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

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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 (Kumar, 2008). Using PDMS microfluidic devices to synthesize polymeric multilayer microparticles has become popular recently. The generation of complex emulsions, such as double and triple emulsions, is also achievable with such devices (Roney et al., 2005). However, limitations with these devices are: the microchannel surface property is crucial to maintain the desired flow within the microdevice; droplets which form within the microchannels require a cross-linking agent to be solidified into particles; 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 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 multilayer polymeric structure, and (2) fast generation of monodispersed particles in nanosize. Compared to conventional methods, SAW atomization is fast and have less limitations in the usage of surfactant and templates. Compared to traditional ultrasonic atomization and electrospraying, SAW atomization driven at much higher frequency is more suitable for shear and heat sensitive drug 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 (Cho et al., 2008; Gelperina et al., 2005). The biodistribution of drug particles can be greatly improved by using particles with size range in submicron or nano-scale, and secondly, nanoparticles can also be modified or coated to target infected organs or tissues (Kumar, 2008; Roney et al., 2005; Fang et al., 2006; Li et al., 2001; Koziaraa et al., 2004). 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 (Zelikin et al., 2008), 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 (Wong, 2009), let alone synthesizing layer-by-layer capsules. For example, the coacervation/precipitation technique requires a careful selection of solvents, which can limits the range of materials that can be applied, especially for the case of synthesizing LbL particles. Spray drying, usually means ultrasonic atomization and electrospray. These methods seems to have less limitation in terms of the choice of solvents, also the usage of templates is not necessary; however, the harsh conditions involved in such technique can cause damage to drugs or biomolecules. For example, the high temperature use in spray drying can cause damage to heat-sensitive biomolecules. Ultrasonic atomization, driven at ~10 kHz order, imposes unavoidable shear forces that can damage many shear-sensitive molecules, for example, DNA. Electrospray, on the other hand, driven at ~kV order voltage, still poses high risks of damaging molecules.

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 (Wong, 2009; Priest et al., 2008). However, this technique share the same limitation as emulsion based method, in addition such technique is limited by: (1) the microchannel surface property is crucial to maintain the desired flow within the microdevice; (2) the need for a cross-linking agent to solidified the droplets that formed from the microchannels; (3) 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 (Friend et al., 2008; Alvarez et al., 2008; Alvarez et al., 2008). Surface acoustic waves (SAWs), with nanometer-order amplitude, can propagate over thousands of wavelengths, typically several centimeters, along the surface in a low loss piezoelectric material like 127.68° y-x cut lithium niobate ($LiNbO_3$ or LN). As its name indicated, the wave amplitude is rapidly attenuated with increasing depth into the LN substrate from the propagation surface. The x-propagating wave speed on the LN substrate c_s is 3965 m/s. When a SAW meets a liquid placed upon the substrate, it diffracts into it at the *Rayleigh* angle, defined by $\theta_R = sin\text{-}1(c_w/c_s) \sim 22°$, where c_w, the sound speed in water, is 1485 m/s. The acoustic energy in the liquid causes the bulk recirculation, known as acoustic streaming, within the drop and a body force that causes the drop to translate in the direction of the SAW propagation. At high powers, though the displacement of the surface is only around 10 nm, when the driving frequency is in an order of 10 MHz, the accelerations of the surface is expected to be as high as in an order of 107 m/s^2, which, when transmitted into a liquid drop, can induce very strong capillary waves on the drop free surface that are destabilized upon sufficient acoustic excitation. In this manner, a forcing mechanism for rapid and efficient atomization is

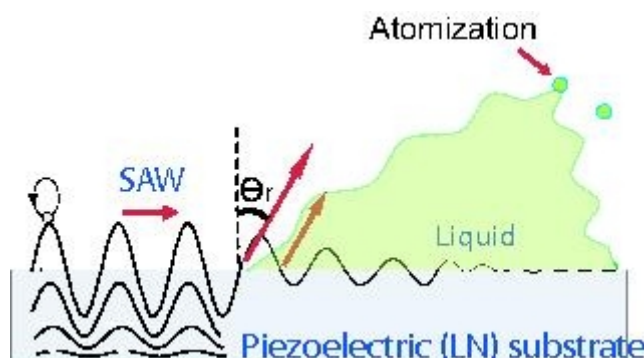formed (Qi et al., 2008), as shown in Fig. 1.

