Allergen-loaded thermo-responsive liposomes for epicutaneous immunotherapy

Jiann Huey Lee, Yingzhu Zhou, Rhiannon P. Kuchel, Rona Chandrawati, N. Alice Lee

School of Chemical Engineering
The University of New South Wales
Sydney, New South Wales, Australia
jiann_huey.lee@unsw.edu.au and *alice.lee@unsw.edu.au*

The rising prevalence of food allergies has become a global concern in recent years as it can be life-threatening. Allergen-specific immunotherapy is the only treatment option showing promising outcomes of desensitisation accompanied by increased clinical thresholds. However, oral immunotherapy is not recommended for highly sensitive individuals with low thresholds due to the risk of fatal reactions. Recently, there has been a growing interest in the use of epicutaneous immunotherapy (EPIT), which involves the repeated delivery of allergens to the skin. This approach aims to modulate the immune response by engaging Langerhans Cells and stimulating the activation of regulatory T cells.[1] One challenge of EPIT is the efficacy of passing macromolecular allergens through the stratum corneum layer without provoking skin inflammation and irritation.[2]

To increase the delivery efficacy of macromolecular allergens across the stratum corneum, nanoformulation incorporated microneedle as a potential delivery vehicle was proposed (Fig. 1). Thermo-responsive liposomes were designed with lipid compositions to achieve a desirable allergen release rate. Liposomes were chosen due to their similarity of structure to skin and their known permeability through skin.

The allergen-loaded liposomes with an average diameter of 161.3 ± 1.3 nm were successfully synthesised and characterised by transmission electron microscope (TEM) (Fig. 2). Evaluation of the stability of the liposomes at 4°C using dynamic light scattering has shown that they were stable with only -0.8% growth in size over one month without compromising their structures. The encapsulation efficiency was lower at 20% for total peanut protein, and higher at 30 and 35% for the major allergens, Ara h 1 and Ara h 2, respectively. The temperature-dependent release rate was attained via tuning the liposomal formulation with different phase transition temperature, showing 50% of the encapsulated allergens was released over three days at 37˚C. The profiles of allergens released from the liposomes was confirmed immunochemically using allergen-specific antibodies and visualised by sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The nanoformulation at 100 µg/mL did not affect cell viability. This study presents a novel application of peanut allergen-loaded liposomes as a promising vehicle for skin delivery in the application of EPIT.

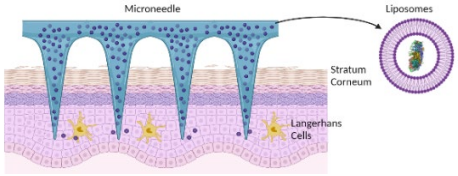

Figure 1: Schematic illustration of liposomes in microneedles. Created with BioRender.com.

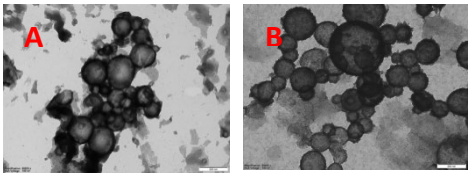

Figure 2: TEM image of DPPC/DOPE/stigmasterol with peanut protein: (A) freshly prepared and **(B)** after one month storage.

References:

[1] Seneschal J, Clark RA, Gehad A, Baecher-Allan CM, Kupper TS. *Immunity* **2012**, 36(5):873-84.
[2] S. Münch, et al. *European Journal of Pharmaceutics and Biopharmaceutics* **2017**, 119: 235-242.