Microfluidic Synthesis of Uniform PLGA Microparticles with the Dolomite Mitos System

Optimizing Particle Size and Distribution for Enhanced Biomedical Applications

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1 Introduction

Poly(lactic-co-glycolic acid) (PLGA) microparticles are extensively utilized in the biomedical field due to their exceptional biocompatibility, biodegradability, and versatility. These properties make PLGA an ideal material for applications such as controlled drug delivery, vaccine delivery, gene therapy, and tissue engineering. However, achieving precise control over the size and morphology of PLGA microparticles is critical for ensuring predictable pharmacokinetics and pharmacodynamics, which are essential for the efficacy and safety of therapeutic interventions. Traditional bulk synthesis methods often fall short in delivering the required precision and uniformity, leading to inconsistent therapeutic outcomes.

Achieving consistent particle size and morphology is the primary challenge in synthesizing PLGA microparticles, as illustrated in Figure 1. Variations in particle size can lead to unpredictable drug release profiles, impacting the safety and effectiveness of the drug delivery system. Microfluidic technology offers a significant advantage by providing unparalleled control over the particle formation process. These systems enable precise tuning of particle size through the adjustment of flow rates and other parameters, which is crucial for producing highly uniform particles and ensuring consistent drug release and therapeutic efficacy.

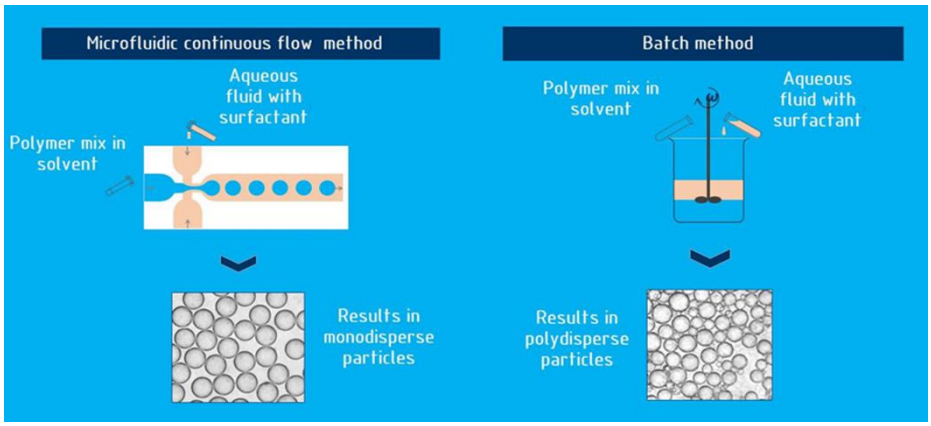

Figure 1 Batch particle synthesis vs microfluidic particle synthesis.

The Dolomite Mitos System, shown as in Figure 2, stands out as a robust and scalable tool for generating PLGA microparticles. It offers advanced capabilities such as precise flow control, temperature regulation, and automated operations, ensuring efficient and reproducible particle generation. The system's adaptability in modifying flow rates, phase ratios, and chip geometries allows researchers to customize particle properties according to specific requirements. Additionally, the Dolomite Mitos System accommodates a wide range of fluids and additives, facilitating the incorporation of diverse components into PLGA particles for various applications.

Figure 2 Dolomite Mitos System with Imaging Pack.

PLGA microparticles have diverse and significant applications in the biomedical field:

- **Drug Delivery**: Encapsulation of drugs within PLGA microparticles enables controlled and sustained release, improving therapeutic outcomes and patient compliance.
- **Vaccine Delivery**: PLGA particles can encapsulate antigens and adjuvants, enhancing the efficacy and immunogenicity of vaccines.
- **Gene Therapy**: PLGA microparticles protect genetic material and facilitate targeted delivery to specific cells or tissues, improving the efficiency of gene therapy.
- **Tissue Engineering**: PLGA scaffolds support cell growth and tissue regeneration, providing structural support for the development of new tissues.

The precision offered by microfluidic systems like the Dolomite Mitos System is crucial for optimizing these applications, ensuring that PLGA microparticles meet the stringent requirements for size, uniformity, and functionality necessary for advanced drug delivery and biomedical technologies.

This application note focuses on the generation of PLGA microparticles using the Dolomite Mitos System, utilizing two Dolomite Application Packs for different PLGA droplet/particle size ranges. The aim is to demonstrate the feasibility and efficacy of employing microfluidic technology for producing uniformly sized PLGA microparticles, thereby contributing to advancements in drug delivery and other biomedical applications. By leveraging the capabilities of the Dolomite Mitos System, researchers can achieve precise control over particle synthesis, ensuring the consistency and reliability needed for advanced therapeutic applications. Notably, the Dolomite PLGA application packs include 3D flow focusing chips, which are ideal for particle synthesis. These chips reduce surface fouling through a unique "pore" structure at the outlet side of the droplet-forming junction. This design helps prevent polymer solutions from fouling the chip surfaces, thus extending the production run duration and chip lifetime. Furthermore, Dolomite's Aqua-Phase is used as the continuous phase to produce PLGA particles, enhancing the stability and consistency of the particle generation process.

