Hybrid Calcium Carbonate-Peptide Nanofiber Microparticles for Mucosal Antigen Delivery

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Statement of Purpose: It is estimated that 70% of pathogens initiate infection via mucosal surfaces, with the respiratory mucosa being especially vulnerable[1]. While most currently approved mucosal vaccines, such as the intranasal FluMist, elicit neutralizing antibodies; $CD8^+$ T cell immunity may also be critical for mucosal protection[1]. Mucosal memory $CD8^+$ T cells respond rapidly to infection at the site of pathogen entry and eliminate infected cells, thereby providing the first line of defense. Mucosal immunity is best induced by inoculation via mucosal surfaces and injected vaccines are often very poor at inducing mucosal immunity and also have associated risk factors[2]. However, the development of mucosal CD8+ T cell vaccines lags far behind its systemic counterparts due to the lack of effective mucosal adjuvants. Bacterial toxins, such as cholera toxin (CT) and the heat-labile enterotoxin (LT) of E coli are the most potent and well studied mucosal adjuvants available, but are too toxic for human use[1]. We have previously reported a self-assembling peptide nanofiber platform that can elicit protective $CD8^+$ T immunity in mice when injected systemically[4]. However, the mucosa is a viscous, protease-rich, harsh environment, which poses a significant hurdle for efficient transport of nanofiber vaccines across the mucosal barrier[1]. Here we report the synthesis and characterization of hybrid microparticles of self-assembling peptide nanofibers and calcium carbonate for efficient delivery of nanofiber vaccines across mucus barriers. We hypothesize that particulate delivery of the nanofibers will prevent their adhesion and degradation at mucosal surfaces, enhance transport, facilitate uptake by dendritic cells, and elicit mucosal CD8+ T cell immunity.

Methods: Self-assembling peptide KFE8 (FKFEFKFE) was linked to the antigenic peptide MHC-class I peptide OVA (SIINFEKL) via a small amino acid linker (GGAAY) using standard Fmoc chemistry and purified by HPLC (>90%). Calcium carbonate/OVA-KFE8 microparticles were synthesized by dissolving OVA-KFE8 nanofibers in 0.33 M calcium chloride at different concentrations and adding 0.33 M sodium bicarbonate solution while stirring vigorously (Fig. 1A). The solution was allowed to settle for 30 minutes and particles were obtained by centrifugation. Encapsulation efficiency, particle yield, and size were calculated and particles were imaged using scanning electron microscopy (SEM). Mice were primed intranasally with 1.5×10^6 microparticles or an equivalent amount of OVA-KFE8 nanofibers to account for equal antigen doses, boosted with half the dose on day 52, and sacrificed on day 56. The lungs were excised, processed, and the levels of tissue OVA-specific CD8+ T cells was analyzed using flow cytometry.

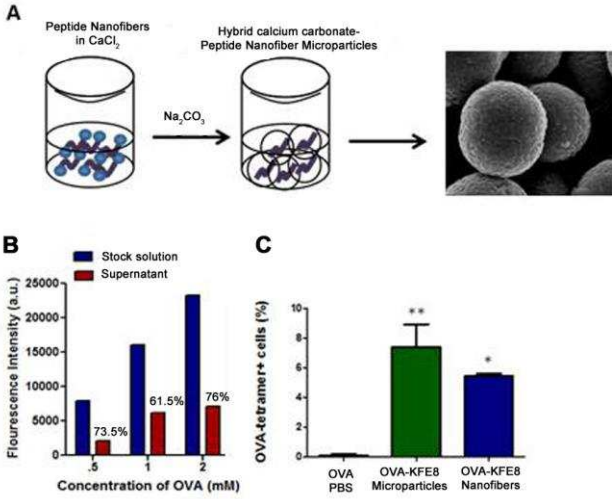

Figure 1. (A) Schematic showing synthesis of hybrid microparticles of $CaCO_3$ and OVA-KFE8 nanofibers and microparticles as visualized by SEM. (B) Encapsulation efficiency of microparticle formulation as a function of OVA-KFE8 nanofiber concentration. (C) Production of lung-resident OVA-specific CD8+ T cells in mice after intranasal vaccination with with composite microparticles or OVA-KFE8 nanofibers alone.

Results: SEM data indicated highly spherical composite microparticles (Fig 1A) and encapsulation efficiency was found to be highest (76%) at 2 mM OVA-KFE8 nanofiber concentration (Fig 1B). While encapsulation efficiency was similar at 0.5 mM and 2 mM, it was observed that the microparticles produced at 0.5 mM concentration were more cuboidal than spherical (data not shown). The average diameter of the microparticles was found to be around 2-2.5 μm (data not shown), which is ideal for phagocytosis by antigen presenting cells such as dendritic cells and macrophages. It was found that the lungs of mice vaccinated with hybrid calcium carbonate-peptide nanofiber microparticles had higher levels of OVA-specific CD8+ T cells compared to mice vaccinated with OVA-KFE8 nanofibers alone suggesting that the encapsulation process enhanced vaccine efficacy.

Conclusions: In conclusion, calcium carbonate-peptide nanofiber composite microparticles can be synthesized high encapsulation efficiency and excellent size distribution. Calcium carbonate encapsulated nanofiber vaccines elicit robust levels of lung resident mucosal CD8+ T cells, which protect against potential infections.

References: 1. Woodrow KA et al. 2012 Annu Rev Biomed Eng 14:17-46. 2. Li et al. 2013 Sci Transl Med 5:204ra130. 3. Chesson et al. (2014) Vaccine 32:1174-80.