Preparation of size-controlled amphiphilic poly(amino acid) nanoparticles for vaccine delivery and adjuvant

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Statement of Purpose: Polymeric nanoparticles (NPs) with entrapped antigens represent an exciting approach to control the release of vaccine antigens and to optimize the desired immune response *via* selective targeting of the antigen to antigen-presenting cells (APCs)[1)]. Dendritic cells (DCs) are highly specialized APCs that can activate naive T cells and hence initiate primary immune responses. Therefore, the delivery of antigens to DCs and the DC activation are one of the most important issues in the development of effective vaccines[2)]. To design optimal drug carriers, polymeric NPs have the advantage of being able to regulate their physicochemical properties, such as particle size, shape, surface charge and polymer composition. In particular, a method for regulating the size of polymeric NPs is essential for effective vaccine delivery, and to elicit a specific immune response. In this study, we prepared size-controlled NPs composed of hydrophobically-modified poly(γ-glutamic acid) (γ-PGA)[3)]. To evaluate their potential applications as vaccine carriers, the size effect on antigen delivery and immunostimulatory activity of the NPs to DCs was investigated, and the NPs were found to be capable of inducing potent immune responses.

Methods: γ-PGA (Mn=3.8×10^4, D:L ratio=6:4) was hydrophobically modified by L-phenylalanine ethylester (Phe). In this study, γ-PGA-Phe with 53 Phe per 100 glutamic acid units of γ-PGA was used (**Fig. 1**). To prepare different-sized NPs, γ-PGA-Phe copolymers were dissolved in DMSO at a concentration of 10 mg/ml, followed by the addition of 0, 0.05, 0.1 or 0.15 M NaCl solutions to the same volume as DMSO. The solution was then dialyzed against distilled water to remove the DMSO. Murine DCs were incubated with different-sized FITC-conjugated γ-PGA-Phe NPs (FITC-NPs). The uptake of NPs was measured by flow cytometry (FCM). To evaluate the size effect on DC maturation, DCs incubated with different-sized NPs (100 μg/ml) were analyzed for their expression of CD40 as maturation markers by FCM.

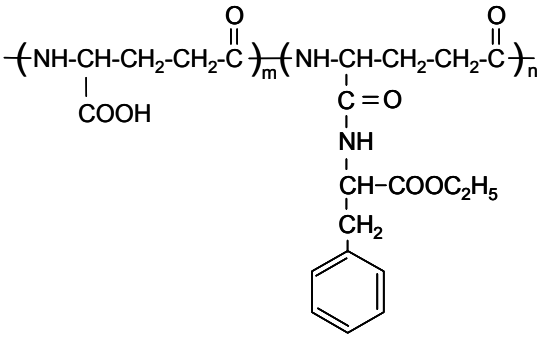
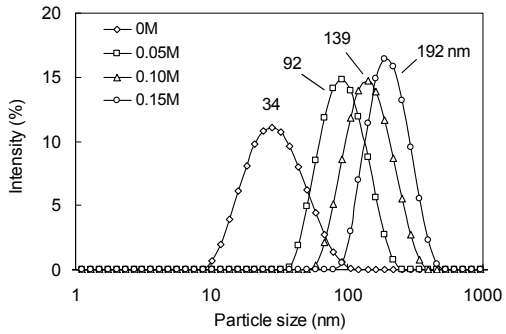


Fig. 1. Chemical structure of γ-PGA-Phe.

Results: The size of γ-PGA-Phe NPs could be easily controlled by NaCl conc. added to γ-PGA-Phe. The mean diameter of the γ-PGA-Phe NPs ranged from 34 nm to 192 nm (**Fig. 2**). The γ-PGA-Phe NPs were efficiently taken up by DCs, the amount of NP uptake increased with increasing the size of NPs. The large-sized (200 nm) NPs were more efficiently taken up into the DCs. In contrast, the size effect on DC maturation was stronger for the smaller sized γ-PGA-Phe NPs (**Fig. 3**). The number and total surface area of the small-sized NPs per unit weight were higher as compared to the large-sized NPs. These results suggest that not only the DC uptake process of NPs but also the surface interactions between NPs and DCs are important for induction of DC maturation.



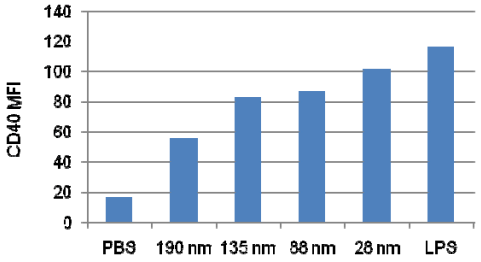


Fig. 2. Size changes of γ-PGA-Phe NPs prepared at various NaCl concentrations.

Fig. 3. Size-dependent maturation of DCs by different-sized γ-PGA-Phe NPs.

Conclusions: Size-regulated γ-PGA-Phe NPs have significant potential as an antigen carrier and adjuvant for DCs. The size-dependent DC maturation may be a result of interactions with NPs and receptors of DC surface. The result would provide guidelines for adjuvant design in the development of an effective vaccine.

References:

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