# MICROFLUIDIC SYNTHESIS OF MULTI-LAYER NANOPARTICLES FOR DRUG & GENE DELIVERY

**Peggy Chan[1], Aisha Qi[2], Semper, Anushi Rajapaksa[1], James Friend[1], Leslie Yeo[1]**

*[1]Micro/NanoPhysics Research Laboratory, RMIT University, Australia*

## ABSTRACT

Multiple layer nanoparticles offers a likelihood of success in drug delivery, as it provides a solution for a more controllable drug release, as with such structures, control over the capsule wall thickness, permeability, stability, and degradation characteristics can be achieved. Using PDMS microfluidic devices to synthesize polymeric multilayer micro/nanoparticles has become popular recently. The generation of complex emulsions, such as double and triple emulsions, is also achievable with such devices [1]. However, limitations with these devices are: (1) the microchannel surface property is crucial to maintain the desired flow within the microdevice; (2) droplets which form within the microchannels require a cross-linking agent to be solidified into particles; (3) the size of the droplets is limited to the size of microchannels, usually around 50-100 mm, which is too large to be used for drug delivery; and (4) the amount of droplets or particles produced is limited as the droplets/particles are formed one by one. Therefore, in this study, we present a novel technique on fast multilayer polymeric nanoparticles synthesis via surface acoustic wave (SAW) atomization using a microfluidic device. We are able to show (1) successful synthesis of nanostructure including multilayer nanoparticles, and (2) fast generation of monodispersed particles in nanosize.

## KEYWORDS

Surface acoustic wave, multi-layer nanoparticles, microfluidic synthesis, gene delivery

## INTRODUCTION

The use of nanoparticles as drug or gene carrier offer several advantages such as better drug stability, feasibility to incorporate both hydrophilic and hydrophobic substances, and their enhanced permeability and retention effect for tumor therapy [2-3]. Multilayer polymeric encapsulation provides a solution for a more controllable in-vivo drug release; multi-functionality can be designed for such structure by using different polymer in different layer each carries a different functionality. In addition, the capsule wall thickness, permeability, stability, and degradation characteristics in such structure can be controlled, and tailored for targeted delivery or successive releasing of drugs. The conventional techniques used for nanoparticle formation and encapsulation all have difficulties in getting nanoparticles with narrow size distribution, unless it is synthesis with the aid of emulsion, surfactant and templates. These methods usually consist of several complicated steps, all of which require well optimized condition to allow for the formation of form homogeneous dispersed single layer particles [4], let alone synthesizing layer-by-layer capsules. Using PDMS microfluidic devices to synthesize polymeric multilayer micro/nanoparticles has become popular recently. The generation of complex emulsions, such as double and triple emulsions, is also achievable with such devices [1,4]. However, this technique share the same limitation as emulsion based method, in addition the size of the droplets is limited to the size of microchannels, usually around 50-100 μm; and (4) the amount of droplets or particles produced is limited as the droplets/particles are formed one by one. Previously, our group showed the synthesis of pure polymeric nanoparticles, protein nanoparticles, and protein loaded nanoparticles via surface acoustic wave (SAW) atomization [5-7]. In this study, we demonstrate that SAW atomization technique can be extended to synthesis multilayer polymeric nanoparticles in a layer-by-layer manner. Herein, we synthesis DNA containing multilayer nanoparticles to demonstrate the flexibility and therapeutic applicability of the SAW atomization approach.

## EXPERIMENT

A single-phase uni-directional transducer (SPUDT) was fabricated using sputtering and standard UV photolithography with wet-etch techniques onto piezoelectric substrate surface. A high frequency electrical signal is supplied to the electrodes, thereby inducing a SAW as the efficient atomization driving source as shown in Fig. 1 [8-10]. Two model polymers, Chitosan (Chi) and carboxylmethyl cellulose (CMC) were employed. Experimental set up is illustrated in figure 2, the first polymer solutions are supply to one end of the device substrate. Atomized polymer aerosols were generated and drive though a drying tube, follow by deposition into the second polymer solution. This suspension can be collected and re-atomized into another polymer solution again, using the same experiment setup and atomization procedures described above to obtain multilayer particles (Fig 2).