Fig. 1: A schematic atomization process (Qi et al., 2008; Qi et al., 2009) SAWs propagating into a drop at a Rayleigh angle, induce subsequent streaming within the drop and destabilize capillary wave on the free liquid surface. When the power is sufficient, capillary wave breaks up into aerosol, which is known as atomization.

Unlike other conventional ultrasonic atomization, SAW atomization works at much higher frequencies ($»10MHz$), meaning that the time period of the molecule exposing to the shear force is much shorter than the molecular relaxation time scale in liquids (Oxtoby, 1981; Hsieh et al., 2001), the shear effect is therefore greatly minimized (Alvarez et al., 2009). In addition, SAW atomization, compared to electrospray, is driven at very low power (~1-3 W), which can hardly cause damages to drugs and molecules (Qi et al., 2009; Qi et al., 2010). 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.

MATERIALS AND METHODS

SAW Microchip fabrication

A single-phase uni-directional transducer (SPUDT) was fabricated using sputtering (Hummerr Tripletarget Magnetron Sputter System, Anatech, USA.) and standard UV photolithography with wet-etch techniques onto a 128± y-cut x-propagating lithium niobate (LiNbO3) piezoelectric substrate surface. To achieve the most efficient atomization with limited power input, an enhanced SAW signal, which is located at the focus of the concentric transducers is also achievable by using curved, focusing electrodes. A focusing SPUDT layout is captured under microscope and presented in Fig 2 (inset). A high frequency electrical signal is supplied to the electrodes, generating mechanical oscillations on the substrate via the inverse piezoelectric effect, thereby inducing a SAW as the efficient atomization driving source (Qi et al., 2008; Qi et al., 2009; Qi et al., 2010).
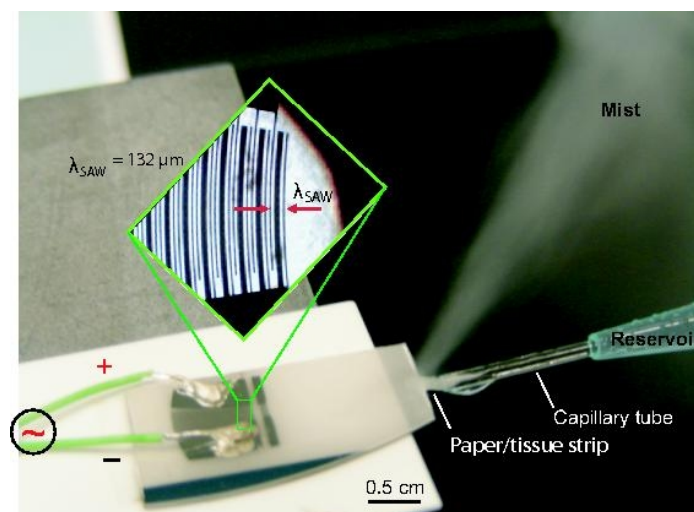
Fig. 2: Photo of a 30 MHz SPIDT SAW device and its electrode layout captured under microscope. A capillary tube was employed as a DNA and polymer supply scheme

Nanoparticle synthesis

A capillary tube with a tiny paper strip placed at one end was used to supply polymer solutions to the device substrate, as shown in Fig 2. As presented in our previous work (Qi et al., 2010), under SAW excitation, a paper can be used as a convenient media to automatically transport solutions from a reservoir to the device substrate for direct and efficient atomization without damaging the biomolecules. Since the paper strip employed in such setup is very small and the flow rate is also high within the paper such that the amount of molecules that could be left inside the paper is negligible.

