PLGA NANOPARTICLES SYNTHESIS USING 3D MICROFLUIDIC HYDRODYNAMIC FOCUSING

INTRODUCTION

Polymers have become increasingly popular drug delivery vehicles for applications ranging from targeted tumour therapy to modulation of the immune system. Polymer-encapsulated or conjugated drugs are frequently more effective than their freely delivered counterparts, since polymer-associated drugs are protected from degradation. This provides longer biological half-life and potentially improved efficacy with reduced systemic side effects [1].

Poly(lactic-co-glycolic acid) is a copolymer of lactic acid and glycolic acid. Depending on the ratio of lactide to glycolide, different forms of PLGA can be obtained. These are usually identified by the monomer ratios used [2].

PLGA is one of the most successfully used biodegradable polymers for the development of nanomedicines because it undergoes hydrolysis in the body to produce the biodegradable metabolite monomers, lactic acid and glycolic acid (figure 1). The degradation rate of PLGA depends on the molar ratio of lactic and glycolic acids in the polymer chain, molecular weight of polymer, the degree of crystallinity and the glass transition temperature (Tg) of the polymer. By manipulating the molecular weight and lactide/glycolide ratio, the degradation time of PLGA and, subsequently, the release profile can be varied accordingly [3].

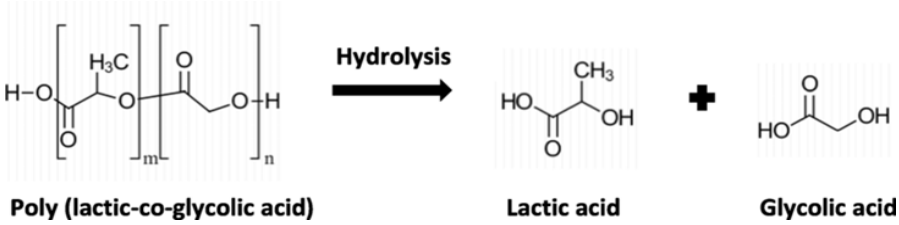

Figure 1: Hydrolysis of PLGA forming the degradation products.

Kharel, Sharad & Gautam, Archana & Dickescheid, Andreas& Loo, Joachim. (2018). Hollow Microparticles as a Superior Delivery System over Solid Microparticles for the Encapsulation of Peptides. Pharmaceutical Research. 35. 10.1007/s11095-018-2461-y.

A wide variety of therapeutic agents, including low-molecular-weight lipophilic or hydrophilic drugs, high-molecular-weight DNA or antisense DNA, can be encapsulated inside NPs. The encapsulated molecules are released at a sustained rate by diffusion or by degradation of the polymeric matrix [4]. This provides a sustained release formulation to elicit enhanced therapeutic efficacy.

Due to their nontoxic behaviour, biocompatibility and biodegradability properties, PLGA nanoparticles have been most extensively studied among all the commercially available polymers. This wide acceptance of the lactide/glycolide has made them a suitable candidate for biomedical applications such as ligament reconstruction, tracheal replacement, ventral herniorrhaphy, surgical dressings, vascular grafts, and nerve, dental and fracture repairs [5].

i. Microfluidic approach for highly-controlled PLGA nanoparticles production

Current methods of particle synthesis rely largely on batch stirred homogenizers (single emulsion, double emulsion...). However, major challenges persist in these systems with regard to process controllability and reproducibility, owing to the rapidity of the involved processes of mixing, nucleation, growth and agglomeration and their complex interactions when they take place concurrently [6].

	STANDARD BATCH METHOD	MICROFLUIDIC METHOD
Particle size distribution	Low	High
Reproducibility	Low	High
Live particle size control	No	Precise
Continuous (in line) production	No	Yes

Table 1: Comparison of PLGA nanoparticles synthesis specification between standard batch method and microfluidic method.

Microfluidic systems make use of the hydrodynamic focusing technique (2D HF to 3D, laminar flow to turbulent jet) to produce PLGA-based nanoparticles.

In the microfluidic solvent diffusion method, nanoparticles are synthesised in a microchannel after mixing between PLGA-acetone solution and water. In this approach, a stable laminar focused stream is created along the central channel meeting adjacent streams flowing at higher flow rates. Flow focusing squeezes the PLGA in an acetone stream between water streams. This results in rapid solvent exchange via diffusion and PLGA nanoparticle precipitation [7-9]. The PLGA containing solvent (acetone) and the antisolvent (water) form an azeotropic mixture.

