# Dendritic Cell Arrays for High-Throughput Immuno-Engineering

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**Statement of Purpose:** Manipulating immune cell responses to boost or repair the body's natural defence mechanisms is a powerful approach for the treatment of diseases as diverse as cancer, infection, and autoimmunity. We have developed a high-throughput approach using dendritic cell microarrays to optimize vaccine formulations. The use of non-living vaccines (e.g., synthetic peptides derived from antigen epitopes) provides multiple advantages over live vaccines. However, current formulations of non-living vaccines have several limitations, including lowered immunogenicity, which requires more frequent administration at large doses along with strong adjuvants. Biomaterial vehicles specifically engineered for peptide vaccines can address these limitations. Phagocytable particles of biodegradable biomaterials allow for co-delivery of antigen and adjuvant along with immuno-functional molecules that can be either surface-tethered or time-released upon degradation. Dendritic cells (DCs) play a critical role as antigen presenting cells in deciding whether a protein/peptide is recognized as an auto-antigen (in the case of autoimmune diseases), part of pathogen (in the case of infection) or self. DCs pass on this information to lymphocytes such as T-cells utilizing surface markers and cytokines. We are engineering high-throughput cellular microarrays in order to optimize particle-based vaccine formulations amenable to needle-free immunization (e.g., oral or epidermal powder immunization). Specifically, we aim to optimize combinations of antigenic peptides, antigen presenting cell-targeting molecules (e.g. CD11c-binding peptides for dendritic cell targeting), immuno-functional molecules (e.g., "danger signals" targeting toll-like receptors or pro-tolerogenic signals such as vasoactive intestinal peptide) as well as chemoattractants to improve antigen-presenting cell recruitment into the vaccination site.

**Methods:** Immature DCs (iDCs) were obtained by culturing precursors isolated from bone marrow of C57BL/6j mice in the presence of 20 ng/ml of growth factor GM-CSF for 10 days with half-media change every alternate day. Rhodomine loaded 75:25 poly-lactic co-glycolic acid (PLGA) microparticles (MPs), 1-5 µm diameter, were made using water in oil in water type emulsion. PD-2/RGD peptide was crosslinked to MPs and micro-arrayed (using the BioRad Calligrapher dip-pen micro-arrayer) on SAMs made of amine terminated silane crosslinked to glass and backfilled with non-fouling pluronic. iDCs were then seeded on adhesive islands. DC maturation was analyzed by measuring IL-12 and IL-10 cytokine production either by sandwich ELISA or by staining for intracellular cytokines after treatment with monensin to block cytokine secretion.

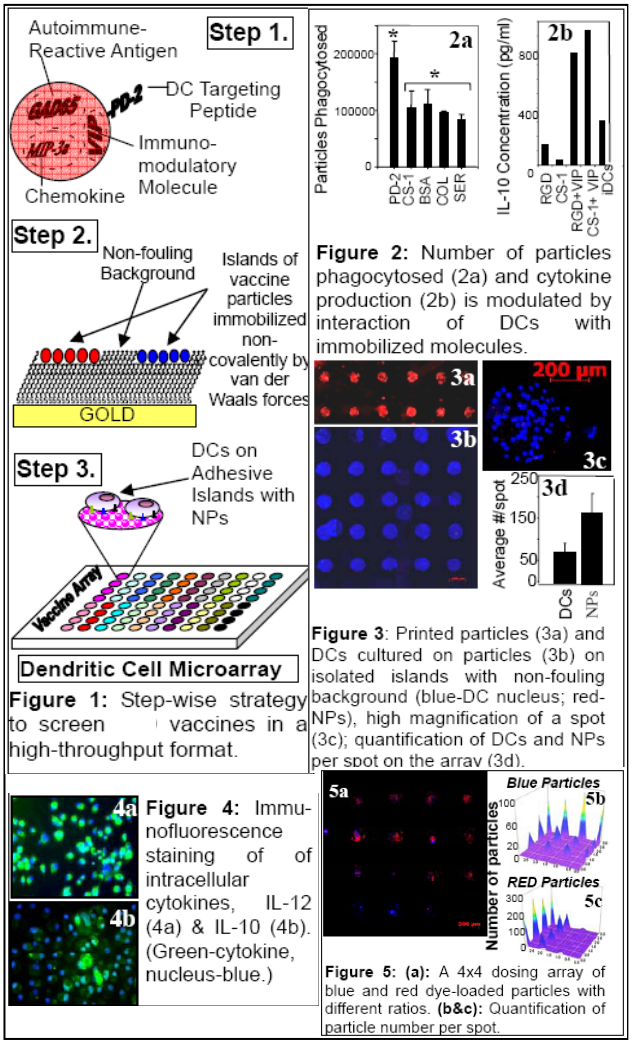


**Figure 1:** Step-wise strategy to screen vaccines in a high-throughput format.




**Figure 2:** Number of particles phagocytosed (2a) and cytokine production (2b) is modulated by interaction of DCs with immobilized molecules.

**Figure 3**: Printed particles (3a) and DCs cultured on particles (3b) on isolated islands with non-fouling background (blue-DC nucleus; red-NPs), high magnification of a spot (3c); quantification of DCs and NPs per spot on the array (3d).




**Figure 4:** Immunofluorescence staining of intracellular cytokines, IL-12 (4a) & IL-10 (4b). (Green-cytokine, nucleus-blue.)




**Figure 5: (a):** A 4x4 dosing array of blue and red dye-loaded particles with different ratios. **(b&c):** Quantification of particle number per spot.

**Results:** Using the steps outlined in **Figure 1**, we have engineered dendritic cell arrays for high-throughput immuno-engineering. PLGA particle uptake by DCs was increased by tethering the DC integrin-targeting peptide PD-2, which targets integrin CD11c (**Figure 2a**). Modulation of IL-12 (not shown) and IL-10 (**Figure 2b**) cytokine secretion by DCs cultured on different peptides suggests that tethered peptides can actuate DCs to exhibit anti-inflammatory or pro-inflammatory response. PLGA particles can be micro-arrayed in a combinatorial fashion (**Figure 5**), and DCs can be selectively cultured on islands with particles. Both cell and particle number can be quantified through image analysis methods (**Figure 3** and **5**). DC cytokine secretion on islands can then be quantified by intracellular cytokine secretion (**Figure 4**).

**Conclusions:** Arrays of vaccine particles with DC-targeting molecules can be used to modulate cell function and allow vaccine optimization.