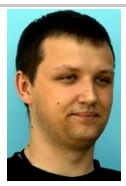
P-075	**Functional nanocapsules for targeted drug delivery**

Szczepanowicz K[1#], Łukasiewicz S[2#], Dziedzicka-Wasylewska M[2], Warszynski P[1*]
[1]Jerzy Haber Inst Catalysis & Surf Chem PAS, Krakow, Poland, [2]Phys Biochem, Fac Biochem, Biophys & Biotechnol, Jagiellonian Univ Krakow, Poland # ncszczep@cyf-kr.edu.pl



INTRODUCTION AND OBJECTIVES

One of the first concept of drug targeting was suggested by Paul Erlich almost a century ago. The hypothetical 'magic bullet' consisting of two principal components was proposed. The first component should recognize the target and bind to it, the second should perform therapeutic action. Recognition can proceed on different levels: a whole organ, specific cells characteristic for the target organ, or on the level of individual components characteristic for the target (for example, cell surface antigens or thrombus ingredients). The most universal form of target recognition is based on the fact that in every single organ or tissue certain compounds (antigens) can be found, that are specific only for the organ of interest. Specific recognizing component, usually antibody (against the target antigen), can be used as a part of the transporting unit, which is capable of the specific interaction with the target antigen. Therapeutic unit (drug, drug carriers like capsules, etc..) can be attached directly to a targeting part. Carriers can be loaded with drugs and then conjugated additionally with the targeting unit (e.g.. antibody). After four decades of research, there have not yet been produced an effective, generally applicable, site-specific drug-delivery system. Nanocapsules are promising candidates for using as therapeutic part of 'magic bullet' and with specially functionalized surface by pegylation and/or by immobilization of antibodies against receptors located in targeted cells allowing drug targeting into pathological area. Therefore, present study describes the development of nanocapsules based on a liquid core with polyelectrolytes shells prepared by LbL technique and their further surface modification by pegylation and immobilization of antibody. Obtained system would be useful for encapsulation of drugs for various fields of medicine.

MATERIALS & METHODS

The polyelectrolytes: poly-L-lysine hydrobromide PLL (MW 15000-30000), poly-L-glutamic acid sodium salt PGA (MW 15000-50000), as well as docusate sodium salt AOT ≥99%, sodium chloride, streptavidin, atto-streptavidin, biotinylated antibody were obtained from Sigma-Aldrich. Chloroform cz.d.a. was purchased from POCH Gliwice. The copolymers: PGA-g-PEG, PGA-g-PEG(-NH_2), PGA-g-PEG(BIOTIN), PLL-g-PEG, PLL-g-PEG(BIOTIN) were synthesized in our laboratory. The distilled water used in all experiments was obtained with the three-stage Millipore Direct-Q 3UV purification system. Capsules were prepared using a method proposed by Szczepanowicz 2010. The oil phase for capsules preparation was prepared by dissolution of AOT in chloroform (340g/dm^3). Polyelectrolytes were dissolved in NaCl solutions (0.015 M) at 2 g/dm^3. Emulsion were formed by addition of AOT/chloroform to polycation (PLL) solution during mixing with a magnetic stirrer at 300 rpm. To encapsulate model drug beta-carotene it was dissolved in chloroform (0,1mg/ml) prior to its emulsification with AOT. The multilayer shells were constructed by subsequent adsorption of polyelectrolytes from their solutions using saturation method. To create pegylated shell, PLL-terminated nanocapsules with five or seven polyelectrolytes layers were coated with PGA-*g*-PEG and their analogous with additional surface groups useful for immobilization of antibody, whereas PGA-terminated capsules with six polyelectrolytes layers were coated with similarly functionalized PLL-g-PEG. Antibody were immobilized by binding to functional groups at capsules surfaces. Figure 1 present one of the selected method of immobilization of antibody using streptavidin bridge. For preparation fluorescently labeled nanocapsules ROD-PAH was used instead one PLL layer.

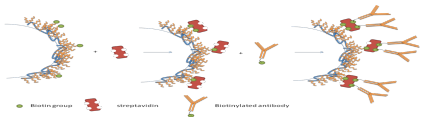


Figure 1 : Scheme of immobilization of antibody using streptavidin bridge

Size (hydrodynamic diameter) and zeta potential of capsules was determined by DLS (Dynamic Light Scattering) and by the microelectrophoretic method using Malvern Zetasizer Nano ZS apparatus. UV-VIS spectrometry was applied to confirm encapsulation of model drugs, spectra were acquired by using an Analytik Jena AG - SPECORD® 40 spectrophotometer. The cytotoxicity of the nanocapsules was checked in a proliferation assay (MTT test). Cells penetration by nanocapsules were observed by the inverted microscope with confocal system (Leica) Two days before experiment, HEK293 cells were seeded on glass coverslips in 10-mm dishes at a density of $1x10^5$ cells/dish. 15 minutes before imagine cells were incubated with FDA and with AOT$(PLL/PGA)_{3,5}$-g-PEG nanocapsules.

