

Glycopolymer-engineered biodegradable microparticles as vaccine against Leishmaniasis

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INTRODUCTION: This work aimed at engineering the surface of biodegradable PLGA microparticles with a glycopolymer for the delivery of synthetic Leishmania antigen promoting T cell mediated specific immune responses. Microparticulate carriers coated with the poly(L-lysine)-*graft*-poly(ethylene glycol) (PLL-g-PEG) brush copolymers reduce their non-specific phagocytosis while polymers functionalized with ligands allows for specific recognition.^{1,2} Herein, a mannose containing synthetic tetrasaccharide, derived from a lypophosphoglycan expressed on the cell surface of *Leishmania donovani*,³ was conjugated to the PLL-g-PEG, which was then adsorbed to poly(D,L lactic-co-glycolic acid) (PLGA) microparticles. The microparticles were also loaded with a model protein to enhance the immunogenicity and characterized with regard to their physico-chemical properties, protein encapsulation and release.

METHODS: Leishmania tetrasaccharide terminated with a mercapto propyl linker at the reducing end was synthesized as previously described³ and conjugated to the polycationic PLL-g-PEG graft copolymer in a ratio ca 20% of PEG-antigen as determined by ¹H-NMR. The PLGA microparticles optionally loaded with 5% w/w ovalbumin, were prepared by static multilamination micromixer technology.⁴ Surface coating was carried out by incubation of the particle suspensions with the polymer solutions in a thermomixer.² Protein loading and release was measured by fluorimetry. Availability of the sugar epitope for biorecognition of a mannose binding lectin Concanavalin A (ConA) was quantified by optical waveguide lightmode spectroscopy (OWLS) on flat surfaces and by FACS analysis on particles using ConA-Alexa Fluor® 488 dye.

RESULTS: OWLS analysis showed specific recognition of the lectin ConA to the glycopolymer while at the same time the surface resisted non-specific adsorption of proteins from human serum. The PLGA microparticles were produced in a size range of 1 – 10 µm. Surface modification was confirmed by the change of the zeta potential values from initial negative charge to neutral or slightly

positive values after incubation with the copolymer. Availability of the antigen on the particles surface was confirmed by FACS analysis showing ConA binding (Figure 1). While PLL-g-PEG coated PLGA microparticles were protein resistant, ConA adsorbed on their surface in the presence of the sugar. Ovalbumin was incorporated within the formulation with an efficiency of 30%. Extent of lectin binding varied with the presence of the protein in the particles (both uncoated and carbohydrate tagged particles). Nonspecific binding might be due to a burst release of the protein during the assay.

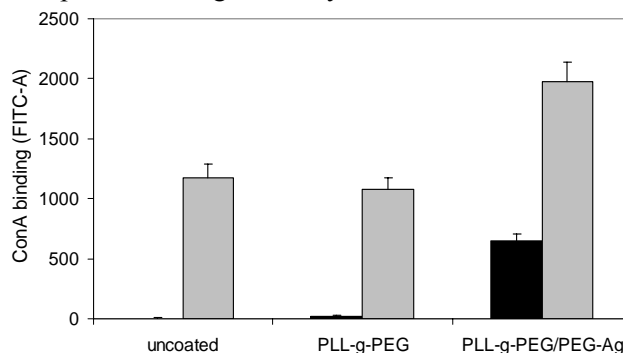


Figure 1. Effect of protein encapsulation and particle coating on ConA binding. (■) PLGA microparticles without protein, (▒) ovalbumin-loaded PLGA microparticles (standard deviation below detection limit for PLL-g-PEG coated particles)

Nevertheless, ConA adsorption was increased on polymer tagged with the antigen indicating specific binding also on the microparticles.

DISCUSSION & CONCLUSIONS : The assembly of a carbohydrate antigen onto PLGA microparticles is feasible through its chemical conjugation to a PLL-g-PEG brush copolymer. These surface modified microparticles could be potentially used as antigen carriers to the immune system.

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