In our experiment setup, a capillary tube filled with paper strip is mounted next to the SAW device, where the paper strip is in contact with substrate. A polymer solution is supplied from the other end of the capillary tube. A funnel was placed above the SAWdevice to collect the aerosols, which, following the air flow provided by a vacuum pump, subsequently passed through a long drying tube. The drying tube was fully embedded in a 300 ml hot water buffer and the temperature within the drying tube is kept between 40–50 degree. The aerosols are dried by evaporation inside the spiral tube, and shrank to small solid particles. These small particles are then deposited into another solution in a glass beaker. Dried particles will be able to bond to molecules with opposite charge instantaneously. Unbound polymers were removed by dialysis prior to sample characterization. As illustrated in Fig 3, if another layer is required, this suspension, can be collected and re-atomized into another polymer solution using the same experiment setup and atomization procedures described above.

Chitosan (Chi) (MW 50k-190k, Sigma), a positive charged natural polysaccharide with low cytotoxicity, (He et al., 1998; Boonsongrit et al., 2006) is employed as a model polymer in this study. Chi can efficiently condenses with plasmid DNA and can also increase permeability of macromolecules across gastrointestinal tract (Mayank & Bhavsar, 2007), thus making it an ideal vehicle for gene delivery and vaccines.

Carboxymethyl cellulose (CMC), is a negative charged polymer derived from cellulose. Polyelectrolyte complex can be formed by the electrostatic interaction between the -COOH group of CMC and the $-NH_2$ groups of chitosan (Anitha et al., 2009; Zhao et al.,

2009). In this study, we select CMC (Molecular weight 90k, Sigma) as the other model polymer. Chi was chosen to be the inner polymer layer, while CMC was selected to be the second layer of the multilayer polymeric nanoparticle to be synthesized by SAW atomization. We also employ Chi to form a third layer with CMC sandwiched between two layers of chitosan.
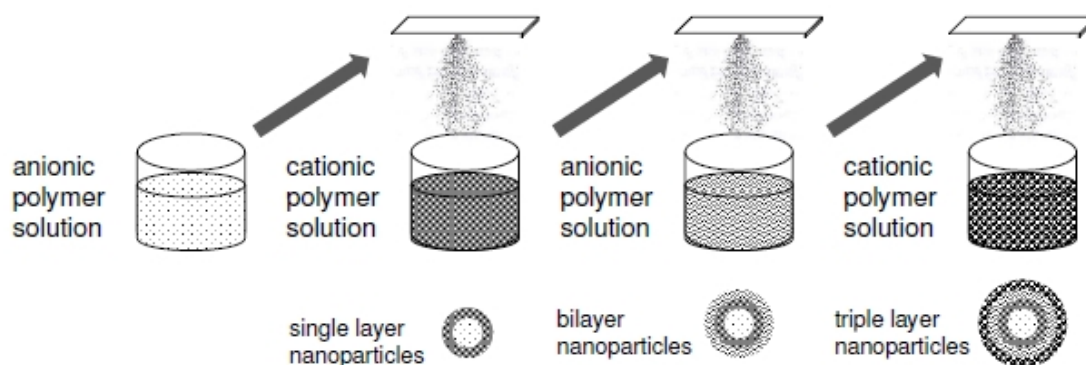

Fig. 3: Schematic diagram of multilayer nanoparticle preparation in layer-by-layer manner

Nano-aerosol atomization rate

In order to measure the amount of aerosol that our SAW microchip is capable of generating, we have set up a capillary flow meter apparatus as shown in Figure 4. The capillary flow meter consist of a glass tube that is marked at every 0.5cm with known inner diameter. We use a tissue paper embedded capillary tube as liquid delivery hose. One end of the wetted paper is placed upon the SAW microchip, while the other end is dipped in a fluid reservoir. A meniscus is formed between the paper and SAW microchip. The SAW wave draw liquid out from the paper as liquid on one end of the paper strip is consumed by atomization. The time for liquid to flow from one marker of the capillary tube to the consecutive marker was recorded, and the flow rate is calculated.

