Electronic Supplementary Material (ESI) for RSC Pharmaceutics.


## SUPPLEMENTAL FIGURES AND TABLES

# Comparison of Emulsion and Spray Methods for Fabrication of Rapamycin-Loaded Acetalated Dextran Microparticles

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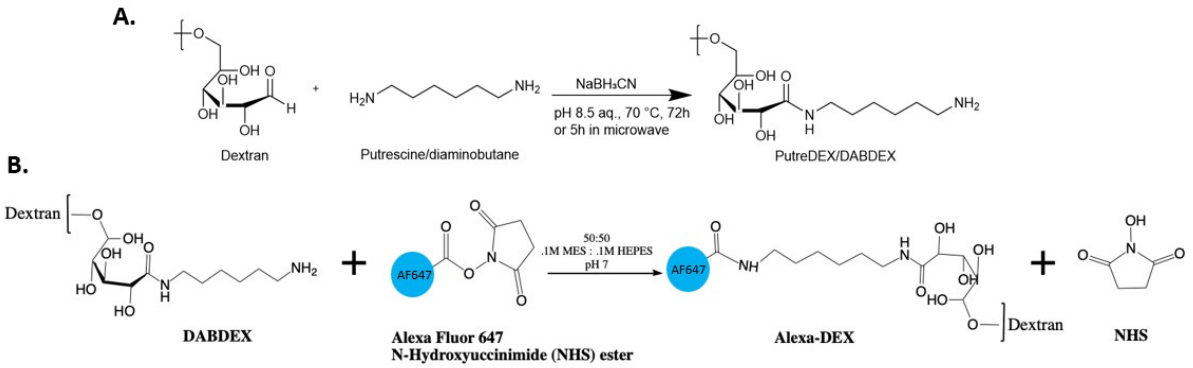


**Figure S1.** Synthesis scheme for AF647-conjugated dextran. A) First, dextran is reacted with EDC (carbodiimide) crosslinker in a microwave reactor, producing dextran with a terminal amine group (DABDEX). B) DABDEX is then reacted with an NHS-ester fluorophore (AF647-NHS) at pH 7, creating fluorescent dextran (Alexa-DEX). Alexa-DEX is then acetalated to form fluorescent Ace-DEX polymer.

**Table S1.** Flow cytometry antibodies, fluorophores, and staining concentrations used in the homogenized Ace-DEX MP trafficking study. All antibodies were titrated in our lab facilities prior to this experiment.

| Marker | Fluorophore | Clone | Staining Concentration (µg/mL) |
|---|---|---|---|
| CD11c | PE | N418 | 1.00 |
| CD19 | PE/Dazzle 594 | 6D5 | 0.50 |
| CD11b | BV711 | M1/70 | 0.40 |
| Ly6G | APC/Fire750 | 1A8 | 0.50 |
| Ly6C | BV605 | HK1.4 | 0.50 |
| MHC II | BV421 | M5/114 | 0.25 |
| CD45 | FITC | QA17A26 | 2.50 |

