Biodegradable polyelectrolyte microcapsules loaded with various therapeutic agents for biomedical applications

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Introduction

Biodegradable polyelectrolyte microcapsules can be fabricated by a simple universal technique based on layer-by-layer (LbL) adsorption of oppositely charged macromolecules onto a surface of inorganic colloid particles (Sukhorukov, 2002; Möhwald et al., 2002). Hollow nano- or microcapsules can be easily prepared by a decomposition of an inorganic core. The capsules can be loaded with bioactive materials, such as protein, DNA, peptides, biologically active herbal extracts etc, and the microcapsule wall can provide desired release properties. The use of various biodegradable polymer materials allows a proper shell design, in order to adjust required stability, biocompatibility and affinity properties of the microcapsules. The microparticles can be easily decomposed to provide the release of the encapsulated biomaterial using ultrasound (De Geest et al. 2007), magnetic field (Lu et al., 2005) or IR-Laser (Skirtach et al., 2006). An another interesting approach to provide a sustained release of biomaterial from the microapsules is based on enzyme-mediated self-degrading microcapsules or digesting polymer shell using enzyme treatment from outside. Several examples for fabrication of polyelectrolyte microparticles loaded with various biomaterials are described in the current paper. The controlled release of proteins, DNA, herbal extracts from microcapsules induced by various enzymes is demonstrated.

Materials and Methods

Chemicals. In this research sodium alginate (Alg) (medium viscosity), poly-L-lysine (PLL) (150 000-300 000) (Fluka) and kappa-carrageenan (Car), Sigma, were used. Samples of low molecular weight oligochitosan (OliChit), 3.5 kDa, DD 89 %, were kindly provided by Prof. A. Bartkowiak (Poland). Calcium carbonate porous microparticles (3-5 μm) were obtained as described earlier (Volodkin D. et al, 2004). Poly-L-arginine (P-Arg, MW 15 000 – 70 000), poly-L-aspartic acid (P-Asp, MW 15 000), ethylenediaminetetraacetic acid (EDTA), DNA (Type IV: from herring testes), $CaCl_2$ and Na_2CO_3 were purchased from Sigma-Aldrich. Pronase® was obtained from Roche (Mannheim, Germany), α- chymotrypsin (Chym) (EC 3.4.21.1), trypsin (EC 3.4.21.4) were from Fluka. and pepsin (800 U/mg) was from Sigma. Herbal extracts of *Calendula officinalis* and *Plantago major* were kindly provided by Dr.B. Feldman (Moscow). A series of stress-protecting peptides (9-14 amino acids) were synthesized at Shemyakin-Ovchinnikov institute (Mikhailova et al, 2005).

Bioencapsulation of biomaterial in polyelectrolyte microcapsules. Two methods (co-precipitation and sorption techniques) were used to get biomaterial-loaded microparticles. DNA and Pronase co-entrapped in microcapsules, were obtained by co-precipitation technique (Method 1) as described earlier (Volodkin D. et al, 2004). The method 1 includes 1) synthesis of $CaCO_3$ microparticles with simultaneous entrapment of biomaterial; 2) formation of multilayer polyelectrolyte membrane

on microparticle surface; 3) dissolution of inorganic core to get hollow microcapsules. Briefly, 2 ml H_20, 0.615 ml 1M $CaCl_2$, 0.615 ml 1M Na_2CO_3 and 500 µl Pronase solution (1, 3, 5 mg/ml) and 500µl DNA solution were mixed and agitated on a magnetic stirrer (20 sec, RT). Then the precipitate was separated from the supernatant, and the latter was tested for its Pronase amount using Coomassie brilliant blue G 250 staining protocol (Bradford,1976). To get 5-8 layers polyelectrolyte microcapsules, alternating absorption of P-Asp and P-Arg from water solutions (2 mg/mL; 0.15 M NaCl) onto plain or loaded $CaCO_3$ particles was carried out. $CaCO_3$ cores were removed by treatment with an ice-cold EDTA solution (0.2 M; pH 7.5). The obtained microcapsules loaded with enzyme and DNA were stored at 4 °C. Another series of microcapsule samples (loaded with herbal extracts, adenovirus DNA, peptides) were also obtained by Method 2 which includes 4 steps: 1) synthesis of macroporous $CaCO_3$ microparticles, 2) sorption of biomaterial in them; 3) formation of membrane on $CaCO_3$ microparticle surface; 4) dissolution of $CaCO_3$ core (Borodina et al, 2007). Briefly, porous $CaCO_3$ particles were incubated with biomaterial (Chym, peptide, DNA, herbal extracts) solutions (Chym, 5 mg/mL, herbal extracts 20 mg/mL, 1:1 w/w, peptides 0.6-0.8 mg/mL, DNA 100-150 µg/mL), then incubated on a shaker at stirring for 2h and washed using a centrifuge (100 *g*, 5 min). The obtained microparticles with entrapped chymotrypsin or extracts were coated with (OliChit-Car) membrane (6-8 layers). Adenovirus DNA (ADV DNA) and peptides were encapsulated in Alg-PLL microcapsules. The $CaCO_3$ core was dissolved by EDTA solution as described above.

