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Supplementary Data

Peptide Nanofiber-$CaCO_3$ Composite Microparticles as Adjuvant-free Oral Vaccine Delivery Vehicles

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Materials and Methods

Confocal Microscopy. Images were collected using a Zeiss LSM-510 META confocal microscope with a 63x oil 1.4 NA objective (Optical Microscopy Core at UTMB). The images were obtained using excitation lines at 364, 488, and 543 nm and three different channels of emission with sequential acquisition. Z-stacks were taken we used step size equal to 1/2 the optical slice size to obtain better rendering. 3D rendering was done using Imaris Premier 7.5 software.

Zeta Potential. Control and composite microparticles were dissolved in PBS buffer with varying pH zeta potential was measured at 25 °C by laser doppler velocimetry using a Zetasizer 2000 (Malvern Instruments, Malvern, UK). Analyses were performed in triplicate.

Bioactivity Studies. OVA, OVA-KFE8 nanofibers were prepared as described and then treated for 24 hours in PBS (pH 7.0), 0.33 M sodium carbonate (pH 11.0), or simulated gastric fluid (pH 1.2) and coated on 96 well plates. Lymphocytes and splenocytes were freshly harvested from OT-II transgenic mice homozygous for I-Ab TCR receptor specific for OVA peptide. Cells were treated with ACK lysis buffer and then pressed through a 70 μM cell strainer after which they were washed twice in cRPMI media and seeded onto 96 well plates with the appropriate treatment. Cells were incubated for 4 hours prior with Brefaldin A at 37 °C and then stained for the intracellular cytokine TNF-alpha-FITC (Clone MP6-XT22), and surface cellular markers CD4-APC-ef780 (Clone GK1.5), CD3-PerCP-Cy5.5 (Clone 145-2C11) and viability dye ef506.

Figure S1. Confocal z-stack imaging of composite microparticles loaded with fluorescent nanofibers. Images indicate that the nanofibers are distributed throughout the particle with higher concentration at the periphery than the center. Each stack depth is 0.35 μm.

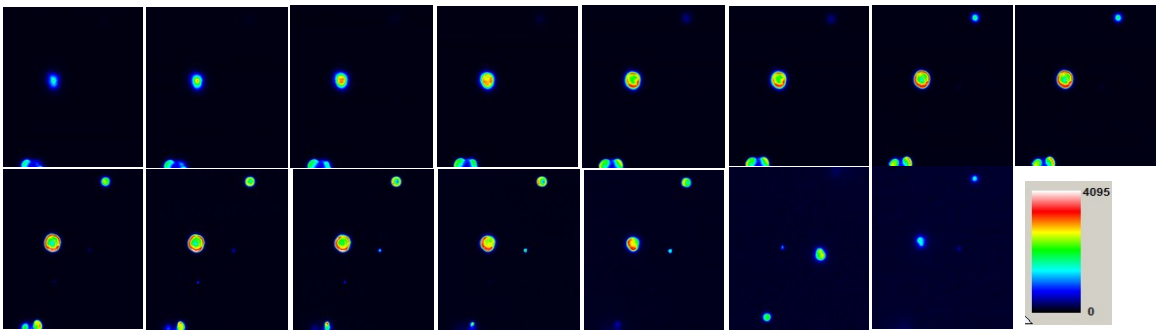

Figure S2. Zeta-potential of composite and control microparticles as a function of pH. Both control and composite microparticles were found to be positively charged at pH 1.0 and negatively charged at neutral pH. Composite microparticles had net higher charge compared to control particles suggesting less adhesion and better penetration through the mucus barrier.

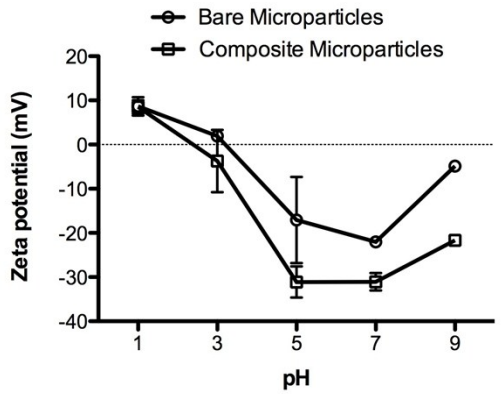

Figure S3. Bioactivity of free OVA antigen and OVA nanofibers after exposure to acidic (SGF pH 1.2) and basic pH (0.33 M Na_2CO_3). The free OVA peptide antigen retained its bioactivity after exposure to at both acidic and basic pH environments. OVA nanofibers had reduced bioactivity at basic pH but maintained their bioactivity at acidic pH.