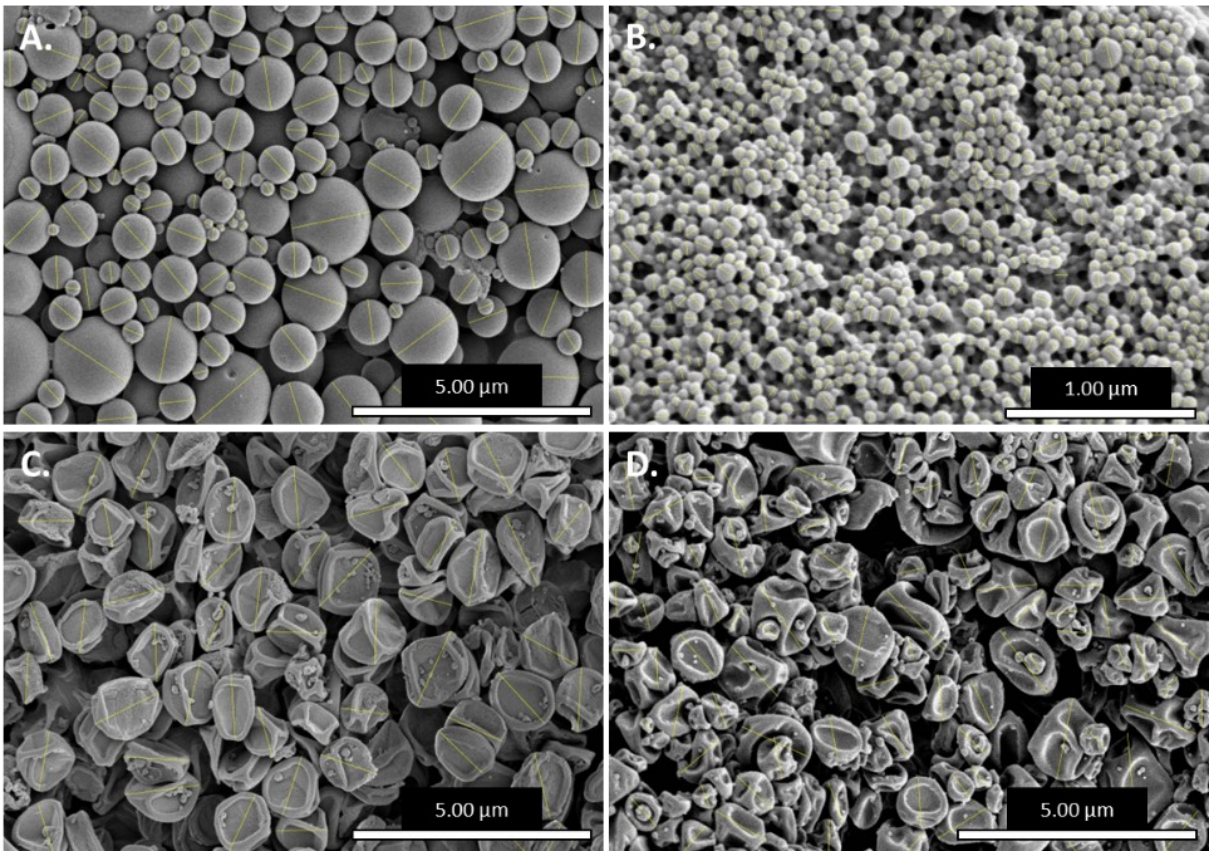


**Figure S2.** Representative ImageJ MP diameter tracing of A) homogenized, B) sonicated, C) electrosprayed, and D) spray dried MPs.