The PLGA particle formation takes place at nucleation spots that are distributed through the mixture. Particle growth then occurs by the addition of PLGA to the surface of the newly formed particles [10].

In standard designs, the focusing is typically bidimensional (two-dimensional 2-D MHF) where the central flow is only focused in the horizontal plane. The simplest design consists of a central flow that is squeezed by two sheath flows from two sides (figure 2). However, one of the challenges for optimal NP synthesis by 2D HFF is that NPs made from polymers with a PLGA block of high molecular weight (>45 kDa) tend to aggregate on the channel walls, resulting in clogging of the channels and, reducing the robustness of operation [12].

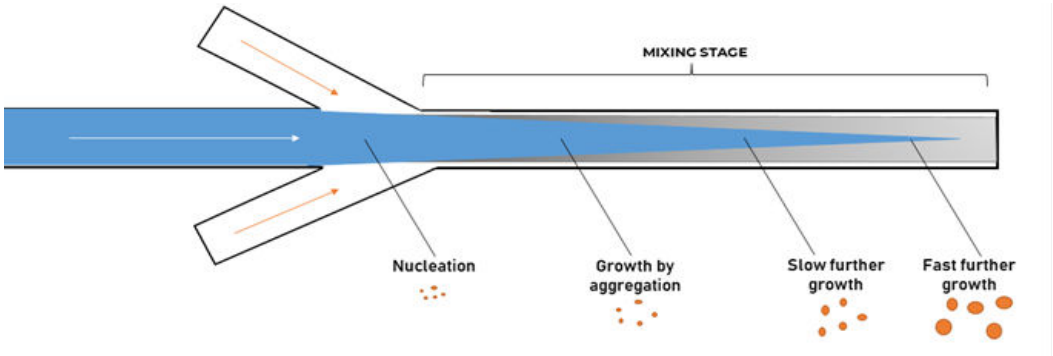

Figure 2: Schematic showing a 2D hydrodynamic flow focusing mediated nanoparticle production strategy.

To overcome these limitations, glass capillary devices have been investigated to generate a hydrodynamically focused 3D stream for nanoparticle synthesis [11].
In 3D hydrodynamic focusing, the aqueous phase fully surrounds the PLGA phase resulting in symmetric mixing of the fluidic inputs. The reagents and precipitating NPs are isolated from the channel walls, therefore aggregation and/or clogging is minimized [12].
By constraining the sample stream in the center of the microchannel where flow velocity is fast and stable, the 3D focused sample stream is expected to have a uniform width and improve the uniformity of the solvent/non-solvent ratio. This allows a robust and predictable nanoparticle synthesis, and facilitates the production of highly uniform nanoscale PLGA nanoparticles (Figure 3) [13-14].

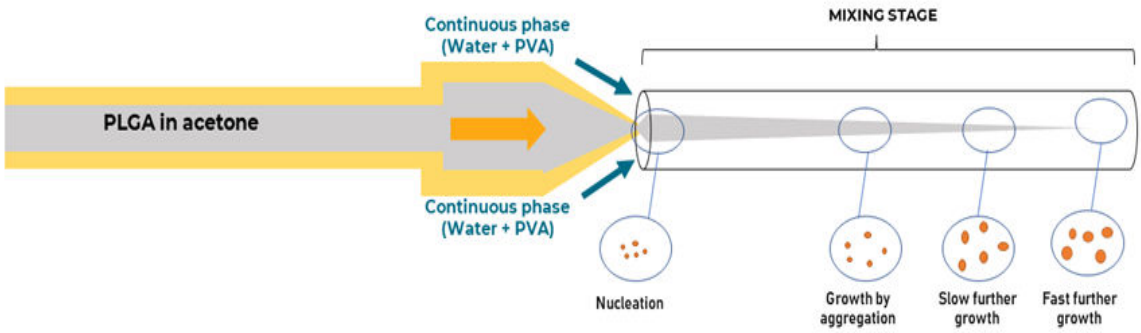

Figure 3. Schematic of 3D coaxial capillary device

In this application note, we show highly monodisperse PLGA nanoparticles synthesis using the RayDrop™ (Raydrop™ is a registered trademark of Secoya Technologies): a commercially-available glass capillary-based microfluidic device, with 3D axisymmetric geometry.

