Dendritic cell microarrays for screening immunomodulatory molecules in the development of tolerogenic vaccines

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Introduction: Modern vaccines are being designed with an emphasis on specific, tailored formulations to elicit more precise and potent immunological responses. One strategy to develop immunotherapeutic treatments involves using biomaterials as carriers to target dendritic cells (DCs). Dendritic cells are the most efficient antigen presenting cells (APCs), capable of orchestrating lymphocyte function and directing the immune response toward either immunity or tolerance. Exploiting this potential, DCs have been modulated *ex vivo* to treat autoimmune diseases such as type-1 diabetes. Polymeric, particle-based approaches have been developed that encapsulate combinations of antigen, adjuvant, chemokines, and other immunomodulating molecules for delivery to DCs via phagocytosis. However, the number of possible immunomodulatory combinations is large, and identifying optimum formulations that are encapsulated in microparticles (MPs) using current immunological methods is challenging due to cost, labor, and time. To address this, cell-based microarrays were developed to screen combinatorial libraries of molecules. Using this platform, both tolerogenic and pro-inflammatory signals were elicited from DCs, providing a robust, miniaturized approach to characterize immunomodulating formulations.

Methods: Arrays consisting of amine islands with a PEG-based non-fouling background were manufactured as described previously from our lab (**Fig. 1a, b**)[1]. Bone-marrow derived DCs were isolated from C57/B6 mice at 8-12 weeks of age. Poly(lactic-co-glycolic acid) (PLGA) microparticles were fabricated using an emulsion-solvent evaporation protocol, encapsulating a small library of immunomodulatory compounds. Dendritic cells were then cultured on the array where they phagocytose loaded MPs. Following 24 h of culture, the microarrays were subsequently stained for costimulatory markers CD80, CD86, activation marker MHCII, inflammatory cytokine IL-12, production of the tolerance inducing enzyme indoleamine 2 3-dioxygenase (IDO), and IL-10, an anti-inflammatory cytokine. Microarrays were then imaged utilizing an automated routine and analyzed. Drug encapsulation efficiency and release was quantified for each compound loaded in PLGA MPs.

Results and Discussion: The tightly controlled specificity of cell attachment allows for co-localization of cells with drug-loaded PLGA MPs (**Fig 1c**). Variable doses of immunomodulatory factors is achievable by controlling the density of MPs per island. Additionally, a library of particles encapsulated with potential tolerance-inducing small molecules has been manufactured and characterized via loading efficiency and release kinetics (data not shown). Smaller particles (1 μm) are readily phagocytosed by DCs and were fabricated for intracellular targeting molecules. Conversely for extracellular targets, larger particles (10 μm) were fabricated, locally releasing their depot. Cell adhesive island spacing was optimized to eliminate potential cross-talk while minimizing inter-spot distance. Microparticle uptake confirmed on the microarray, and further was observed to follow a dose-dependent relationship. Utilizing this platform, DCs were screened in high-throughput against a panel of immunomodulating factors. Dendritic cell activation was quantified and confirmed in the presence of LPS-loaded MPs, though DC activation varied in the presence of other immunomodulating factors. Ongoing work is focused on identification of molecules or combinations thereof that possess the ability to induce tolerogenic DCs. Promising formulations are then co-cultured with lymphocytes, and proliferation is evaluated to assess their tolerogenic capacity.

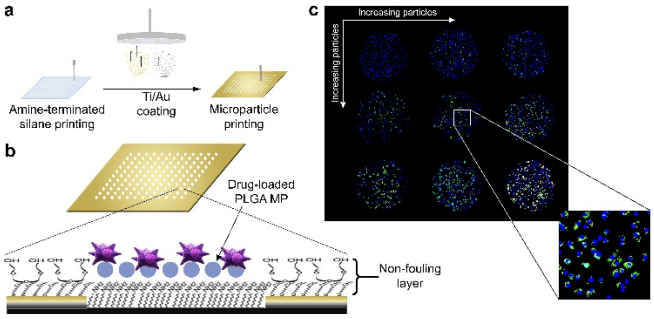

Figure 1. Design of microarray. (a) Production of microarray and, (b) schematic illustrating surface chemistry of final array configuration. (c) Micrograph of a microarray seeded will cells stained for nucleus illustrating specific adhesion to the drug-eluting islands.

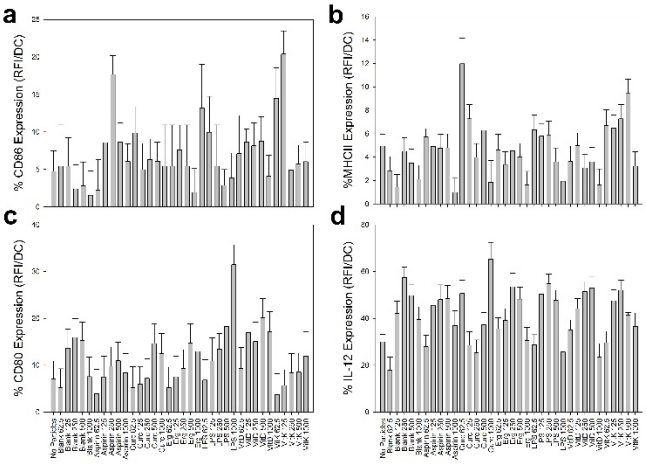

Figure 2. DC activation quantified on the microarray platform. Dendritic cell activation is characterized by expression of **(a)** CD86, **(b)** MHCII, **(c)** CD80, and **(d)** IL-12 secretion in the presence of variable concentrations of immunomodulatory factors.

Conclusion: A recent strategy to ameliorate autoimmune diseases such as type-1 diabetes (T1D) involves inducing specific immunological tolerance via upregulation of tolerance-promoting dendritic cells (DCs). The ability to screen vaccine formulations intended to increase this tolerogenic phenotype on patient-derived DCs, however, represents a substantial barrier to this approach. We show that utilizing the cell-based microarray described here to investigate these immunomodulatory effects adds a valuable tool to the existing immunological methodologies, and provides a means to robustly investigate libraries of immunomodulating factors.

References:

[1] Acharya AP et al. Biomaterials. 2009; 30(25):4168-77