RESULTS & DISCUSION

For preparation of the suspension of nanocapsules, 0.1 ml AOT in chloroform solution (340 g/dm^3) was added to 200ml of aqueous PLL solution (c=0,1 g/dm^3) during continuous mixing. Chloroform was evaporated from suspensions of nanocapsules after preparation. The average drop size measured by DLS was around 70nm and zeta potential was 77 ± 8mV. The formation of the multi-

layer polyelectrolyte shells on such prepared cores were performed by subsequent adsorption of polyelectrolytes from their solutions. Typical zigzag dependence of the zeta potential of capsules on the adsorption of layers was obtained, which indicated that polyelectrolytes multilayer shells were successfully constructed. The average size of nanocapsules obtained with five PLL/PGA bilayers, as measured by DLS was 100nm. To encapsulate model drugs, beta-carotene was dissolved in chloroform prior to emulsification. Comparison of UV-Vis absorption spectra of capsules contained model drugs with the empty ones, shown in Figure 2 provided the evidence of encapsulation.

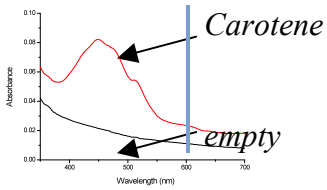


Figure 2 : UV-Vis absorption spectra of empty capsules and containing beta-carotene.

Pegylated nanocapsules were synthesized by adsorption of pegylated polyelectrolytes PGA-*g*-PEG and PLL-g-PEG at the surface of nanocapsules with seven and six polyelectrolytes layers. The measured zeta potential of pegylated nanocapsules was close to zero (-7± 4 mV and 4± 4 mV). To determine whether that strong decrease of zeta potential of capsules with pegylated shells does not induce their aggregation the stability test was performed. Freshly prepared nanocapsules were stored in 0.015M NaCl solution at room temperature for up to 90 days. We did not observe significant changes in size and zeta potentials for up to 90 days, which indicated that PEG corona at the capsule surface provides sufficient steric stabilization. Pegylated nanocapsules with special (-NH_2, -BIOTIN) surface groups useful for immobilization of antibody were synthesized using PGA-g-PEG(-NH_2), PGA-g-PEG(BIOTIN), and PLL-g-PEG(BIOTIN). The measured zeta potential of pegylated nanocapsules with introduced surface groups was close to zero, capsules were stable up to 90 days, which means that introduced groups (-NH_2, -BIOTIN) don't affect on stability of nanocapsules. To BIOTIN groups streptavidin was attached by forming biotin-streptavidin bond, which allowed specific binding of the biotinylated antibodies to the remanding binding site of streptavidin. Biotinylated antibody was succesfully immobilized, which was confirmed by fluorescence measurmants. As the alternative way for immobilization of antibodies, preparation of pegylated shell with -NH_2 surface groups by using PGA-g-PEG-NH_2 was proposed. The antibodies could be easily attached to these groups by the peptide bond. Analysis of internalization of AOT(PLL/PGA)$_{3,5}$PGA-g-PEG and AOT(PLL/PGA)$_3$PLL-g-PEG nanocapsules in HEK 293 cells indicate that tested nanocapsules can easily penetrate cells. Figure 3 presents selected image of HEK 293 cells after incubation with fluorescently labeled nanocapsules.

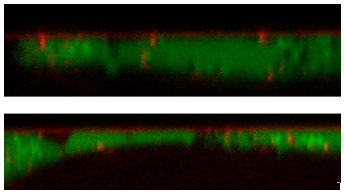


Figure 3 : Vertical scan of HEK293 cell (green) after 15min incubation with AOT(PLL/PGA)$_{3,5PGA}$-g-PEG (red).

We tested also cytotoxicity of the nanocapsules in co-culture with a human cell line HEK 293 using MTT assay. The results of this bioassay have shown that the capsules can be used in high concentrations without harming the cells.

CONCLUSIONS

The procedure of preparation of biocompatible nanocapsules was proposed. The oil cores stabilized by an AOT/PLL surface complex were encapsulated by layer-by-layer adsorption of biocompatible polyelectrolytes, PGA and PLL. The average size of the obtained capsules was 100nm. Functionalization of capsules surfaces was achieved by pegylation of external layer using PGA-g-PEG, PLL-g-PEG and by immobilization of antibodies. For binding of antibody we proposed the method of preparation of pegylated shell with biotin surface groups using PGA-g-PEG(BIOTIN) or PLL-g-PEG(BIOTIN). To these groups streptavidin was attached by forming biotin-streptavidin bond, which allowed specific binding of the biotinylated antibodies. As the alternative way for immobilization of antibodies, preparation of pegylated shell with –NH_2 surface groups by using PGA-g-PEG-NH_2 was proposed. Nanocapsules were biocompatible when tested for cytotoxicity in a cellular co-culture.

REFERENCES

- Szczepanowicz K. et al. (2010) *Formation of Biocompatible Nanocapsules with Emulsion Core and Pegylated Shell by Polyelectrolyte Multilayer Adsorption*. Langmuir, 26 (15), pp 12592–12597

ACKNOWLEDGMENTS

The work was partially financed by MNiSW 'JUVENTUS' project and N N401 009640 grant and NCN research grant