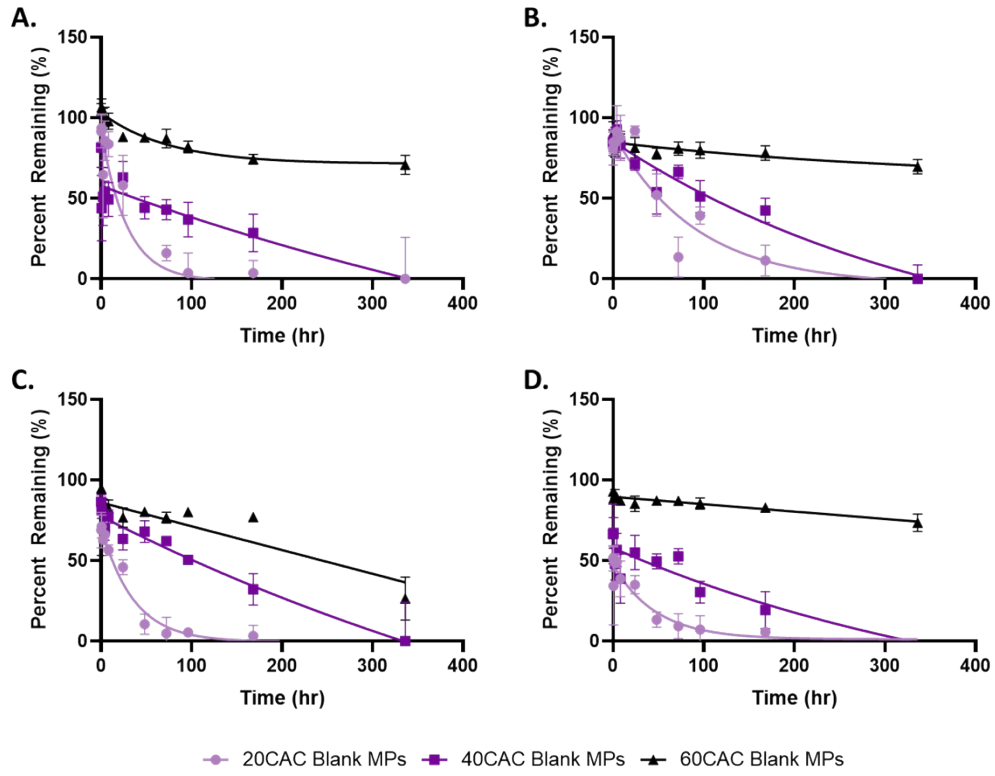


**Figure S3.** MP degradation profiles of A) homogenized, B) sonicated, C) electrosprayed, and D) spray dried blank Ace-DEX MPs by CAC. Degradation was measured over two weeks at pH 7.4 and 37°C. Samples were normalized to a fully degraded sample to calculate the percent remaining. Data are presented as average ± standard deviation (n = 3). Non-linear curves were interpolated using a one-phase decay model on GraphPad Prism.

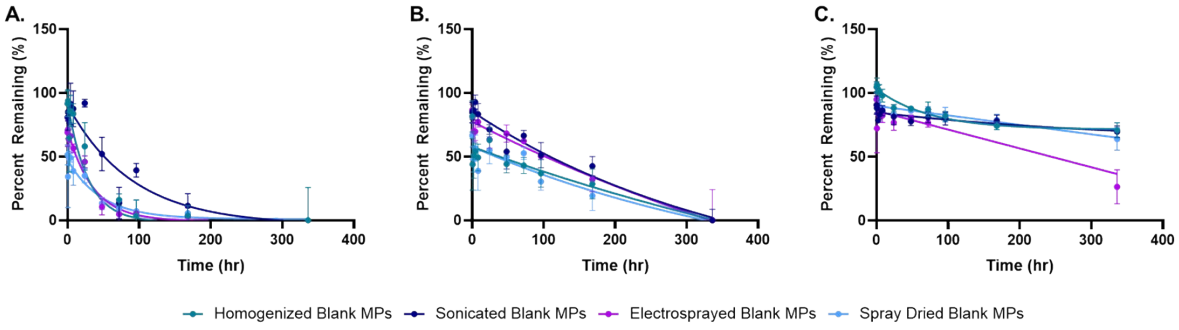


**Figure S4.** MP degradation profiles of A) 20 CAC, B) 40 CAC, and C) 60 CAC blank Ace-DEX MPs by CAC. Degradation was measured over two weeks at pH 7.4 and 37°C. Samples were normalized to a fully degraded sample to calculate the percent remaining. Data are presented as average ± standard deviation (n = 3). Non-linear curves were interpolated using a one-phase decay model on GraphPad Prism.

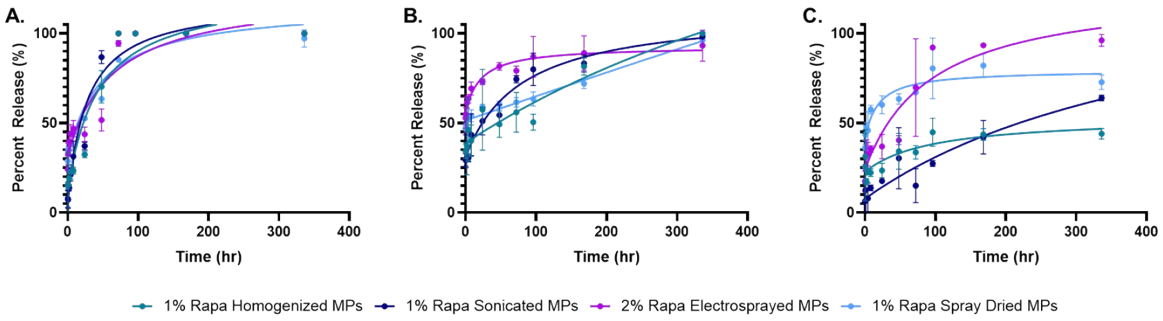


**Figure S5.** Rapamycin release profiles of A) 20 CAC, B) 40 CAC, and C) 60 CAC Ace-DEX MPs by fabrication method. Release was measured over two weeks at pH 7.4 and 37°C. Data are presented as average ± standard deviation (n = 3). Non-linear curves were interpolated using a Pade (1,1) approximate on GraphPad Prism.

