Polyelectrolyte microparticles as biomimetic mucosal delivery vehicles facilitate antigen presentation by dendritic cells

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Vaccination is regarded as the most efficient and cost-effective way to prevent infectious diseases. Vaccine design nowadays focuses on the implementation of safer recombinant subunit vaccines. However, these recombinant subunit antigens are often poor immunogens and several strategies are currently under investigation to enhance their immunogenicity. The encapsulation of the antigens in biodegradable microparticulate delivery systems seems a promising strategy to boost their immunogenicity. Here, we evaluate the capacity of polyelectrolyte microparticles (PECMs), fabricated by single step spray-drying, to deliver antigens to porcine dendritic cells (DCs) and how these particles affect their functional maturation. As clinically relevant model antigen F4 fimbriae, a bacterial adhesin purified from a porcine-specific enterotoxigenic *E. coli* (ETEC) strain, was chosen. PECMs were loaded with the F4 fimbriae by co-spray-drying these antigens with the polyelectrolytes dextran-sulphate and poly-L-arginine and the sacrificial template mannitol. SIRPα$^+$ monocytes were enriched from peripheral blood by immunomagnetic selection and cultured with IL-4 and GM-CSF to generate porcine monocyte-derived DCs (MoDCs). These *in vitro* generated immature MoDCs were incubated with F4 fimbriae or F4-fimbriae-loaded polyelectrolyte microparticles (F4-PECMs) and their maturation was assessed by confocal and live cell imaging, flow cytometry, T-cell proliferation assays and cytokine ELISAs. The F4-PECMs were efficiently internalised by porcine MoDCs and enhanced the expression of DC activation markers. This phenotypical maturation correlated with an increased secretion of the pro-inflammatory cytokines IL-6 and IL-1β. More importantly, F4-PECMs elevated the T-cell stimulatory ability and antigen presentation capacity to CD6$^+$ T-cells of MoDCs. Moreover, PECMs efficiently promoted the CD8$^+$ T-cell stimulatory activity of dendritic cells, indicating an enhanced ability of the DCs to cross present the encapsulated antigens via MHCI. In conclusion, single step spray-dried antigen-loaded polyelectrolyte microparticles efficiently enhanced the functional maturation and increased the cross-presentation ability of porcine DCs. Our results confirm recent data obtained in rodent models that these PECMs boost the immunogenicity of vaccine antigens. In addition, this antigen delivery system allows not only the co-encapsulation of immune potentiators, such as PRR ligands, but also the functionalisation of the microparticles' surface with targeting ligands to enhance the delivery of these carrier systems to the immune system. Our results could accelerate the development of veterinary and human subunit vaccines based on polyelectrolyte microparticulate delivery systems to combat a variety of extra- and intracellular pathogens.