Particle characterization

In order to demonstrate the formation of bonding between polymeric layers, we characterize the properties of these LbL nanoparticles after each polymer atomization step using fourier transform spectroscopy (FTIR), size distribution and zeta-potential measurements. FTIR spectrum was employed to examine the chemical bonding between each polymer layer, thus providing evidence of polymer layers formation. Further evidence of the presence of polymer layering was obtained using zeta-potential measurements (Zetasizer Nano S, Malvern, UK). Particle size distribution was obtained using the Zetasizer Nano S (Malvern, UK). Particle size and morphology are further characterized using atomic force microscopy (AFM).

RESULTS AND DISCUSSION

The capacity the SAW microchip in terms of atomizing nano-size aerosol is measured as the flow rate of liquid being drawn by the SAW microchip during atomization. This

atomization rate is plot as a function of the input power for the SAW as shown in Fig 4. We observed that the atomization rate increases with increasing power, as expected given the larger energies delivered to the liquid to induce atomization by interfacial destabilization, this observation is in agreement with our earlier observations (Qi et al., 2009). The SAW microchip can generate aerosol up to ~200 μl/min with a power supply as low as 4 W, despite the miniaturize size of the chip, indicating that SAW atomization is an efficient means to generate nano-size aerosol compare to conventional electrospraying (Tang & Gomez, 1995).

(a)

(b)

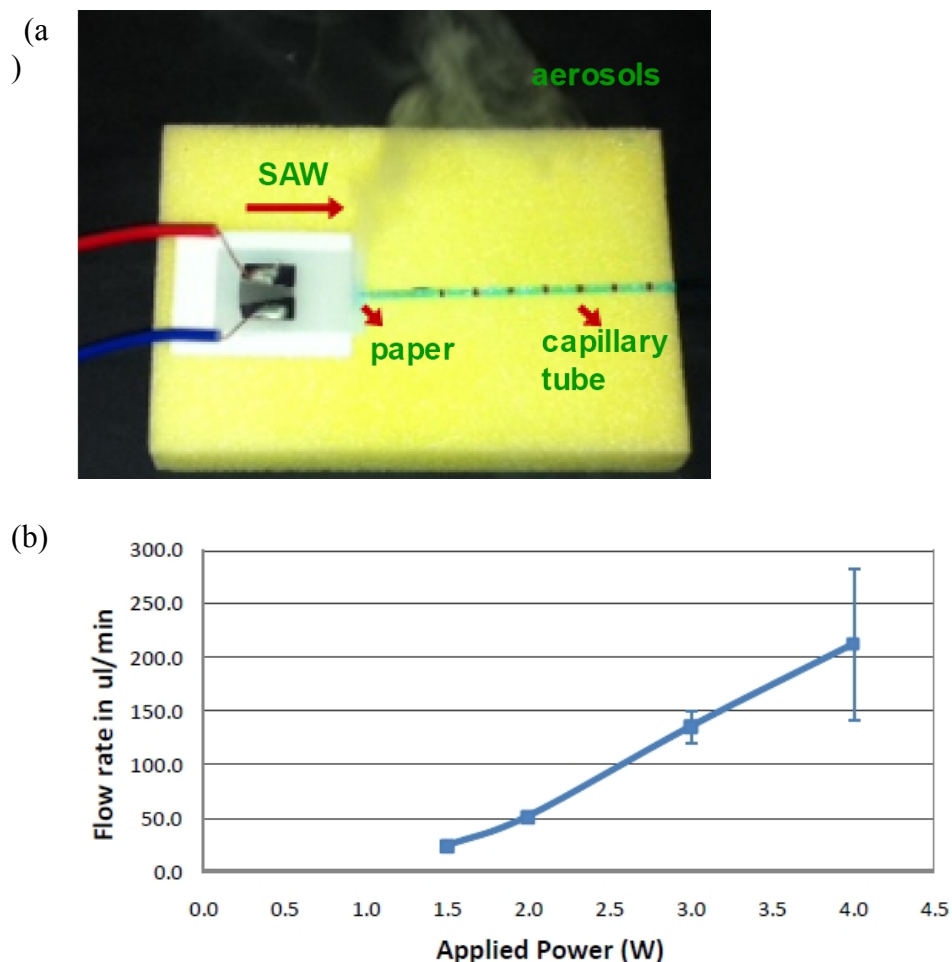

Fig. 4: (a) Photo ofcapillary flow meter set-up used to measured nano-aerosol atomization rate (b) Nano-aerosol atomization rate as a function of applied power. The trend line is included to aid visualization

