Parallel Particle Production & Cellular Arrays For Particle-Vaccine Development And Optimization

Abhinav Acharya[1], Natalia Dolgova[2], Jamal Lewis[2], Michael Clare-Salzler[3], Chang-Qing Xia[3], Benjamin G Keselowsky[2]

1University of Florida, Material Science and Engineering, Gainesville, FL, USA ;
2University of Florida, Pruitt Family Department of Biomedical Engineering, Gainesville, FL, USA
3University of Florida, Department of Pathology, Immunology and Laboratory Medicine, College of Medicine, Gainesville, FL, USA

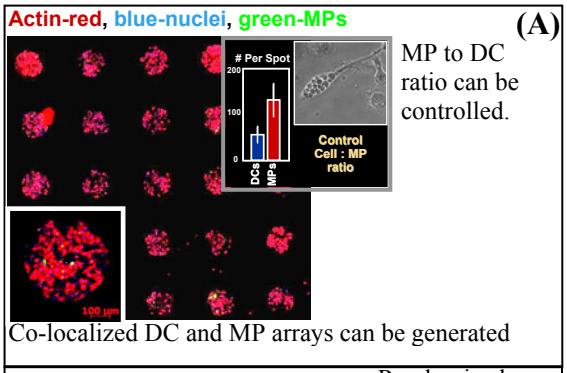
Introduction: High-throughput screening of synthetic vaccines incorporated in a targeted drug-delivery vehicle for cells modulating the immune response is attractive for drug development and discovery. Although, there are now scores of known antigenic epitopes and adjuvants present, there has not emerged an analogous systematic examination of the functional responses of immune cells toward combinatorial vaccine formulations generating synergistic immune responses in a HTP-manner. To address this concern we have developed a technique to characterize particle-based vaccines targeted toward dendritic cells in a high-throughput method.

Methods: Bone marrow-derived dendritic cells (DCs) were arrayed and cultured on poly lactide co-glycolide microparticle (PLGA-MP) vaccines encapsulated with different factors, printed on chips in a square matrix. The DCs were then immunofluorescently stained for tolerogenic or pro-inflammatory markers and quantified using image analysis. Additionally, small volume micro-batches of MPs were generated in a high-throughput manner in a 384 well polystyrene plate. Solid/Oil/Water based emulsion-diffusion process was utilized to generate these particles with polystyrene compatible oil and PVA as surfactant using programmable contact pin miniarrayer.

Results: A) DCs could be co-localized and cultured on the printed particle arrays and MP to DC ratio can be controlled. **B)** DCs were cultured on randomized arrays of poly I:C encapsulated MPs, stained and quantified for surface expression of MHC-II. It was observed that there was minimum cross-talk between the islands and the DCs expression of MHC-II follows the poly I:C gradient. **C)** MPs with different combination of different tolerogenic factors, rapamycin(RAPA), TGF-β1, Vitamin D3(VD3), IL-10, hemoglobin(Heme) were printed in an array format in MP-dilutions and in triplicate. DCs were cultured on the arrays and intracellular cytokine production of IL-10 was quantified. Interestingly, the highest MP-dose of TGF-β1 and VD3 had synergistic effects. **D)** ~1 µm MPs were generated using parallel particle production (PPP) method in a 384 well plate having smooth surface morphology as determined by SEM. **E)** 216 unique MPs were generated by encapsulating combinations of 6 dilutions of 3 fluorescent dyes. Particles generated using PPP technique, were printed on a microscope slide and imaged using Typhoon 9410 fluorescent imager.

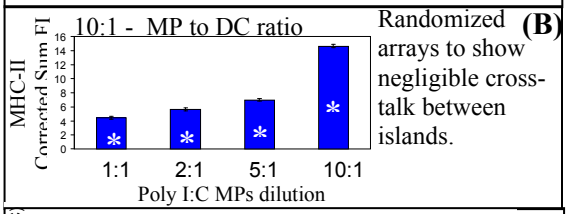
Conclusion: 1. We could successfully make co-localized MP and DC-arrays. **2.** We tested multiple combinatorial particle based vaccines targeted to DCs and observed unexpected synergistic effects between co-encapsulated/ co-delivered factors. **3.** We can generate 384 different phagocytosable particle-based vaccines encapsulating multiple combinations of therapeutic drugs.

Reference: Acharya AP et al 2008, Acharya AP et al 2009.

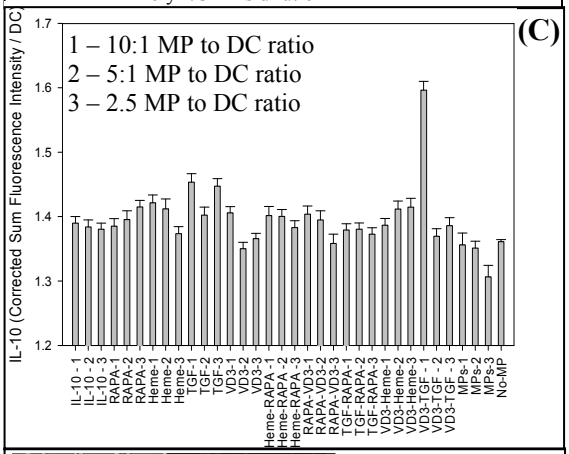


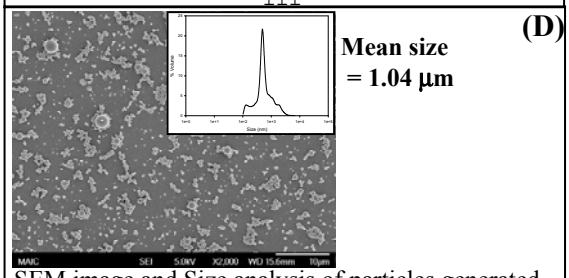
MP to DC ratio can be controlled.

Co-localized DC and MP arrays can be generated

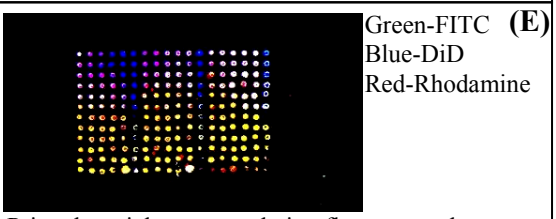


Randomized arrays to show negligible cross-talk between islands.





SEM image and Size analysis of particles generated using PPP technique



Printed particles encapsulating fluorescent dyes generated via PPP technique