# Microfluidic Nanoparticle Physicochemical Property Effects on Inflammatory Cytokine Modulation in Macrophages

**Jacob Shaw[1], Nhu Truong[2], Sheneil K. Black[2], Ryan M. Pearson[2,3]**

**[1]Department of Microbiology and Immunology, University of Maryland School of Medicine, [2]Department of Pharmaceutical Sciences, University of Maryland School of Pharmacy, [3]Marlene and Stewart Greenebaum Comprehensive Cancer Center, University of Maryland School of Medicine**

**Statement of Purpose:** Nanoparticle (NP)-based therapeutics have garnered significant attention as a versatile drug delivery system. Due to their inherent modularity, NPs can be engineered to exhibit controlled drug release properties, improved circulation times, specific cell targeting, and reduced toxicity profiles. Among these NPs, biodegradable polymers such as poly(lactic-co-glycolic acid) (PLGA) or poly(lactic acid) (PLA) are ideally suited due to their low toxicity and tunable physicochemical characteristics. Recently, our group reported that cargo-less immunomodulatory NPs (iNPs), prepared from PLA, possess surface chemistry and polymer composition-dependent properties that aid in the reduction of pro-inflammatory cytokine expression in response to Toll-like receptor agonists [1].

Conventional methods for the bulk synthesis of polymeric NPs rely on sonication, however, this process is limited by its high batch-to-batch variability, poor polydispersity, and low recoveries. Microfluidic devices have emerged as high-throughput alternatives to conventional NP synthesis, with improved polydispersity, reproducibility, and production efficiency. By precisely and systematically controlling the formulation parameters, a physiochemically diverse NP library can be produced. Herein, we describe the development of a high-throughput microfluidic method for the production of cargo-less immunomodulatory nanoparticles (iNPs) and evaluation of the formulation-dependent anti-inflammatory properties in modulating lipopolysaccharide (LPS)-induced macrophage responses.

**Methods:** iNPs were prepared using a microfluidics platform (Dolomite, Royston, UK). A three-inlet hydrodynamic flow-focusing configuration was utilized to generate iNPs with varying sizes and surfactants. Two converging streams of aqueous surfactant solutions encountered the polymer (organic; acetone) solution at the chip junction to facilitate the nanoprecipitation of iNPs. iNPs were generated using PLA and six types of surfactants (one neutral and five anionic) that differed regarding their chemistries: poly(vinyl alcohol) (PVA), poly(ethylene-alt-maleic acid) E400 (E400), PEMA E60 (E60), poly(acrylic acid) (PAA). iNPs were characterized for size, polydispersity, and zeta potential using dynamic light scattering. Flow rate ratio (FRR)-dependent particle size was determined by adjusting surfactant:polymer flow rates. Two NP FRRs for each surfactant were chosen for *in vitro* examination. MTS assay for cell proliferation was used to assess cytotoxicity in murine bone marrow-derived macrophages (BMMØ). iNP immunomodulatory capacity was assessed by proinflammatory cytokine TNFα release in BMMØ after LPS stimulation measured by ELISA. Bay 11-7082 IKK inhibitor was used as a negative control.

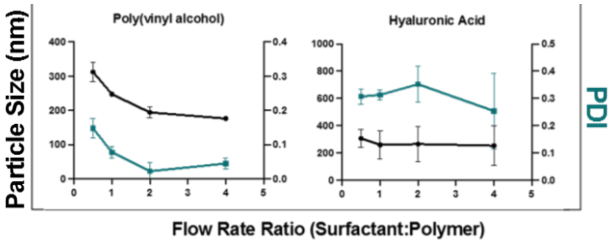


**Figure 1.** Representative iNP size and polydispersity (PDI) dependence on FRR and surfactant type.

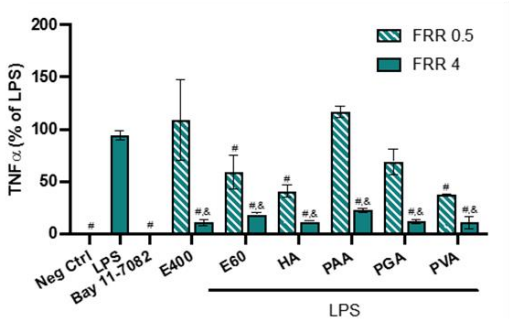


**Figure 2.** TNFα ELISA results for iNPs as a function of FRR. BMMØs were treated with iNPs for 3 hrs prior to LPS stimulation and culturing for 48 hrs.

**Results:** iNP size can be specifically tuned using FRR of surfactant:polymer solutions, which controls the mixing of solutions and particle precipitation. As the FRR decreased, the particle size and PDI increased, with the exception of HA (**Figure 1**). All iNPs evaluated did not significantly affect cellular viability in BMMØ. In general, smaller iNPs led to greater suppression of proinflammatory cytokine TNFα secretions and iNP zeta potential did not correlate with cytokine suppression. FRR used in the generation of iNPs was determined to be a critical factor for the measured reductions in proinflammatory cytokine secretions. Between the two FRRs, all iNPs exhibited a significant enhancement in cytokine suppression when produced at a FRR of 4 (**Figure 2**).

**Conclusion:** Our results demonstrate that microfluidic devices are a useful platform in the generation of NP libraries to evaluate the impact of formulation parameters on their physicochemical properties. FRR was found to be a critical feature associated with the immunomodulatory potential of iNPs potentially due to the nucleation rate during nanoprecipitation.

**References: [1]** L.M. Casey, S. Kakade, J.T. Decker, J.A. Rose, K. Deans, L.D. Shea, R.M. Pearson, Biomaterials. 2019;218:119333