**P-004** **Polymer brushes for enhanced biocompatibility of immunoisolating microcapsules**

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## INTRODUCTION AND OBJECTIVES

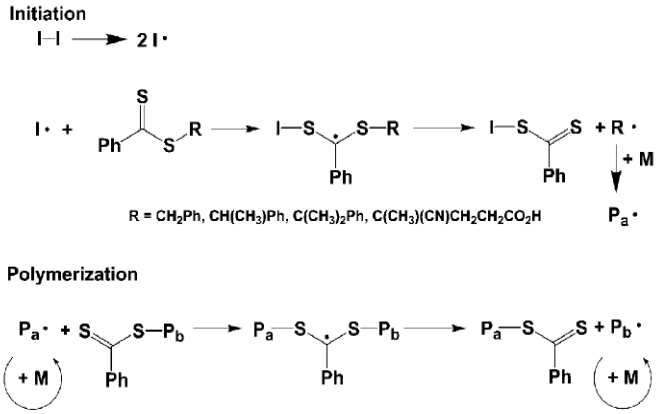
Diabetes represents a major public health problem in industrialized countries (Kleinman J.C. 1988). Transplantation of microencapsulated pancreatic islets is a promising approach for the cure of this disease. Long term survival of microencapsulated grafts has been demonstrated, but the reproducibility 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) forms the polyelectrolyte complex with alginate, another 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.

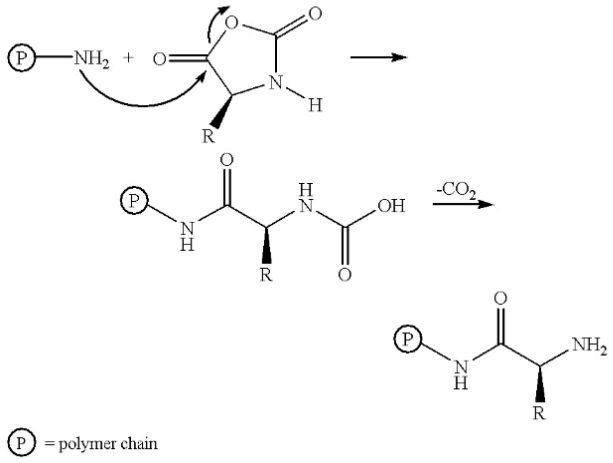
## MATERIALS AND METHODS

In our research we use two different block copolymers. The first monomer (M) (Aldrich), was distilled under vacuum before use. The initiator, α, α'-Azobis-isobutyronitrile (AIBN) (Aldrich, 99% purity) was recrystallized from methanol and dried under vacuum. Solvent 1,4-dioxane was dried by distilling over $CaH_2$ prior to use. The homopolymer α-methoxy-ω-aminopoly (ethy1ene glycol) (PEG) with different chain lengths is commercially available (Iris Biotech GmbH). In order to get rid of the small amount of water prior to use, PEG was purified by azeotropic disti-llation with toluene. Synthesis of N-carboxyanhydride of 6-(benzyloxycarbonyl)-L-lysine (Lys(Cbz)-NCA) was carried out by the Fuchs-Farthing method using triphosgene (Harada A. 1994). Tetrahydrofuran (THF), n-hexane, N,N-dimethyl formamide (DMF), and chloroform were doubly distilled by general methods (Harada A. 1994). Trifluoroacetic acid, anisole, methanesulfonic acid and triethylamine were used without purification.

In order to control the size of the first block, homopolymer was synthesized by controlled radical polymerization ( RAFT ) (Davis K. A. 2002):




The block copolymer was obtained by ROP of Cbz-lysine N-carboxyanhydride:




Block copolymer PEG-b-Plys was obtained by ROP of N-carboxyanhydride lysine in a presence of α –me-thoxy-ω-aminopoly(ethy1ene glycol) as an initiator.

The 6-(benzyloxycarbonyl) group from the polylysine block was removed according to literature procedures (Harada A. 1994).

Biocompatibility tests are currently in progress and we expect to have the first results soon.

