Degradation and immunological signatures of emulsified particles based on bioresorbable polymers

Chung-Hsiung Huang, Chiung-Yi Huang, Ming-Hsi Huang

National Institute of Infectious Diseases and Vaccinology, National Health Research Institutes, Miaoli 35053, Taiwan (E-mail: huangminghsi@yahoo.com.tw)

Emulsion-based adjuvants have been demonstrated to be an effective tool in increasing vaccine efficacy. The objective of this study is to provide more information on the contribution of bioresorbable polymeric emulsifiers and core oils of degradable emulsions to the modulation of immune systems (**Fig. 1**). Firstly, we investigated how the degradation properties of the polymeric emulsifier can affect the stability of the resulting emulsion[i]. Secondly, we aimed to elucidate the roles of the core oil in vaccine immunogenicity[ii]. Three amphiphilic bioresorbable copolymers derived from lactide (LA), ε-caprolatcone (CL) and poly(ethylene glycol) (PEG) were investigated for their emulsifying properties and degradation characteristics. Polymers consisted of 80 wt. % hydrophilic PEG block and 20 wt. % lipophilic PLA, PCL, PLACL block were synthesized by ring-opening polymerization of LA and/or CL in the presence of monomethoxy PEG. By possessing similar hydrophilic-lipophilic balance values (HLB of 16), these polymeric emulsifiers have an equivalent ability to stabilize squalane/water interfaces during emulsification. Degradation of polymers in aqueous solution and within emulsion was carried out in water at 37°C selected to mimic the human body conditions. Our results demonstrated that polymer matrices within the emulsion exhibited lower degradation rates than the corresponding polymers in aqueous solution. Moreover, the degradability intrinsic to each polymer is the predominant cause of destroying the emulsion. In parallel, we conducted a histological TUNEL staining study of the tissues at the injection site to investigate the adjuvanticity of emulsions *in vivo*. The results showed that subcutaneous vaccination of mice with ovalbumin (OVA) plus a PEG-*b*-PLACL-emulsified squalene-in-water emulsion recruited cell infiltration around the emulsion depot, and a numerous percentage of those cells were TUNEL-positive apoptotic cells. We further investigated the expression of MHC class II and co-stimulatory molecules on APCs harvested from the LNs of vaccinated mice. Consistently, high levels of CD40, CD86 and MHC II expression on $CD11c^+$ LN cells were observed for mice vaccinated with emulsion-adjuvanted OVA compared with OVA alone. However, the potency was rather reduced when the emulsion of the core oil replaced as squalane, the saturated form of squalene. The information gathered from this study is of great interest in pharmaceutical applications, especially for the design of sustained delivery systems.

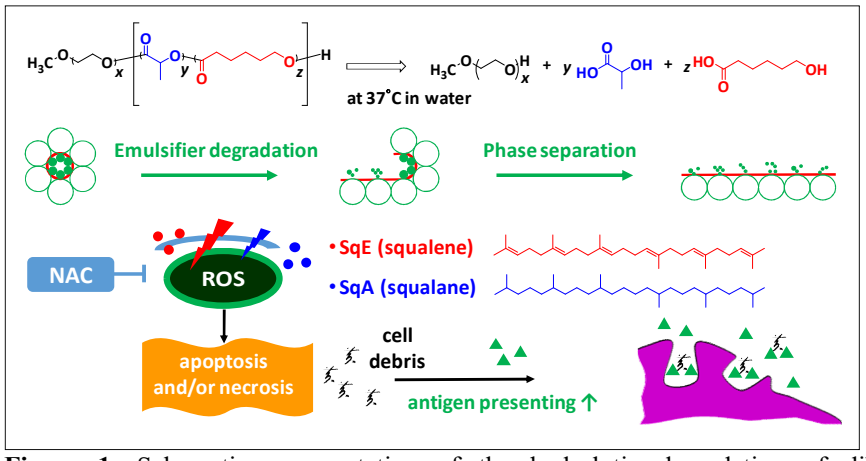

Figure 1. Schematic representation of the hydrolytic degradation of diblock bioresorbable polymers, PEG-*b*-PLA, PEG-*b*-PCL, and PEG-*b*-PLACL in aqueous solutions or within emulsions at 37°C, and how emulsion adjuvants interacting with immune cells and the roles of the core oil in antigen presenting.

[i] C-Y. Huang, M-H. Huang, *Polym. Degrad. Stab.* **2017**, *139*, 138-142.

[ii] C-H. Huang, C-Y. Huang, M-H. Huang, *Mol. Pharmaceutics.* **2018**, *15*, 420-429.