Results and Discussion

The data on encapsulation efficacy for various biomolecules which were encapsulated are summarized in Table 1. As can be seen, the efficacy depended on the method used (method 1 or method 2), as well as on the properties of biomolecules encapsulated (a charge, hydrophobic-hydrophilic properties, molecular weight etc). Thus, for peptides it did not exceed 70% while for both types of DNA is was very high (96-98 %). It is also obvious from the demonstrated results, that Method 1 was more effective than Method 2 for such low molecular weight compounds as peptides and extracts.

Encapsu-lated biomaterial	Type of membrane	Me-thod	Encapsu-lation efficacy, %
DNA+Pro-nase	PArg/ PAsp	1	98
ADV DNA	Alg/PLL	2	95
Chymo-trypsin	OliChit/ Car	1	80
Herbal extracts	OliChit/ Car	1	63
Herbal extracts	OliChit/ Car	2	25
Peptides	Alg/PLL	1	40-70
Peptides	Alg/PLL	2	20-40

Table 1. Encapsulation of various biomolecules in biodegradable microcapsules.

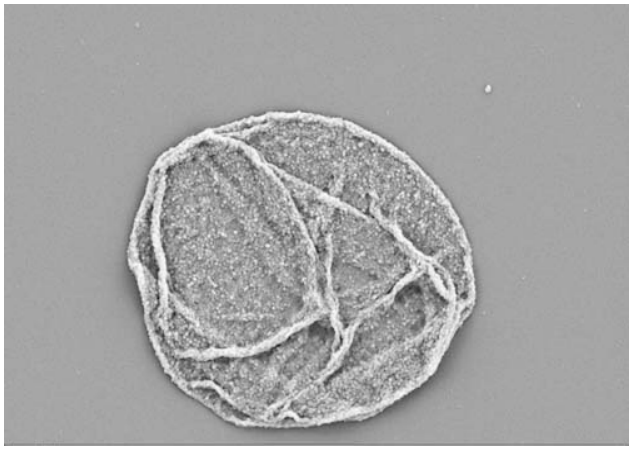


Figure 1. SEM image of $(P\text{-}Asp/P\text{-}Arg)_6$ hollow microcapsule. Bar shows 1 µm.

Since polymer membrane is based of biodegradable polymers, the release of biomolecules from microcapsules can be induced by appropriate enzyme able to digest the polymer shell. The enzyme can start working from "inside" of microcapsule (being previously co-encapsulated with biomolecule which is to be released), or from "outside", for instance after introducing microcapsules into an animal/human body. Both approaches were proposed. In the case of DNA-Pronase co-encapsulation we observed the degradation of the polymer shell as soon as microcapsules were taken from the fridge (where they were kept), and $CaCO_3$ core was dissolved at RT (Figure 2). The DNA release kinetic could be controlled by varying Pronase concentration. Thus, in the case of 0.277 pg pronase/capsule, DNA was completely released in 40 hrs, while for the microcapsules with pronase content of 0.808 pg/capsule the DNA release time was 2.5 hrs. Similar results (bigger enzyme concentration resulted in faster release) were obtained for $(OligoChit/Car)_8$ microcapsules loaded with herbal extracts which were degraded by treatment