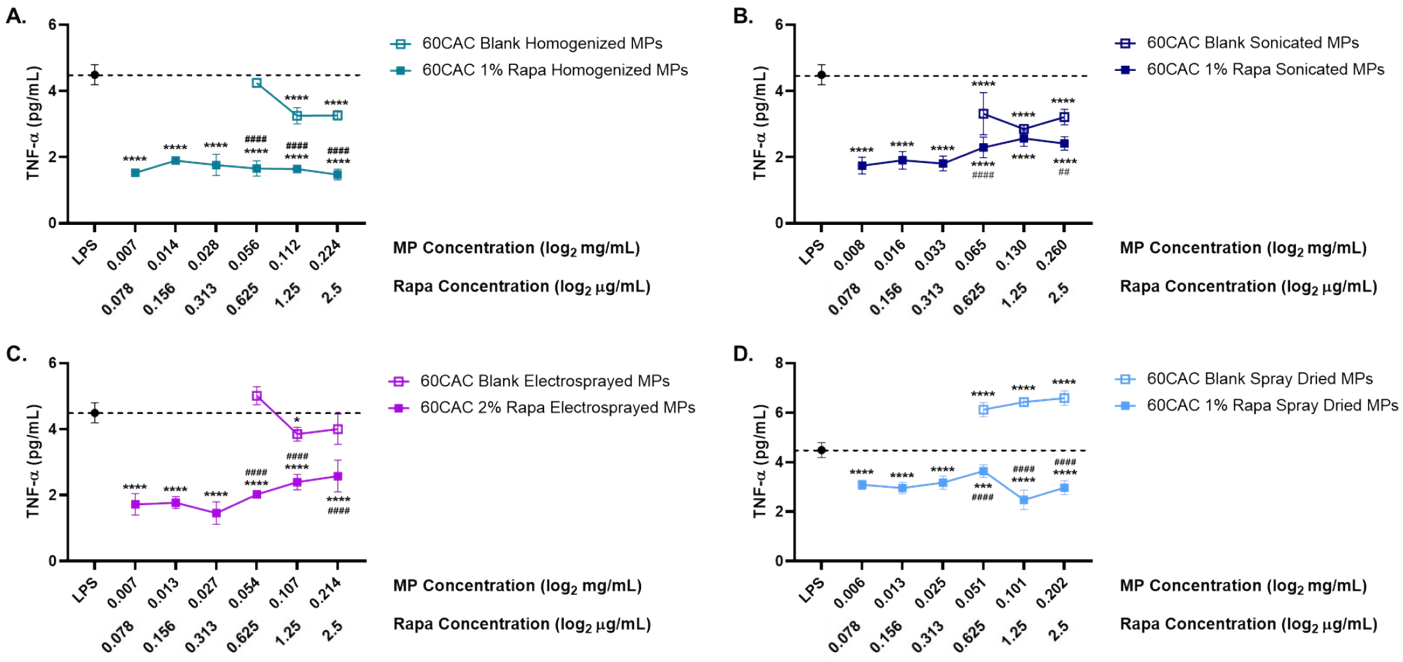


**Figure S6.** Inhibition of LPS-induced inflammation following treatment with rapamycin-loaded Ace-DEX MPs. Macrophages were stimulated with 20 ng/mL LPS for 1 hour, followed by concurrent treatment with the indicated concentrations of soluble or encapsulated rapamycin for 24 hours. The "LPS" control (black) represents cells only receiving LPS stimulation. A-D) TNF-α content from cell supernatants in the indicated MP groups, including blank MPs. Data are presented as an average ± standard deviation (n = 3). Statistical significance is presented as *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001 for a two-way ANOVA test applied between MP groups and LPS alone (*) or for a two-way ANOVA test applied between rapamycin-loaded and blank MPs (#).

