Peptide Nanofiber-Calcium Carbonate Composite Microparticles for Mucosal Vaccine Delivery

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Statement of Purpose: The development of mucosa-targeting vaccines lags far behind its systemic counterparts partly due to lack of effective mucosal vaccine adjuvants[1]. Self-assembling peptides that form β-sheet rich nanofibers have been reported to elicit strong immune responses in mice when linked to peptide or protein antigens[2]. This adjuvanting capability was not restricted to primary sequence of the self-assembling domain, position of the epitope, linker sequence, mouse strains, or, the route of immunization[3,5]. Immunization with peptide nanofibers bearing disease-relevant epitopes has been shown to be protective in murine models of malaria and cancer[4,5]. Preliminary investigations self-assembling peptides as mucosal adjuvants indicated that the nanofibers elicited weak antibody responses and were inefficient at penetrating the mucosal barrier into the lamina propria, which is rich in mucosal antigen-presenting cells. Here we report the synthesis of and characterization of composite microparticles of self-assembling peptide nanofibers and calcium carbonate for enhanced transport across mucosal barriers. We hypothesize that particulate delivery of the nanofibers will prevent their adhesion and degradation at mucosal surfaces in the lumen, enhance transport into the lamina propria, facilitate uptake by dendritic cells, and enhance mucosal antibody responses.

Methods: Self-assembling peptide KFE8 linked to the antigenic peptide OVA was synthesized using standard Fmoc chemistry and labeled with rhodamine. OVA-KFE8 nanofibers were prepared as reported previously[3]. Peptide nanofiber-calcium carbonate composites were synthesized by adding 0.33 M sodium bicarbonate solution to OVA-KFE8 peptide nanofibers dissolved in 0.33 M calcium chloride and stirring vigorously for 30 sec (Fig. 1A). The solution was allowed to settle for 30 minutes and particles were obtained by centrifugation. Nanofiber encapsulation efficiency, particle yield, and size was calculated and particles were imaged using scanning electron microscopy (SEM). Bone marrow dendritic cells (BMDC's) were obtained from the femurs of naïve B6 mice using standard protocols. Particle uptake by BMDC's was confirmed by confocal microscopy.

Results: SEM data indicated highly spherical composite microparticles (Fig. 1B), which were dense (Fig. 1C) compared to control microparticles (Fig. 1D) suggesting encapsulation of the nanofibers within the particle core. Encapsulation efficiency was found to be highest (73%) at 2 mM peptide concentration (Fig. 1E), which also yielded microparticles with a narrow size distribution (Fig. 1F). Mean particle diameter was found to be 2.2 μm which is within the range for phagocytosis by antigen-presenting cells. In *in vitro* cultures, composite microparticles were efficiently phagocytosed by BMDC's, which exhibited a

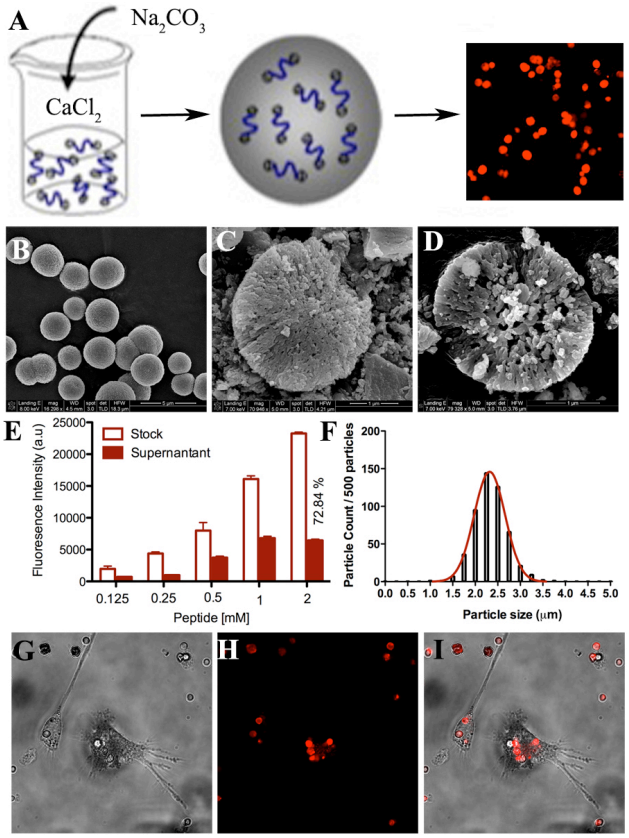

Figure 1. Schematic showing synthesis of composite microparticles (A). SEM image (B) and cross-section (C) of composite microparticles. Control particles without OVA-KFE8 have a highly porous core (D). Encapsulation efficiency as a function of nanofiber concentration (E) and size distribution of composite microparticles (F). BMDC uptake of composite microparticles (G, H, I).

mature morphology (Fig. 1G-1H) suggesting that vaccination with the composite microparticles can induce robust immune responses. Studies are underway to investigate the uptake of the composite microparticles in the mucosa and antibody responses after oral vaccination in mouse models.

Conclusions: In this study, we have synthesized composite microparticles of peptide nanofibers and calcium carbonate with high encapsulation efficiency and narrow size distribution. The composite microparticles are efficiently phagocytosed by BMDC's and could be efficient adjuvants for inducing strong mucosal immunity.

References: 1. Woodrow KA et al. (2012) *Annu Rev Biomed Eng* 14:17-46. 2. Rudra JS et al. (2010) *Proc Natl Acad Sci* 107(2):622-27. 3. Rudra JS, et al. (2012) *ACS Nano* 6(2):1557-64. 4. Rudra JS, et al. (2012) *Biomaterials* 33(27):6476-84. 5. Huang ZH, et al. (2012) *J Am Chem Soc* 134(21):8730-33.