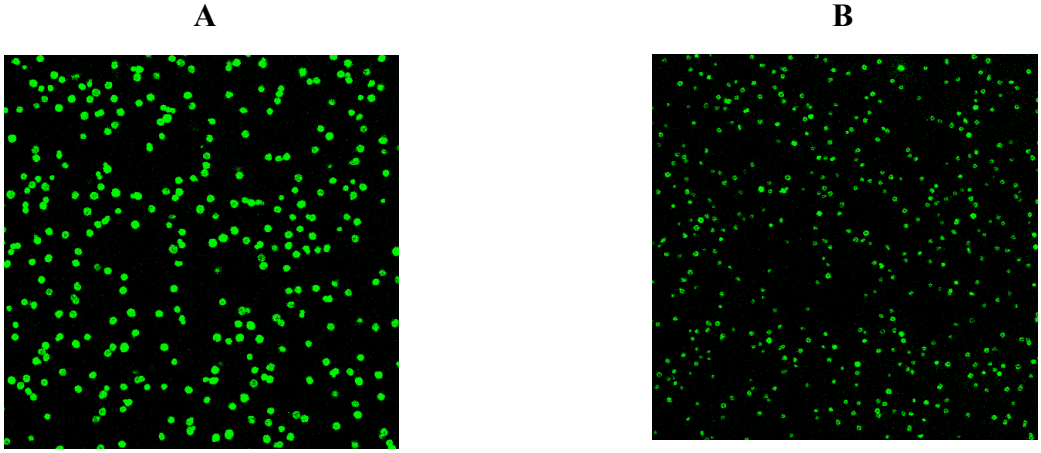
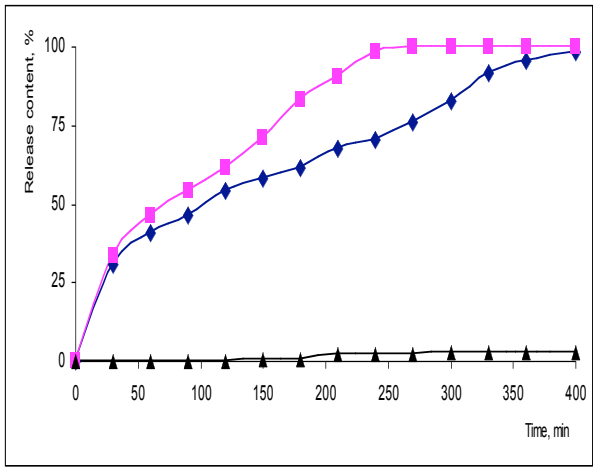


Figure 2. Confocal images of the self-degrading $(PAsp/PArg\text{-}FITC)_5$ microcapsules loaded with Pronase and DNA before (A) and after dissolution of the core (B) due to Pronase activity.



with pepsin (Figure 3). Pepsin is known to digest chitosan which was used as polycation in these microcapsules. $(Alg\text{-}PLL)_6$ microcapsules we could degrade with trypsin which digested PLL in a polymer shell. As can be seen from Fig. 3, the increase of pepsin concentration resulted in the increase of herbal extracts release. Thus, after 3 hrs of microcapsule incubation in the pepsin solution, 85 % and 55 % of the extracts (of initial concentrations in microcapsules) were found in supernatants when we used pepsin concentrations 10^{-4}M and 10^{-6} M, relatively.

Figure 3. Herbal extracts release from micro-capsules induced by the treatment with pepsin (concentrations 10^{-4} M (■) and 10^{-6} M (♦). The control microcapsules were without pepsin (▲).

The complete release of herbal extracts (100 %) was observed in 3.5 and 6 hrs, respectively.

Conclusion

Thus, we developed biodegradable polyelectrolyte microcapsules based on natural (alginate, carrageenan, chitosan etc) and synthetic (P-Arg, P-Asp, PLL) polycations and polyanions. The microcapsules can be used for encapsulation of a wide spectrum of biomolecules like DNA, proteins, peptides etc. The microcapsules membrane can be easily destroyed by using appropriate enzymes (trypsin, pepsin, pronase etc), in order to release encapsulated biomaterial. The release kinetics of the biomolecules can be controlled by varying the enzyme concentration. The enzyme could be co-encapsulated with biomolecule in one microcapsule or can occur out of microcapsule. The described microcapsules could be promising for development of novel formulations with controlled drug release.

Acknowledgments

The research was supported by Russian Foundation for Basic Research (grant 07-03-00812) and by Moscow government (Program "Development and practical use of novel methods and compounds for prophylactics, diagnostics and treatment of oncological diseases and infections"). The authors are very thankful to DAAD and FEBS for the support of PhD student Tatiana Borodina.

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