Fourier transform spectroscopy (FTIR) was employed to examine the chemical bonding between each polymer layer, thus providing proof of the formation of the different polymer layers. Figure 5 shows the FTIR spectrum of polymeric particles. Curves 1 and 2 represent pure CMC and pure Chi molecules, respectively, while curves 3 and 4 show the varied spectrum of chitosan-CMC-chitosan triple-layer particle and chitosan- CMC double-layer particle, respectively. From curve 1, the bands at 1154, 1058, and 1026 cm^{-1} are corresponding to the polysaccharide skeletons of CMC. In Chi spectrum (curve

2), the characteristic bands at 1640 and 1558 cm$_{-1}$ are assigned to the amide I and amide II, respectively. The bands at 1052 and 1020 cm^{-1} are the characteristic of the polysaccharide skeleton of Chi (Rosca et al., 2005). Clearly, curves 3 and 4 show characteristic spectrum different from those of Chi and CMC. The amide I band at 1640 cm^{-1} in the double layer and triple layer nanoparticles have shifted to 1660 and 1650 cm^{-1}, respectively, reflecting the interactions between the -COOH groups of CMC and the -NH$_2$ groups of Chi. The shift in polysaccharide skeleton characteristic bands in the nanoparticles also suggested that ionic complexation between the Chi and CMC has successfully formed.
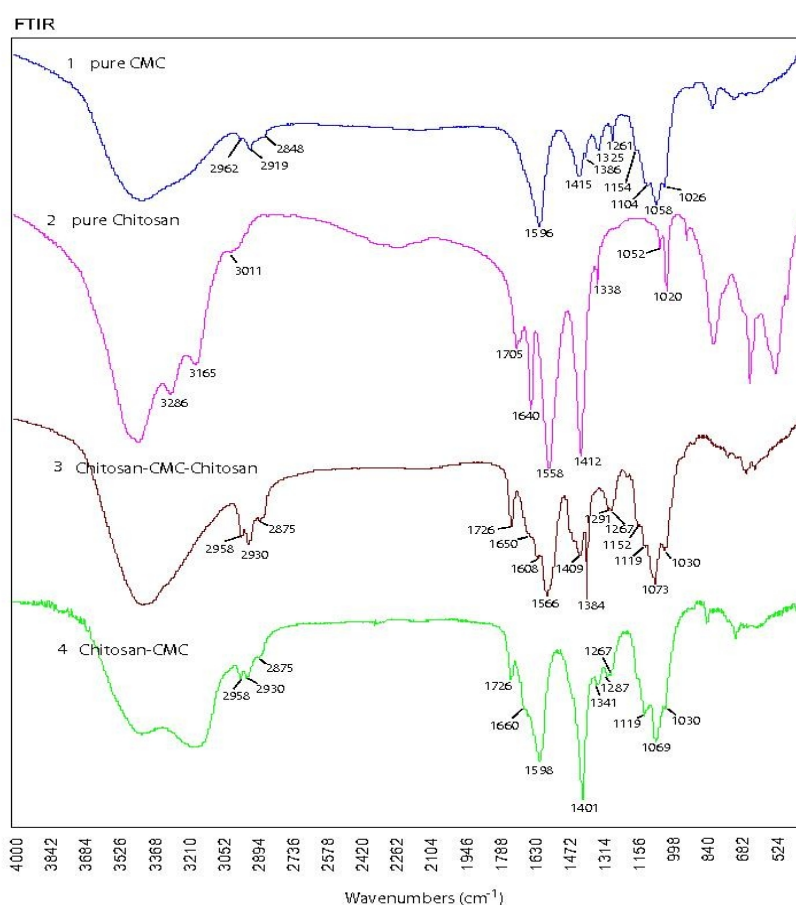