MATERIALS AND METHODS

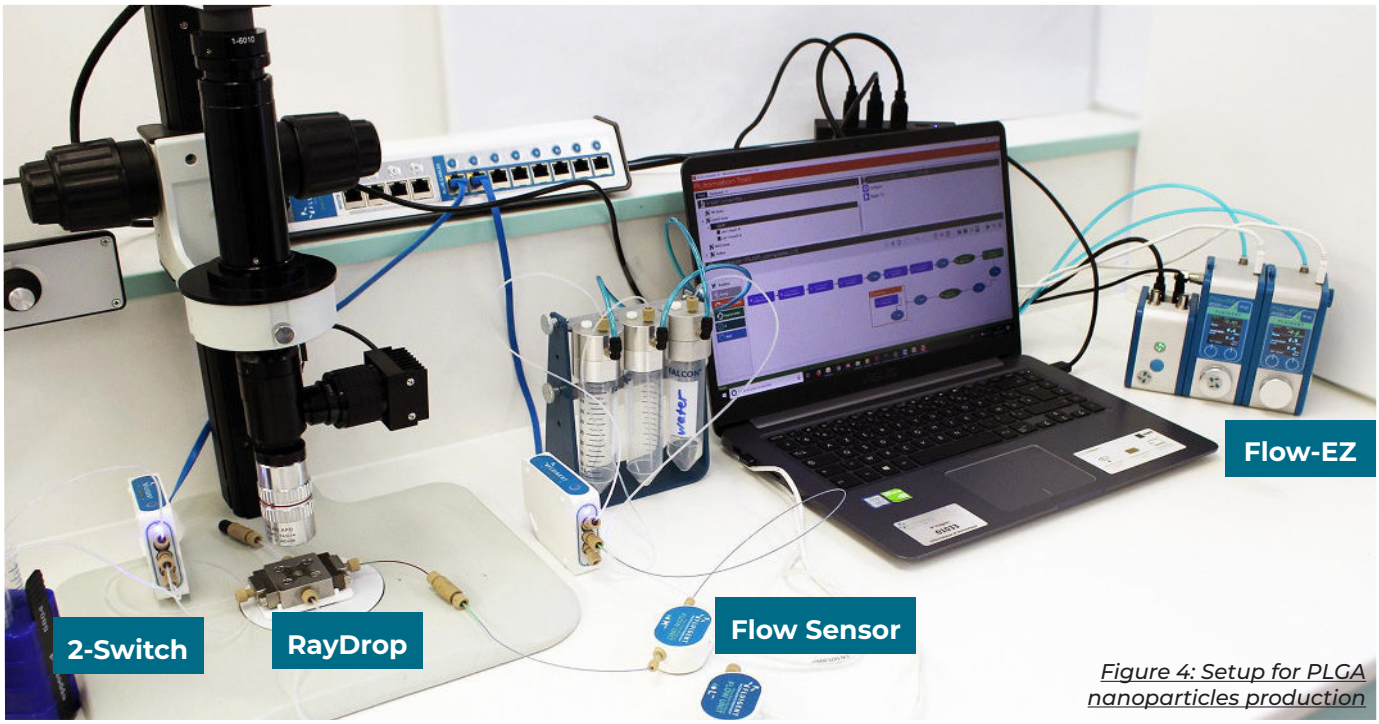

Figure 4: Setup for PLGA nanoparticles production

I. Materials

i. Microfluidic system

Pressure controller

Two **Fluigent Flow EZs** with 2 bars are shown above.

Flow sensor

The **Flow Unit** is a flow sensor that allows real time flow rate measurement. By combining a Flow Unit with the Flow EZ, it's possible to switch from pressure control to flow rate control, allowing for the generation of highly monodispersed droplets over a long period of time. Two Flow Unit M are used to monitor and control the flow rates of the dispersed and continuous phase during the run.

2-Switch

A **3-port/2-way microfluidic valve** is used to switch between pure acetone and acetone + PLGA solution. This allows for better reproducibility by priming the system and adds a cleaning step during the experiment.

Microfluidic device

RayDrop™ Single Emulsion with a 30 µm injection nozzle and 150 µm collection capillary (Secoya Technologies).
The Raydrop™ Single Emulsion is a versatile microfluidic device that can be used for highly precise fluid mixing. It's based on the alignment of two glass capillaries immersed in a pressurized chamber containing the outer phase. The inner phase exits the capillary through a 3D-printed nozzle, placed in front of a second capillary, where it's surrounded by the outer phase. This non embedded implementation of axisymmetric flow-focusing is referred to as coflow-focusing, and allows one to generate a hydrodynamically focused 3D stream. This prevents wettability issues that could appear in other microfluidic chips and allows for the generation of highly monodispersed nanoparticles with any type of fluid.

ii. Reagents

Outer phase

Deionized water and 1% Polyvinyl alcohol Mw 9000 – 10000 80% hydrolyzed (Sigma Aldrich) filtered with 0,2 µm polypropylene syringe filter, in a 50 ml falcon reservoir.
Connexion to the Raydrop : tubing PEEK "1/16" OD, 250 µm ID, 80 cm length
Flow-unit M 268 Scale factor (SF) =1 (calibration water).