## RESULTS AND DISCUSSION

The polymerization of the first monomer was carried out at $80^0$C under conditions mentioned in Table 1. In the same table the molecular weights and polydispersity index of the synthesized polymers are presented:

**Table 1: Experimental conditions for the homopolymerization of NVP in 1,4-dioxane solution in the presence of DPCM at $80^0$C, $M_w$, $M_n$ and PDI of synthesized PVP determined by GPC, [AIBN]/[DPCM]= 0,125**

| Sample | [M]/[RAFT agent] Molar ratio | $M_n$ g/mol | $M_w$ g/mol | PDI |
|---|---|---|---|---|
| M-1 | 50 | 2 500 | 2 600 | 1.04 |
| M-2 | 125 | 2 500 | 2 800 | 1.12 |
| M-3 | 200 | 4 400 | 5 400 | 1.23 |
| M-4 | 400 | 3 200 | 3 800 | 1.19 |

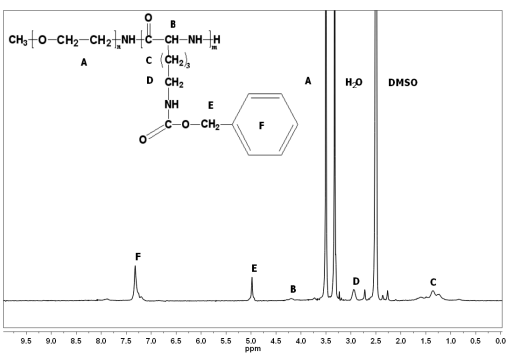
Both homopolymers with $NH_2$ end groups were used as initiators for ROP of Lysine-NCA. Molecular weights and polydispersity of the block-copolymers, determined by GPC, are presented in Table 2.

**Table 2: $M_w$, $M_n$ and PDI of synthesized block copolymers**

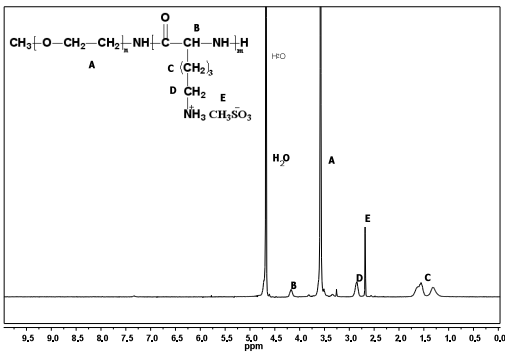
| Sample | $M_n$ g/mol | $M_w$ g/mol | PDI |
|---|---|---|---|
| (M-b-Plys) | 7 300 | 11 500 | 1.57 |
| (PEG-b-Plys) | 9 700 | 11 300 | 1.16 |

Deprotection of the polylysine block of the synthesized copolymers was performed in the presence of trifluoroacetic acid, anisole and methanesulfonic acid. The counterion of deprotected PEG-b-Plys was then remo-ved by using an anion exchange column (Amberlite IR-402), followed by freeze-drying (Harada A. 1996). The completion of the deprotection reaction was confirmed by $^1$H-NMR.

**A)**




**B)**




**C)**

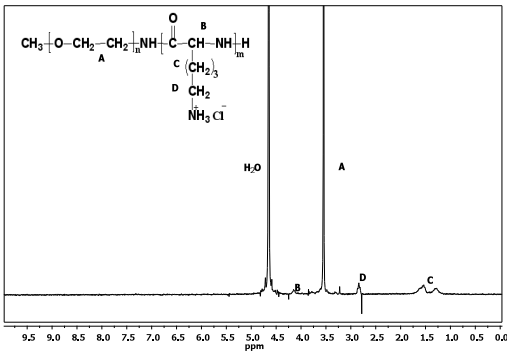



**Figure 1: $^1$H-NMR spectrum of protected PEG-b-Plys (A, $CDCl_3$), deprotected PEG-b-Plys (B, $D_2O$) and deprotected PEG-b-Plys after anion exchange (C, $D_2O$)**

## CONCLUSIONS

Polylysine block-copolymers were successfully synthesized via controlled polymerization. To further characterize these block-co-polymers, biocompatibility studies have been started and results should be forthcoming soon.

## REFERENCES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