Title	**Cationic microparticle carrier system as adjuvant approach for pandemic influenza vaccines; proof-of-principle with adsorbed whole inactivated H5N1 influenza.**
Keywords (up to 5)	PLGA, Adjuvant, Influenza, Pandemic, WIV
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Abstract	For the development of pandemic vaccines, adjuvants have been identified as essential for their immune potentiating effect and as a dose-sparing approach. In this work, cationic poly lactic-co-glycolic acid microparticles (PMPs) are evaluated as an antigen carrier system for whole inactivated influenza (wH5N1)[1]. Negatively charged wH5N1 is adsorbed onto cationic PMPs by electrostatic interaction. Two reagents have been evaluated, branched polyethylenimine (PEI)[2,3] and DEAE-dextran (DEAE)[4]. Depending on the nature of the cationic reagent, it can be added to the 'oil' phase (O) or the outer aqueous phase (W2) in a 'double-emulsion solvent evaporation' particle manufacturing method. Size and distribution was characterized using laser diffraction and particle morphology observed by SEM. To identify the adsorbed virus a fluorescent lipid probe Octadecyl Rhodamine B Chloride (R18) was used. The immunogenicity of the PMPs with adsorbed wH5N1 was assessed *in vivo* by serum hemagglutination inhibition (HAI). Cationic PMPs with PEI have a size of 21 µm, span 1.2 and PMP-DEAE particles are 25 µm, span 1.7. Particles with PEI have a surface charge of 13-17 mV, while DEAE-dextran between 30-57 mV. *In vivo* results indicate an initial peak (HI titer 185) is attained with the PMP-DEAE formulation. A stable HI titer of 25 is measured on day 70 and 105, comparable to a single administration of wH5N1. Two manufacturing protocols for cationic PMPs within a particle range of 20-30 µm have been established. The role of cationic PMPs as antigen carrier is confirmed by confocal imaging. The potent and immediate response observed with wH5N1 adsorbed onto PMP-DEAE suggests possible interactions with PMP surfaces have not affected its antigenicity. Such a potent response is desired in a pandemic situation, indicating the formulation has potential to elicit an adjuvant effect. The current proof-of-concept can be further elaborated with the aim to obtain broad cross-protective immunity.
References	1 Pavot, V. *et al.* Poly(lactic acid) and poly(lactic-co-glycolic acid) particles as versatile carrier platforms for vaccine delivery. *Nanomedicine (Lond)* **9**, 2703-2718, doi:10.2217/nnm.14.156 (2014). 2 Oster, C. G. *et al.* Cationic microparticles consisting of poly(lactide-co-glycolide) and polyethylenimine as carriers systems for parental DNA vaccination. *J Control Release* **104**, 359-377, doi:10.1016/j.jconrel.2005.02.004 (2005). 3 Negash, T., Liman, M. & Rautenschlein, S. Mucosal application of cationic poly(D,L-lactide-co-glycolide) microparticles as carriers of DNA vaccine and adjuvants to protect chickens against infectious bursal disease. *Vaccine* **31**, 3656-3662, doi:10.1016/j.vaccine.2013.06.011 (2013). 4 Wischke, C., Borchert, H. H., Zimmermann, J., Siebenbrodt, I. & Lorenzen, D. R. Stable cationic microparticles for enhanced model antigen delivery to dendritic cells. *J Control Release* **114**, 359-368, doi:10.1016/j.jconrel.2006.06.020 (2006).