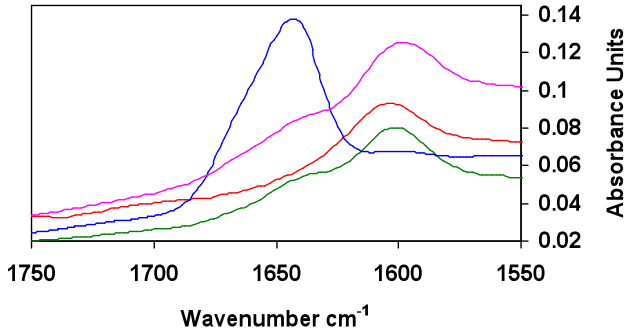


Figure 2: hATR spectra of alginate beads (red), PLL solution (blue), PLL capsules (pink) and capsules with PEG_{113}-PLL_{100} (green)

Figure 2 shows the spectra of alginate beads, a solution of PLL, PLL capsules and of the block copolymer absorbed by the beads. The most indicative spectral area is between 1750 and 1550 cm^{-1}, where the carbonyl absorptions of the COO^- of alginate and the Amide I of PLL show up. The shoulder in the pink and green spectra clearly indicates the presence of PLL (PLL capsules) and PLL block (capsules with PEG_{113}-PLL_{100}) in the sampling depth of the hATR technique (~ 1 µ). On the other hand hATR spectra of the capsules with high molecular weight PEG blocks and short PLL blocks did not show significant difference in comparison to the spectra of the alginate beads (Figure 3). This similarity between capsules' and beads' spectra can be due to the presence of PEG on the surface which is impossible to identify by this method and very small PLL block. Therefore possible surface structures of our capsules are 2 and 3 (Figure 1).

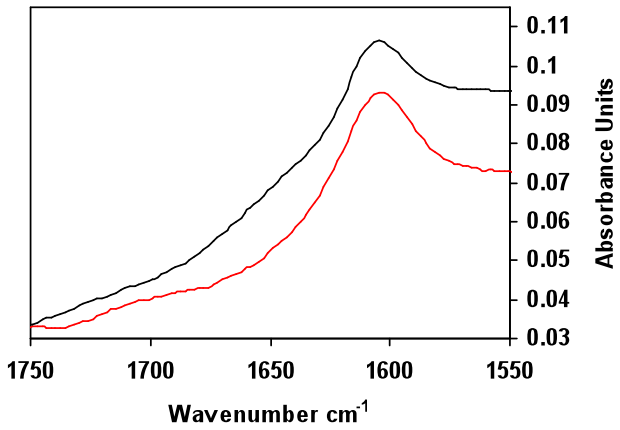


Figure 3: hATR spectra of the alginate beads (red) and the capsules with PEG_{454}-PLL_{10}(blue)

Fluorescent labelling of PEG

Fluorescent labelling of the end-methoxy groups of PEG blocks was used as a method to demonstrate the location of the PEG blocks on the capsules' surface. PLL capsules were used as a negative control.

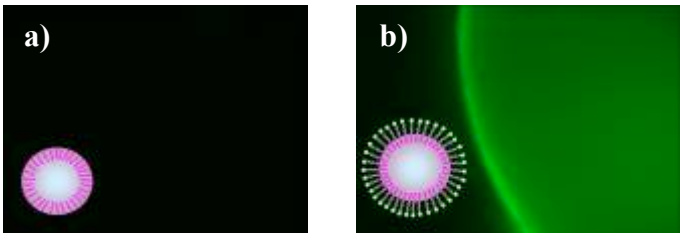
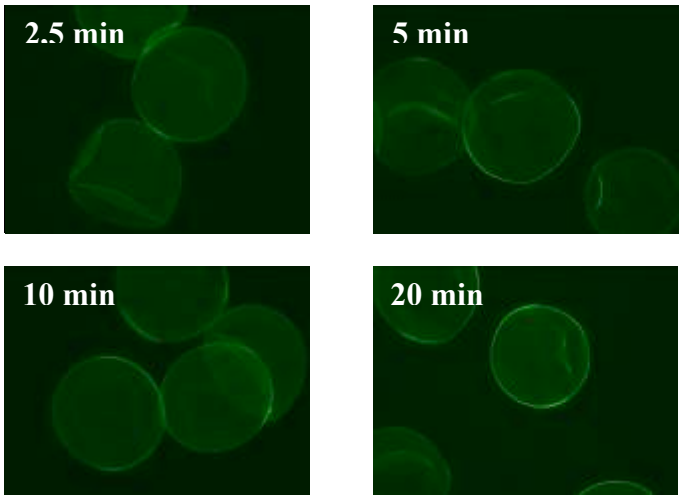


Figure 4: Fluorescent labeling: a) PLL capsules-negative control; b) PEG_{113}-PLL_{200} capsules

Figure 4 shows clearly the presence of PEG blocks on the surface of the capsules, therefore the most likely surface structure of capsules is illustrated on the picture 2, Figure 1. Furthermore we measured qualitatively the influence of the absorption time in polymer solution on the surface coverage with the block copolymer (Figure 5).



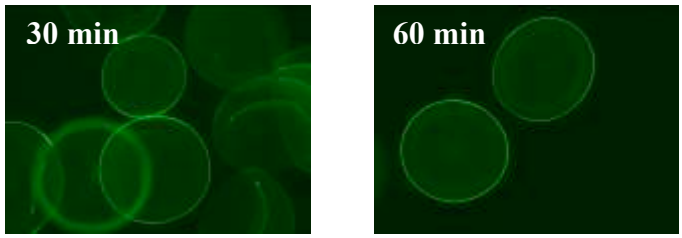


Figure 5: Influence of different absorption time on the surface coverage with PEG_{113}-PLL_{200}

With increasing the absorption time the amount of polymer on the capsule's surface is increasing too. This trend was noticed in the first 20 to 30 min depending on the block sizes of the block copolymers. After this time saturation of the surface was achieved. Currently running experiments will show us if this time is enough for block copolymers to stretch away from the surface and to form a polymer brush.

During the staining procedure the capsules with PEG_{454}-PLL_{10} first got swollen and then they got dissolved which indicates that the PLL_{10} block is too short in comparison to PEG block to obtain stable capsules. These results are in agreement with hATR results (where PLL band was hardly visible in the spectra, Figure 3, black). The other samples with the same PLL block and lower PEG block (x=22 or 113) were homogeneous microcapsules which indicates that both blocks penetrated into capsules (Illustrated on Figure 1, picture 3).

CONCLUSIONS

A qualitative surface characterization of the PEG-PLL capsules was successfully done by hATR and fluorescent labelling of the PEG blocks. hATR results confirmed the presence of PLL blocks. Since PEG does not have any pronounced separate bands in IR spectra, it is not possible to identify the position of this block with this method. Fluorescent labelling of methoxy-end group of PEG block showed that PEG block stays at the surface of the capsules and it does not penetrate into the bead completely. In the case of a too short PLL block all block copolymers penetrate into alginate or they attach to the surface and form unstable capsules (PEG is too big). In most cases an absorption time of 20-30 minutes seems to be enough for full coverage of the surface of the beads.

REFERENCES

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- 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