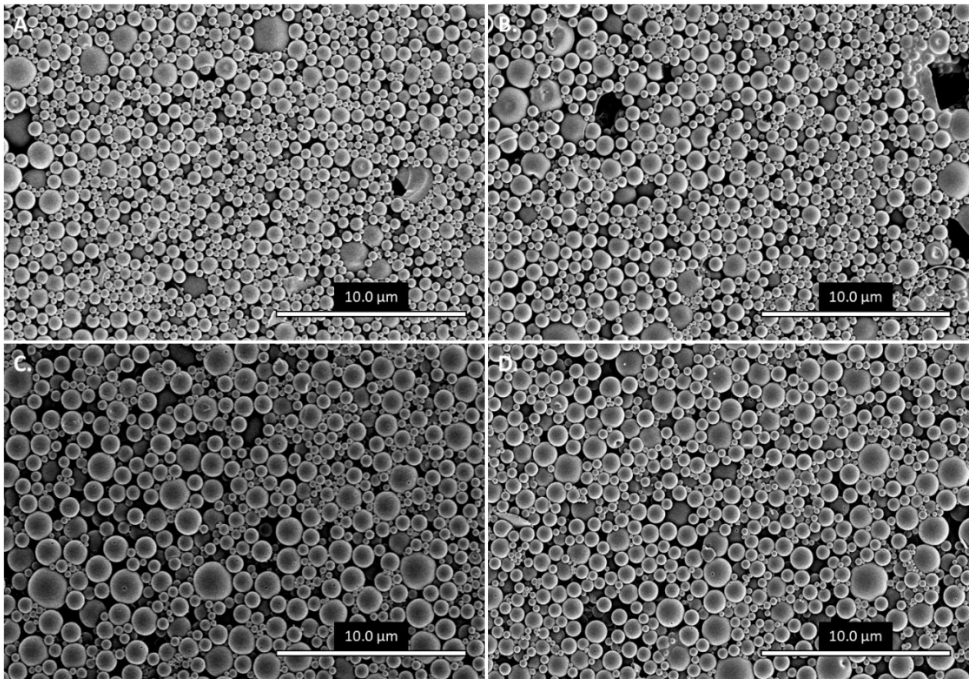


**Figure S7.** Representative scanning electron micrographs of A) 0.5%, B) 1%, C) 3%, and D) 5% rapamycin Ace-DEX MPs. The approximate scale is indicated by the scale bars in the lower right.

**Table S2.** Physiochemical characterization of 0.5, 1, 3, and 5% rapamycin Ace-DEX MPs. Effective particle diameter (dehydrated) and polydispersity index (PDI) were measured by ImageJ tracing of scanning electron micrographs, with a minimum of 50 MP traces collected for each particle group (n ≥ 50). Particle surface charge was measured by electrophoretic light scattering (n = 3). Data are presented as average ± standard deviation.

| Fabrication Method | Theoretical Loading | CAC (%) | Diameter (nm) | Zeta Potential (mV) |
|---|---|---|---|---|
| Homogenization | 0.5% w/w | 60 | 461 ± 199 | -7.8 ± 4.4 |
| | 1% w/w | | 666 ± 341 | -9.8 ± 2.3 |
| | 3% w/w | | 578 ± 368 | -9.6 ± 0.7 |
| | 5% w/w | | 594 ± 335 | -10.6 ± 0.2 |