Inner phase

Technical acetone, and PLGA Resomer 756 1 % (Sigma Aldrich) filtered with 0,2 µm polypropylene syringe filter, in a 15 ml falcon reservoir.
Connect to a switch connected to the M on IPA calibration with SF=1,2.
Total resistance tubing : PEEK "1/16" OD, 125 µm ID 2m length.

Inner phase to initiate and clean

Technical acetone filtered with 0,2 µm polypropylene syringe filter, in a 15 ml Falcon reservoir. Connect to a switch connected to a flow-unit M (the same as acetone + PLGA)

II. PLGA nanoparticle synthesis and size analysis

PLGA dissolved in acetone is injected through the nozzle, and mixing with water occurs by diffusion at the interface between the two fluids.
The microfluidic method offers the potential of a controlled system in which the organic and the aqueous phases are mixed in a microfluidic chip with precision settings, such as the total flow rate (TFR) and the flow rate ratio (FRR).
The TFR is the total flow rate at which both the fluids are mixed in the microfluidic platform, and the FRR is the volumetric ratio of the mixed organic and aqueous phases. This leads to precise NPs size control and a high degree of particle uniformity (polydispersity index below 0.2) [15]. These parameters are utilized in this application note.

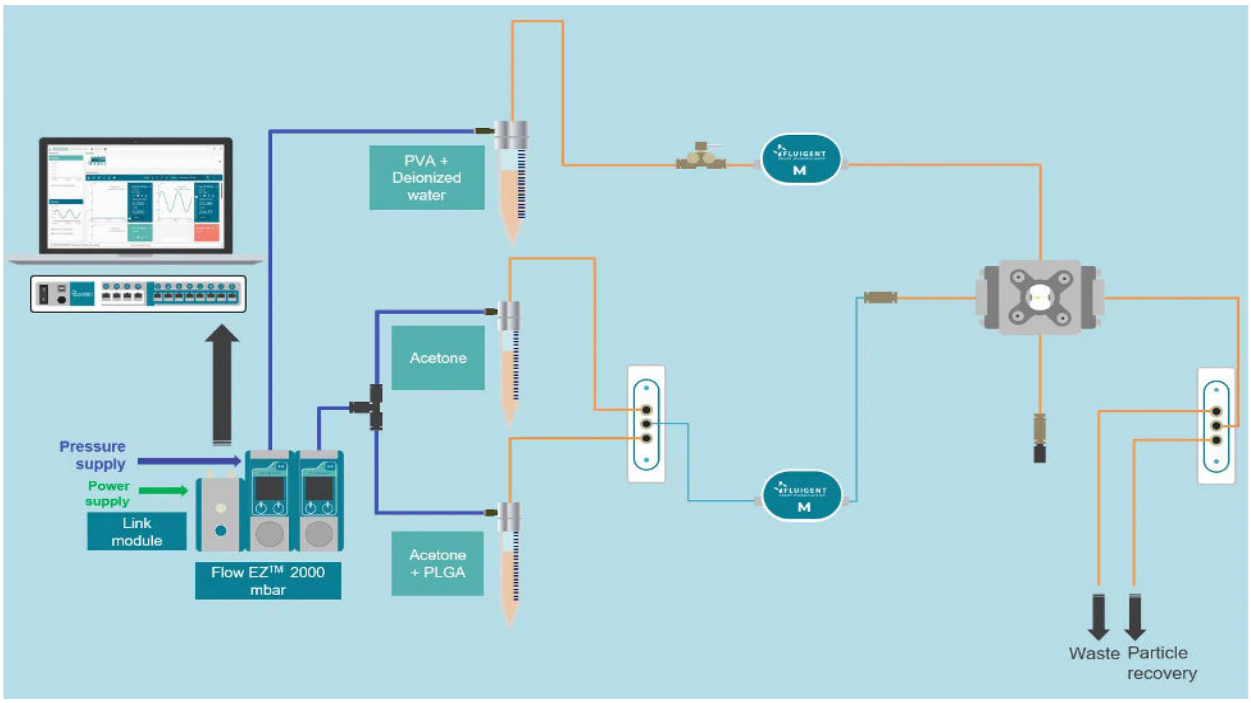

Figure 5: Scheme of the setup for PLGA nanoparticles production

The PLGA production system is illustrated in figure 5. Two reservoirs of 15 mL containing acetone, and PLGA in acetone are connected to a 2-SWITCH, which is connected to the microfluidic device via 1/16 in. PEEK tubing (inner phase). One reservoir of 50 mL containing water + PVA is connected to the second inlet of the device (outer phase). The tubing passes through flow units allowing flow rate measurement and control. A shut-off valve is placed on the continuous phase flow path to close the system when not in operation. Outlet tubing can be connected to a second 2-SWITCH to switch between waste and particle recovery.
It is also possible to work with L-Switch or HPLC-type injection loop to inject a solution into the system.

i. How to synthesise PLGA nanoparticles?

1. In the initial state, the Raydrop is filled with the continuous phase and the 2-switch for acetone or acetone + PLGA choice is switched off.

2. A continuous phase flow rate is applied. Then, the 15 mL reservoir containing acetone is selected by the 2-SWITCH and acetone is delivered as the inner phase. As a result, a co-flow of acetone and Water/PVA is formed.

