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# Chitosan Coated Plga-Microspheres - A Modular System for Targeted Drug Delivery

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**INTRODUCTION:** Microparticulate drug and antigen delivery systems are of considerable therapeutic interest. Currently, this field is dominated by the use of poly(lactide-*co*-glycolide) (PLGA) type microspheres (PLGA MS). However, because of the deficiency of suitable functional groups on their surface, conventional PLGA MS lack the possibility of surface modifications for specialized targeting or biomimetic purposes. Such modifications are thought to improve greatly the effectiveness of microparticulate delivery systems.

To solve this problem, we propose a solvent extraction process to coat conventional PLGA particles by means of a biocompatible cationic polyelectrolyte, e.g. chitosan, using a static micromixer. This one-step procedure can easily be performed aseptically. In a second step the functional groups of the hydrocolloidal chitosan shell provide the possibility to bioconjugate various bioactive ligands, e.g. sugars, antibodies or peptides, to the surface via covalent coupling. Such modular synthetic carriers may pave the way to targeted delivery of microparticulate drug delivery systems to specialised cells, e.g. phagocytic antigen-presenting cells[1].

**METHODS:** PLGA microspheres were prepared by solvent extraction using a static micromixer[2]. The polyelectrolyte shell was formed by incorporating chitosan of different molecular weights into the $W_2$-phase. The surface charge of the resulting particles was assessed by zeta potential measurements. The particle size distribution was determined by laser light scattering using a Fraunhofer diffraction model. N-hydroxysuccinimidyl-poly(ethylene glycol)-biotin (NHS-PEG-biotin) served as model ligand and was covalently linked to the chitosan's free amino groups on the particle surface under mild conditions. To assess the presence of PEG-biotin on the particles´ surface, particles were incubated with a fluorescent streptavidin conjugate and visualized by confocal laser scanning microscopy (CLSM). To account for false-positive results due to adsorption of the fluorescent marker, chitosan-free PLGA particles were treated the same way and compared to chitosan-coated particles. Particle morphology was analysed by scanning electron microscopy (SEM).

**RESULTS:** The zeta potential of the microparticles increased with higher chitosan concentrations in the external aqueous $W_2$ phase (***Fig. 1***). The mean diameter was well reproducible and adjustable within the range of approx. 1 to 10 µm.

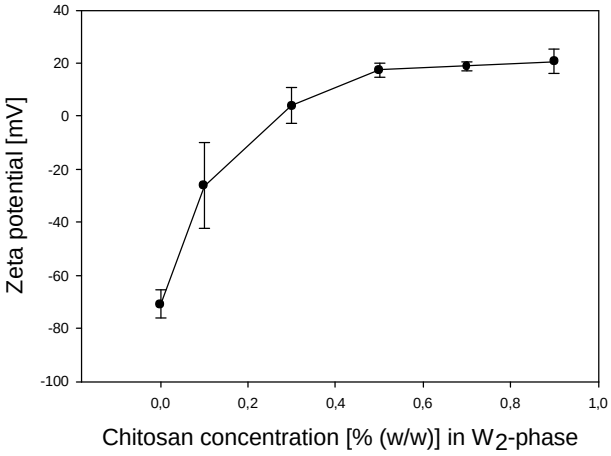


*Fig. 1: Zeta potential [mV] of chitosan-modified microspheres in 1 mM KCl as a function of chitosan concentration [% (w/w)] in the $W_2$ phase.*

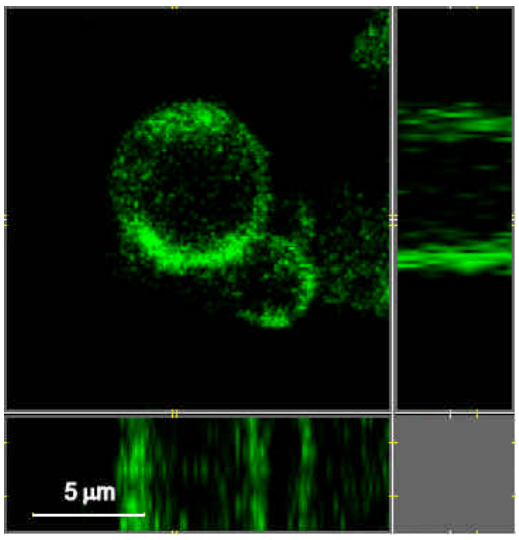


*Fig. 2: Confocal laser scanning micrograph of chitosan coated PLGA microparticles after coupling with PEG-biotin and incubation with fluorescent streptavidin*

As proof-of-concept for the accessibility of the primary amino groups of the chitosan coatings for covalent conjugation purposes, PEG-biotin was coupled to the surface. Subsequent incubation with fluorescent streptavidin and inspection of the particles by CLSM demonstrated the fluorescent character of the surface modification and the principal proof-of-concept of our approach (***Fig. 2***). The chitosan coated microparticles were spherical and showed smooth surfaces without pores as assessed by SEM (not shown).

**DISCUSSION & CONCLUSIONS:** The increase in zeta potential from –70.8 mV (chitosan-free PLGA particles) to +20.5 mV with increasing chitosan concentrations in the $W_2$-phase used for particle preparation strongly suggests that the polycationic chitosan was firmly adsorbed to the particle surface. This finding was confirmed by X-ray photoelectron spectroscopy (data not shown). The coupling of biotin via a NHS-PEG-linker showed that the amino groups of chitosan represent suitable sites for covalent bioconjugation of different ligands. The process allows the production of particles with a mean diameter between 1 and 10 μm, a useful size range for the phagocytosis by phagocytes like dendritic cells or macrophages.

Preparation of these novel modular micro-particulate delivery systems is straightforward. Particle size and surface characteristics can be easily adjusted and controlled, and drugs and antigens can be readily embedded. Thus, these novel carriers may serve as multifunctional delivery system for various applications. Its applicability as a modular delivery platform will be further investigated.

**REFERENCES:** [1] S. Faraasen, J. Vörös, G. Csúcs, M. Textor, H.P. Merkle, E. Walter (2003) *Pharm Res* **20**:237-246. [2] S. Freitas, A. Walz, H.P. Merkle and B. Gander (2003) *J Micro-encapsulation* **20**:67-85

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