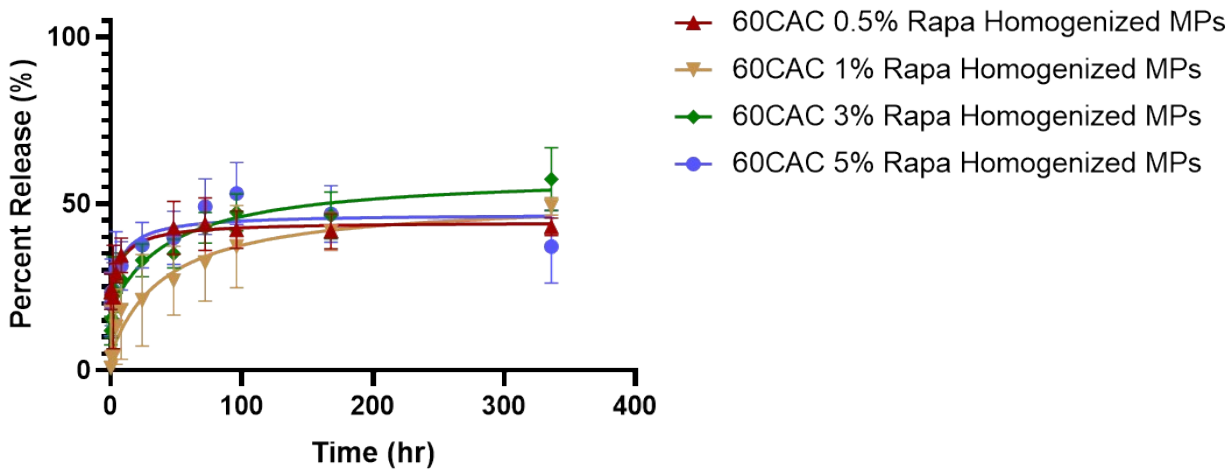


**Figure S8.** Rapamycin release profiles of 0.5, 1, 3, and 5% rapamycin homogenized Ace-DEX MPs. Release was measured over two weeks at pH 7.4 and 37°C. Data are presented as average ± standard deviation (n = 3). Non-linear curves were interpolated using a Pade (1,1) approximate on GraphPad Prism.

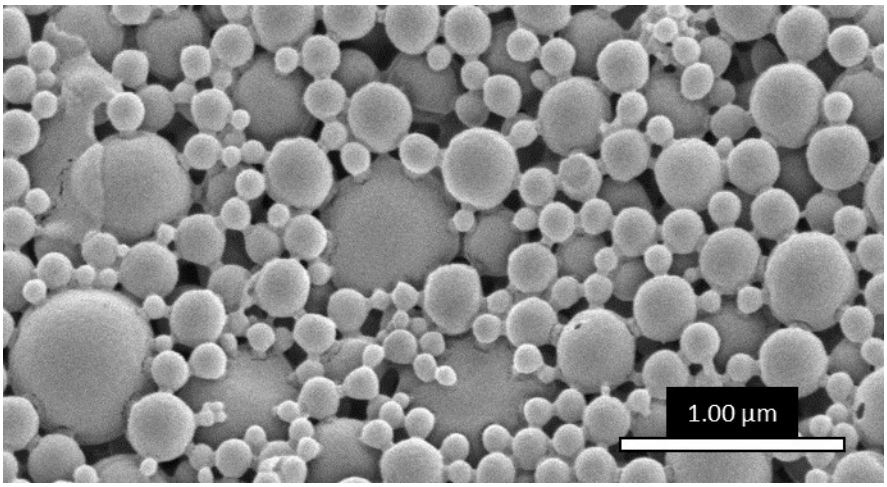


**Figure S9.** Representative SEM image of homogenized Alexa-DEX MPs. The approximate scale is indicated by the scale bar in the lower right.