3. As soon as a steady co-flow is reached (figure 6), the solution of PLGA in acetone can be substituted by changing the 2-SWITCH to the second position (PLGA/acetone).

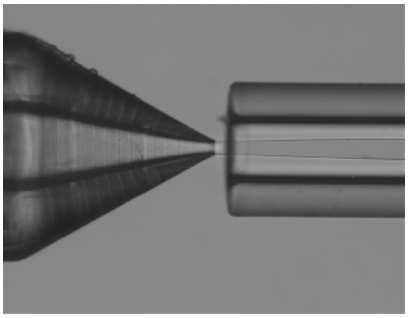

Figure 6. Steady co-flow of acetone and water/PVA.

4. With a flow rate of 20 µL/min and with the tubing described in *'Reagents'*, approximately 70 s are necessary to change liquid and therefore start producing PLGA nanoparticles.

5. Pressure and/or flow rate can be then adjusted to target the stream diameter desired TFR and the related PLGA nanoparticle size.

6. To stop the experiment, pure acetone is injected by switching the 2-SWITCH to first position (at 10 µL/min the liquid replacement takes approx. 140 s). The dispersed phase is then stopped, and the 2-switch is switched off (to avoid flow-back)

7. The shut-off valve on the continuous phase is switched off and the pressure on the continuous phase is stopped.

For more information about assembly, filling and cleaning using the Raydrop™, please refer to the Good Practice Guide on droplet generation process. In this application note, we determine the effects of the FRR and the TFR on PLGA nanoparticle size.

$Q_{PVA/Water}$ (µl/min)	**$Q_{PLGA/Acetone}$** (µl/min)	**TFR** (µl/min) = $Q_{PLGA/Acetone}$ + $Q_{PVA/Water}$	**FRR** [-] = $Q_{PVA/Water}/Q_{PLGA/Acetone}$
23	6	29	3.83
70	7	77	10
62	8	70	7.75
45	25	70	1.8

Table 2. Summary of the flow rates used, with their respective FRR and TFR.

ii. PLGA nanoparticle size analysis

Analysis using a Dynamic Light Scattering system (DLS, Nanoflex 180 from Microtrac), assuming n=1.50 for PLGA, n =1.33 for the water phase and a spherical shape for the PLGA NP. For each sample, 3 measurements of 30 s each are performed and the mean value is calculated.

RESULTS & DISCUSSION

Table 2 shows the used pressures, flow rates, TFR and FRR of the PLGA/acetone solution. We observe from the image (figure 6) a well-centered and steady inner flow, confirming enhanced flow stability using the Raydrop™.

Dispersed phase (1% PVA / Deionized Water)		**Phase PLGA (1% PLGA / Acetone)**		**TFR** (µl/min)	**FRR** [-]
P (mbar)	**Q (µl/min)**	**P (mbar)**	**Q (µl/min)**		
350	23	150	6	29	3.83
1510	70	446	7	77	70
1750	62	294	8	70	7.75
1032	45	334	25	70	1.8

Table 3. Summary of the flow rates used, with the TFR, FRR and their corresponding micrographs.