FTIR spectrum was employed to examine the chemical bonding between each polymer layer. Visual distinction between successive layers is facilitated by selectively tagging each polymer molecule.The fluorescently-labelled multilayer nanocapsules were then synthesized using these tagged polymers in the dark via the same atomization-evaporation-resuspension process described above. The sequential bonding of each successive polyelectrolyte layer onto the surface of the nanocarrier was detected by selectively labeling the polymers. Fluorescence measurements were carried out on a multimode spectrophotometer. Further evidence of the presence of successive polyelectrolyte layering was obtained through zeta-potential measurements. Particle size distribution was



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obtained using the Zetasizer Nano S. The pDNA release study was carried out under near-physiological conditions, samples were subsequently extracted at specific time intervals for testing. The samples were quantified using a Quant-iT PicoGreenr dsDNA reagent.

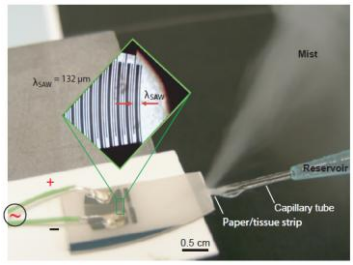


Figure 1. A photo showing a 30 MHz SPUDT SAW device and its electrode layout captured undermicroscrope

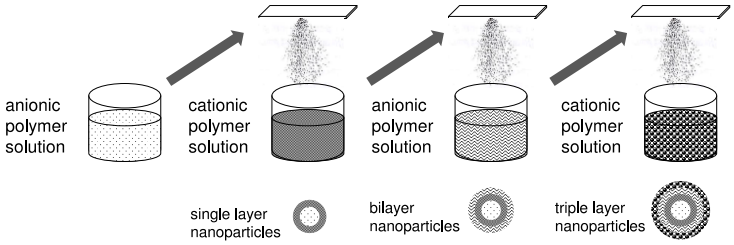


Figure 2. (left) Experimental set-up for aerosol atomization and particle collection, (right) schematic diagram of multiparticle preparation in layer-by-layer manner.

**RESULTS AND DISCUSSIONS**

FTIR spectrum showed that ionic complexation has successfully formed between each polymer layer. Fig. 3 shows the change of zeta-potential after different polymeric layers was added on the nanoparticle by SAW atomization. Samples containing plasmid DNA exhibited negative charge due to the phosphate group present in each nucleotide. Nanoparticle containing DNA and Chi exhibited positive charge due to the present of positively charged Chi on the nanoparticle surface. Nanoparticle containing DNA/Chi core and CMC outer layer display negative charge due to the present of negatively charged CMC on the nanoparticle surface. The reversal of zeta potential is observed as the nanoparticle is further deposit into a complementary polymer solution, suggesting that a stepwise formation of layer on the nanoparticles.

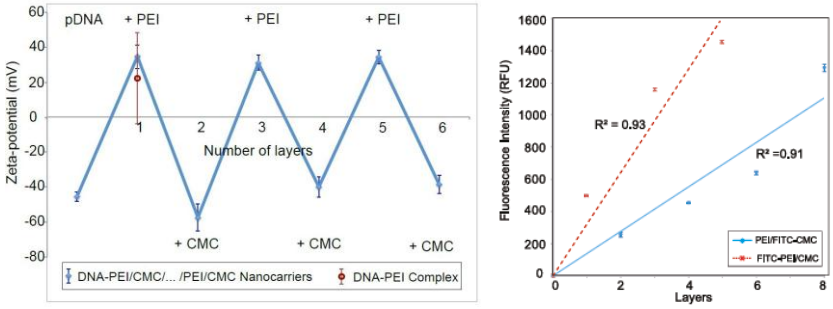


Figure 3. (left) The zeta-potential of multilayer polymer nanoparticles with Chi or CMC as outer layer. (right) intensity measurements of the fluorescence emitted by successfully deposited in turn.