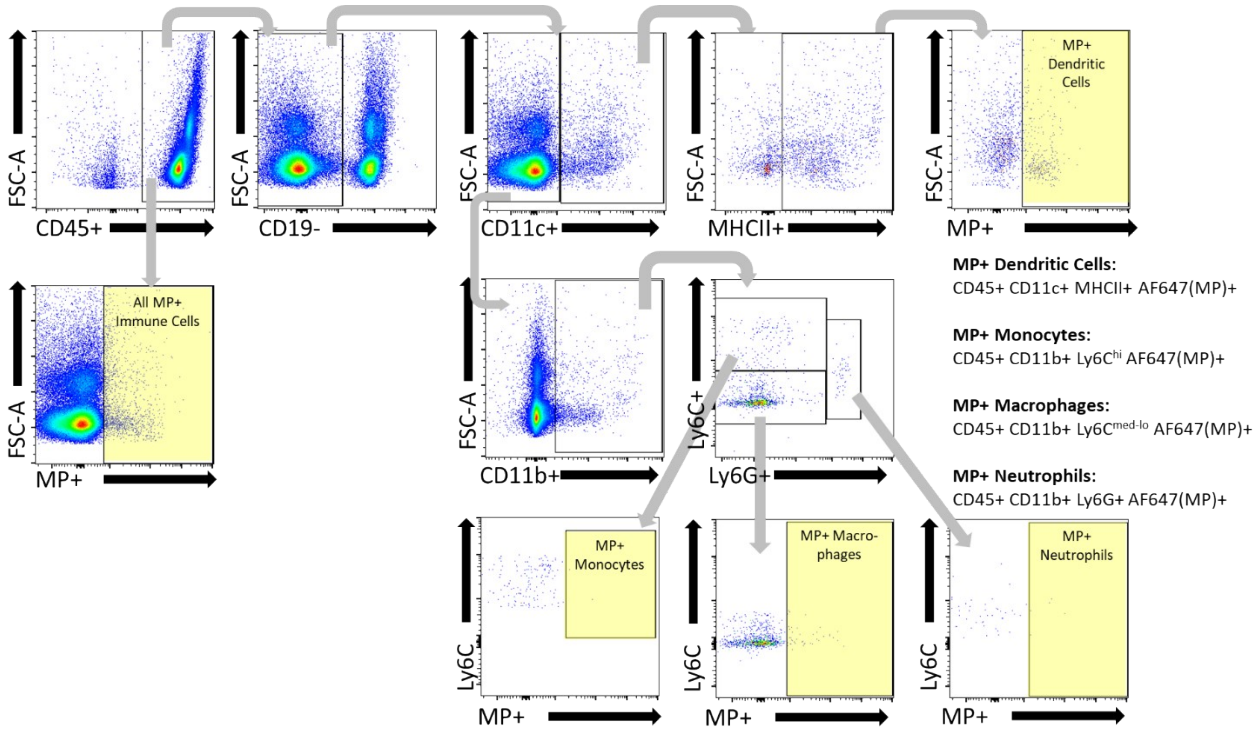


**Figure S10.** Gating strategy for trafficking study. The first gate reflects live cells. Cell phenotype identification is shown on the right.

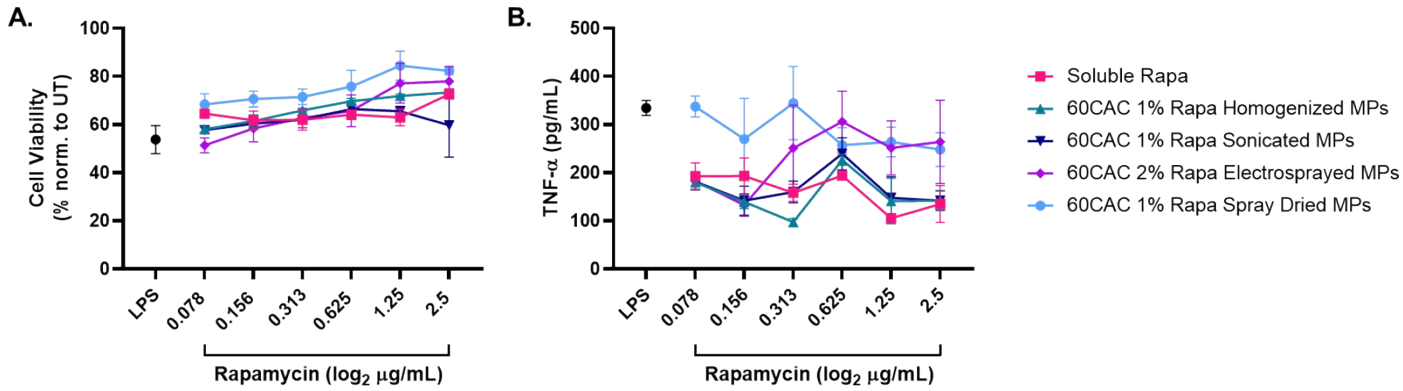


**Figure S11.** Inhibition of LPS-induced inflammation following treatment with rapamycin-loaded Ace-DEX MPs of different fabrication methods. Dendritic cells were stimulated with 20 ng/mL LPS for 1 hour, followed by concurrent treatment with the indicated concentrations of soluble or encapsulated rapamycin for 24 hours. The "LPS" control (black) represents cells only receiving LPS stimulation. A) Cell viability measures collected via lactose dehydrogenase (LDH) assay. Viability is quantified as 100% - LDH Cytotoxicity. B) TNF-α content from cell supernatants, as measured by TNF-α ELISA. Data are presented as an average ± standard deviation (n = 3).