The PDI obtained for each DLS measurement varies between 0.05 and 0.1.
Figure 7 shows the PLGA nanoparticle median diameter as a function of the FRR. We can observe that PLGA nanoparticles with a diameter ranging from ~110 nm to ~250 nm were generated under these conditions.
In addition, we observe that when the FRR increases, the PLGA nanoparticle size decreases. In fact, a diameter of 110 nm is obtained using a FRR of 10, while a diameter of 250 nm is obtained using a FRR of 1.8. This result is in compliance with microfluidic methods and is attributed to the increasing degree of focusing experienced by the center stream of PLGA [17].
This level of control of nanoparticle size would not have been possible using traditional methods. With the RayDrop™, it's possible to obtain a wide range of sizes depending on the parameters used.
PLGA nanoparticles ranging from approximately 110 nm to 250 nm have been generated, confirming the potential of the Raydrop™. It's worth noting that it's possible to reach a broader nanoparticle size range by using other values of FRR.

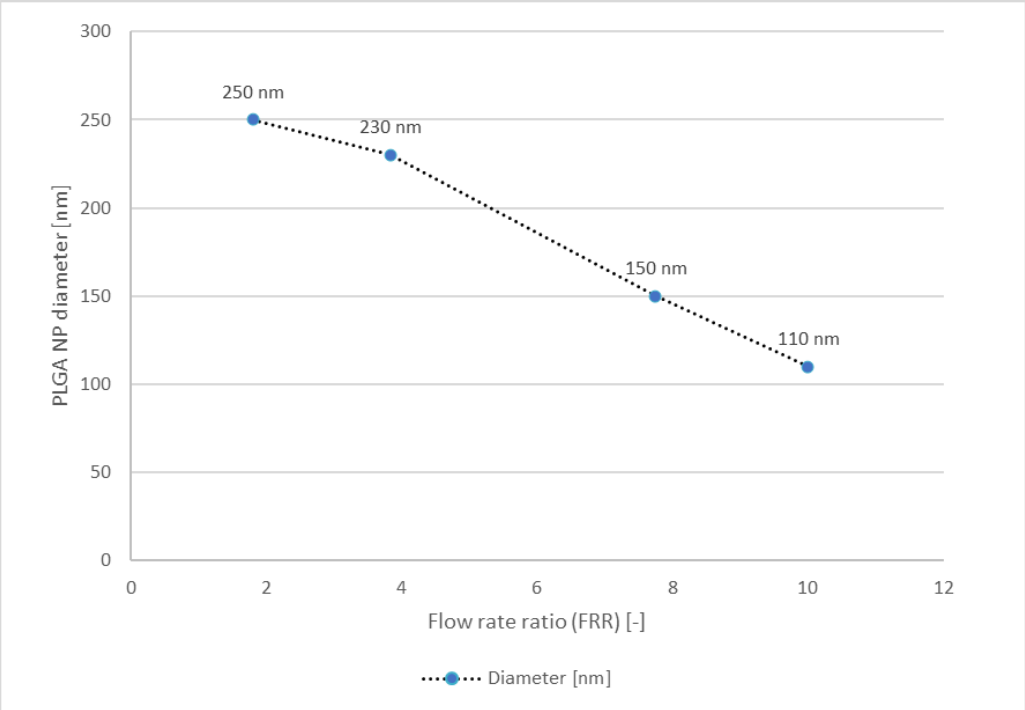

Figure 7. PLGA nanoparticles mean diameter as a function of the flow rate ratio (FRR).

CONCLUSION

The PLGA nanoparticle size can be controlled by tuning the synthesis method and parameters of operation.

Conventional fabrication methods for these particles are suitable for mass production but show a lack of controllability and reproducibility. Microfluidics has been investigated to produce PLGA nanoparticles with high throughput, monodispersity and reproducibility.

In this application note, we have demonstrated the production of PLGA nanoparticles using a microfluidic system (3D microfluidic hydrodynamic flow) consisting of pressure-based flow controllers and the RayDrop™ microfluidic device with standard configuration.

PLGA nanoparticles ranging from 110 to 250 nm were generated. This size range is optimal for various biological applications, such as tumour targeting, as it falls within the compatible size range. The Polydispersity Index (PDI) ranges from 0.05 to 0.1. Sizes can be adjusted by controlling the device flow input parameters, particularly the flow rate ratio (FRR). In this way, the ability to synthesize PLGA nanoparticles in a more controllable and reproducible way opens up possibilities for custom tuning surface properties.

A full-featured, cost-effective and readily available platform for the production of monodisperse PLGA nanoparticles is now available. This allows for control of nanoparticle size and frequency by adjusting flow parameters.

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