# High Throughput Fabrication of Polymeric Microparticles

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## INTRODUCTION

A popular and extremely attractive method for releasing biologically active materials is through polymeric microparticles fabricated via a double emulsion procedure[1]. The particles offer protection to the encapsulated materials, which have the potential to be sensitive to physiologic environments, and maintain the ability to release continuously or intermittently over periods of days to months[2]. The double emulsion technique allows for practically any combination of water soluble small molecule drug, protein, DNA, etc, to be loaded into particles made from a variety of polymers. This flexibility allows for combination therapies involving several agents, which may have synergistic effects. However, varying all of the available parameters to fully optimize a therapy can be a daunting task given that this process usually takes approximately 4-5 hours at a time. Further complicating this scenario is the possibility that some proteins[3] and plasmid DNA[4] can become deactivated in the particle microenvironment, requiring the need for additional stabilization agents. Even a scenario where only a few variables are explored could require hundreds of particle formulations. Furthermore, we have recently synthesized a library of over 2000, structurally-diverse poly(β-amino ester)s (PBAE), all of which may have potential to enhance particle delivery capacity[5]. Clearly, to make progress in screening even a portion of this library, especially if it is desired to vary any other parameters, it would be necessary to develop rapid methods for synthesizing these formulations on a smaller scale. Here we describe a new high-throughput method for fabricating microparticles by the double emulsion procedure which enables rapid screening.

## METHODS

Fabrication of Particles: Microparticles were prepared by the following modification of the double emulsion procedure: A solution (12μL) of aqueous "drug" (10 mg/ml of pCMV-Luciferase plasmid DNA or rhodamine conjugated dextran), EDTA (1 mM), and D(+)-Lactose (300 mM) was added to 0.25 ml of $CH_2Cl_2$ solution with polymer at varying degrees of composition (50 mg/ml) in a deep, 96 well plate with a staggered formation (Figure 1). To emulsify these immiscible phases, we utilized a 24 tip, probe sonicator attachment (Sonics and Materials Inc; Danbury, Conneticut) at a setting of 47 % amplitude for 10 seconds. The resulting emulsion was then immediately transferred to a solution of poly(vinyl alcohol) (120 μL into 1.5 ml, 1% PVA (w/w), 0.25M NaCl) in deep, round bottom 24 well plates using a 96 tip fluid handling robot. The contents of this plate were then sonicated at a setting of 37% amplitude for 20 seconds to form the final water-in-oil-in-water emulsion. The suspensions were then placed on a rotating plate and allowed to stir for 3 hours to allow for solvent evaporation. The plate was centrifuged at 1200 rpm for 10 min at 4ºC followed by washing 3X to remove excess PVA. After the final wash, particles were suspended in water, frozen, and lyophilized for 3 days. Characterization: Size distributions were measured via volume displacement/impedance using a Multisizer 3 with a 30-200 μm orifice tube (Beckman Coulter; Miami, FL). Morphology of microsphere surfaces was imaged using scanning electron microscopy (SEM). To determine if the process yielded active plasmid DNA encapsulate, we incubated microparticles with a P388D1 macrophage cell line as previously described[6]. A titration of the soluble, lipid-based transfection agent, Lipofectamine 2000 (Invitrogen), was prepared with DNA as a positive control.

## RESULTS & DISCUSSION

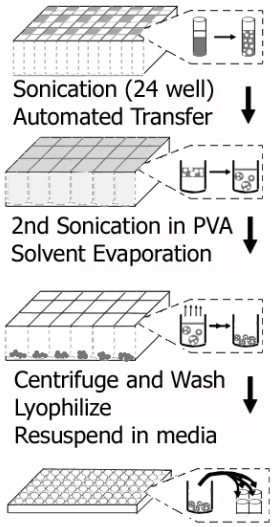


**Fig 1: Schematic of high throughput fabrication.**

Particles prepared using the said technique had relatively high surface integrity with minor flaws as seen using SEM microscopy. Sizes of particles were inversely dependant upon the concentration of PVA used in the outer aqueous phase as determined by volume displacement/impedance. PVA concentrations of 0.5% yielded particles with mean diameters of 4 μm, while concentrations of 5% PVA resulted in particles with a mean diameter in the nanometer range. There was no statistical difference in mean particle diameters between random wells of the periphery versus the center of the plate. Fluorescence microscopy revealed that particles containing rhodamine conjugated dextran encapsulated relatively high quantities of material indicated by localized and bright fluorescence associated with the particles. This result remained consistent throughout all the wells of the plate. Besides quantity of encapsulate, it is extremely important for any new fabrication technique to allow for encapsulation of a material in its biologically active state. To evaluate the activity of encapsulated material, we used PLGA blended with various PBAEs to encapsulate luciferase encoded plasmid DNA and deliver it to a P388D1 macrophage cell line. The results of this assay conform to the results obtained previously using PBAE as a delivery enhancer in a similar optimum polymer ratio range[7]. These results prove that active plasmid can be successfully encapsulated.

## CONCLUSIONS

The speed in which this technique allows for microparticles to be fabricated provides a valuable tool to study variations in particle formulations in many ways including high throughput testing for release, bioactivity, and *in vitro* efficacy. The disclosed fabrication method allows for a researcher to prepare hundreds of separate microparticle formulations per day, and therefore enables the screening of large polymer and encapsulate libraries.

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