

## Yeast particles targeted to APN as antigen delivery system to porcine immune cells

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### Abstract

Neonatal and newly weaned piglets are highly susceptible to enterotoxigenic *E. coli* (ETEC) infections, which cause huge economical losses in the pig industry. Oral vaccination seems to be the most efficient and economic strategy to prevent intestinal diseases. Current oral vaccines include live attenuated or inactivated organisms have been widely used, however, they are often inadequate or have known safety issues. The implementation of safer recombinant subunit vaccines is currently under investigation, however, the development of effective vaccine subunits encounters multiple challenges, such as instability and limited immunogenicity. The encapsulation of antigens in microparticles is a promising approach to overcome these problems, as they can protect the antigens against degradation as well as carry potent adjuvants or immune modulators to enhance the immunogenicity. Yeast particles (YP) are innovative hollow and porous microparticles consisting of a  $\beta$ -glucan cell wall. B-glucans are key constituents of the cell walls of fungi and major 'microbe-associated molecular pattern' (MAMPs). These MAMPs are very interesting in the development of a vaccine for their adjuvant effect on the immune system, resulting in the activation of dendritic cells (DCs), the secretion of specific cytokines/chemokines and oxidative burst generation. In addition, these microparticles can be engineered to target receptors located on enterocytes to enhance the translocation of the particles through the epithelial barrier. We aimed to evaluate the capacity of these yeast particles as oral antigen delivery system. First, we loaded the hollow yeast particles with BSA-FITC as antigen model and investigated the adjuvanticity of these particles. We demonstrated that these particles are able to stimulate the antimicrobial response of neutrophils. Next, we targeted these particles to aminopeptidase N (APN), which is a very interesting target by its location on intestinal epithelial cells as well as on immune cells. The effect of these APN-targeted particles on porcine cells was analysed. Targeting to APN resulted in an enhanced endocytosis by intestinal epithelial cells and by monocyte-derived dendritic cells (MoDCs). Moreover, these APN-targeted particles were able to increase the antimicrobial activity response in porcine neutrophils. These findings suggests that targeting of yeast particles to APN is an efficient way to deliver antigens to immune cells located in the intestinal mucosa.