Fig. 5: FTIR spectrum of polymeric particles. Curves A and B are original CMC and chitosan samples, respectively. Curve C represents triple layer particles while curve D shows the spectrum of bilayer particles

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, as shown in Fig. 6(a). Representative samples 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. As showed in Fig 6(b) AFM image, 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.
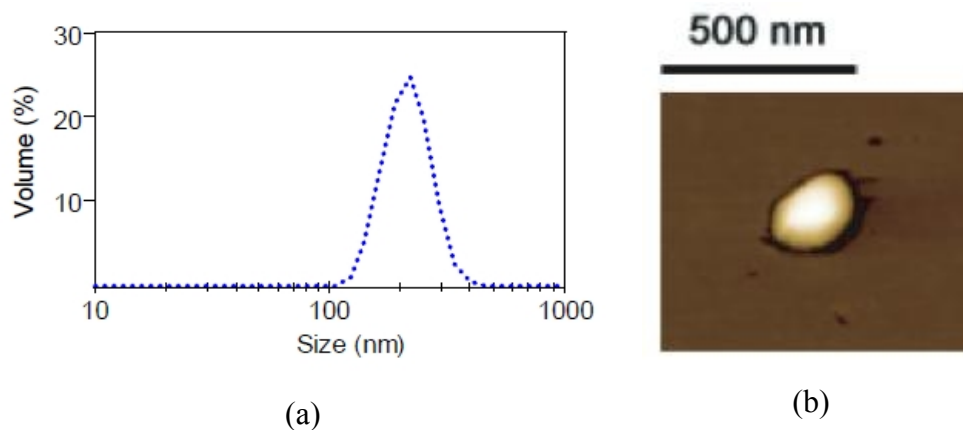

(a) (b)

Fig. 6 (a) Particle size and size distribution, and (b) representative AFM image of nanoparticles generated by SAW microchip. The nanoparticles are composed of Chi core with CMC outer layer.

The variation of Zeta-potential of polymeric particle with different layers serves as another proof of bondings. Table 7 lists 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.

Nanoparticle	Zeta potential (mV)
pDNA	-44.7±3.2
pDNA/Chi	6.84±4.0
pDNA/Chi/CMC	-18.2±1.5
pDNA/Chi/CMC/Chi	38.5±2.1

Tab.7: The zeta-potential of multilayer polymeric nanoparticles with Chi or CMC as outer layer

CONCLUSION

This study demonstrated using SAW atomization as a fast and efficient technique to synthesize multilayer polymeric nanoparticles for drug encapsulation usage. A serial of characterization have been conducted and shown the successful bonding between each polymeric layer. The size distribution obtained shows those synthesized multilayer

polymeric particles are in a narrow range (around 200 nm), which meets the requirement of many drug administrations (<1 mm). 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, has much less damage to drugs and vaccines, making it suitable for a wide range of drug deliveries and vaccines.

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BRIEF BIOGRAPHY OF PRESENTER

Dr Peggy Chan received degree in bioprocess engineering, and Ph.D. in chemical engineering from University of New South Wales, Australia. Later she worked as a post-doctoral fellow in the drug & gene delivery group at Institute of Bioengineering & Nanotechnology, A-Star Singapore. She was actively participated in developing nano-biomaterials for gene, siRNA and drug delivery. Later she was promoted to research scientist. Her research activities include developing novel injectable hydrogels for therapeutic protein delivery and tissue engineering applications. Some of these researches have gained considerable attention for their novelties, which were reported in top journals and patent application. Dr Chan works as a Lecturer at Department of Chemical Engineering, Monash University since March 2009. Her recent research interests are in the microfluidic biomaterials area, particularly in the development of microengineered scaffold and microfluidic synthesized biomaterials for drug delivery.