Further evidence of the presence of multiple polyelectrolyte layers can be obtained by fluorescently labeling either PEI or CMC (while leaving the other complementary polymer pair unlabeled). Figure 2c shows the fluorescence intensity of the nanocarriers as additional layers of PEI and CMC are alternately deposited. We observe the fluorescence intensity to gradually increase, albeit nonlinearly, with the increase in nanocarrier mass upon the addition of each labeled polymer layer which binds via electrostatic interaction to the underlying polymer.

Nano-sized particles are advantageous for a wide range of drug delivery administrations. We examined the size distribution of synthesized polymeric particles to see if the size obtained is in the required range. Representative samples (Fig. 4) containing chitosan as the inner core and CMC as the outer layer exhibited a hydrodynamic size of 198.2±7.4 nm with narrow size distribution. AFM data reveal that the nanoparticles exhibited oval shape, possibly due to the rigid and extended conformation of CMC. Particles with narrow size distribution offer various practical advantages compare to particle with similar average size but boarder size distribution such as better controlled drug release.

*In vitro* release profile of encapsulated pDNA from multilayer nanocapsules indicating that the release occurred in a steady and sustained manner (Fig. 5). The initial burst over a transient of approximately 5 h in both sets of data

can be attributed to any unbound or excess pDNA adhering loosely to the outer shell of the nanocapsules. In any case, this is followed by a slower and more uniform continuous release phase. It is also clear that sample B, wherein the nanocapsules include an additional Chi outer layer, shows a much slower release compared to sample A, as expected. The ability therefore to tune the release to some extent with the number of polymer layers deposited over the drug molecule is therefore attractive from the standpoint of tailoring the drug carrier to the desired release dynamics.

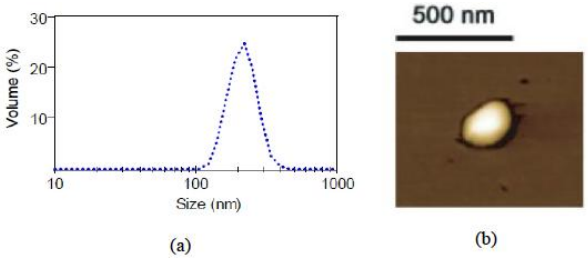


Figure 4. (a) Particle size and size distribution, and (b) representative AFM image of nanoparticle generated by SAW microchip. The nanoparticles are composed of Chi core with CMC outer layer.

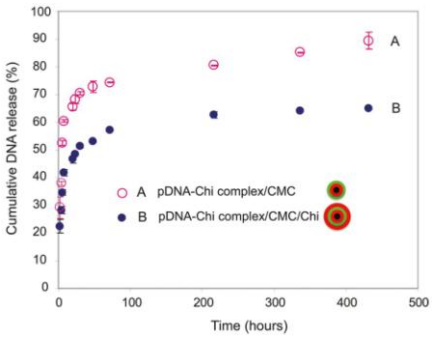


Figure 5. Release profile of pDNA encapsulated in the Chi/CMC bilayer and Chi/CMC/Chi trilayer nanoparticles.

## CONCLUSION

This study demonstrated the use of SAW atomization as a fast and efficient technique to synthesize multilayer nanoparticles for drug encapsulation usage. A serial of characterizations have been conducted and shown the successful bonding between each polymeric layer. Furthermore, unlike many conventional methods in producing polymeric particles, the usage of surfactant and templates are not required in SAW atomization. Compared to traditional spray drying methods, SAW atomization, driven at much higher frequency and lower power, produces much less damage to drugs and DNA vaccines, making it suitable for a wide range of drug and vaccines deliveries.

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## CONTACT


Peggy Chan peggy.chan@rmit.edu.au