2 Methods

2.1 Dolomite Mitos System Set up with PLGA Application Packs

The Dolomite Mitos System (Part number: 3201105) was employed to generate PLGA beads using the PLGA Particles 10-60 µm Application Pack (Part number: 3201094) and the PLGA Particles 40-140 µm Application Pack (Part number: 3201095). This setup effectively facilitated the generation of PLGA beads through precise microfluidic control. Figures 3 and 4 illustrate the schematic diagrams of the system setup for each application pack.

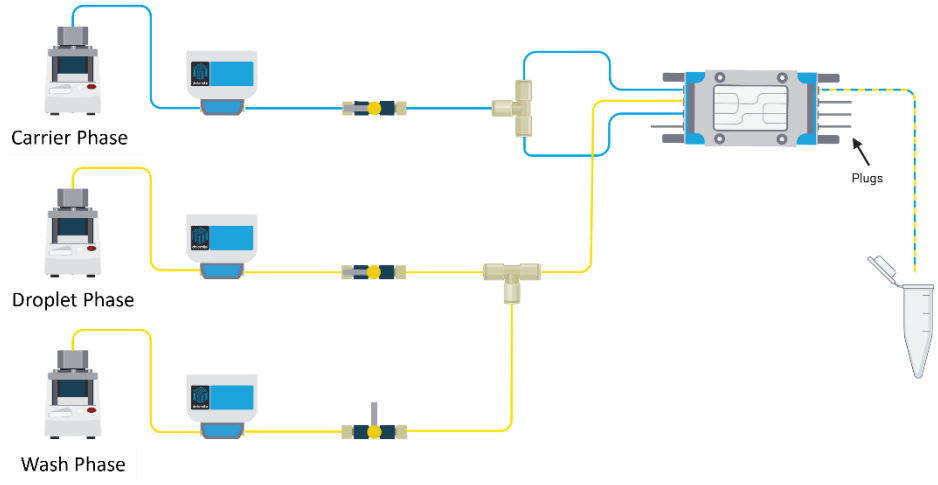

Figure 3 Schematic drawing for the Dolomite Mitos System with the PLGA Particles 40-140 µm Application Pack (Part number: 3201095).

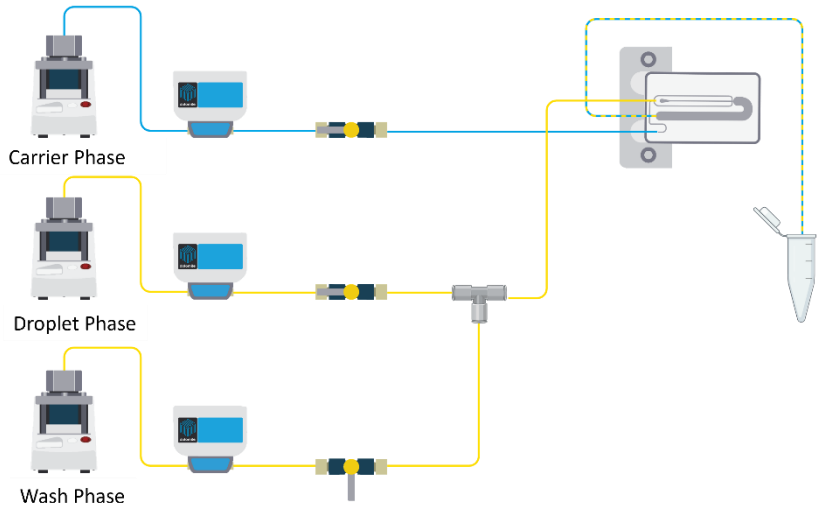

Figure 4 Schematic drawing for the Dolomite Mitos System with the PLGA Particles 10-60 µm Application Pack (Part number: 3201094).

P-Pumps Configuration: Three precision P-Pumps were utilized to ensure the accurate delivery of fluids.

- Pump 1: Administered the carrier phase, Aqua-Phase (Part number: 3200775 for a 500 mL pack).

- Pump 2: Managed the droplet phase, using PLGA solution (CAS: 26780-50-7) in ethyl acetate (CAS: 141-78-6).
- Pump 3: Flush pump. Pure ethyl acetate as wash phase.

Fluidic Connections: The fluidic connections were meticulously assembled using specialized tubing kits for each application pack. The connections included FEP tubing, two-way in-line valves, T-connectors, and specific connectors for each type of chip.

Imaging and Documentation: Junction imagery was captured using the Dolomite Imaging Pack (Part number: 3201107, shown in the right of Figure 2) equipped with a high-speed microscope and camera to document and analyze the bead formation process.

2.2 Reagents

Measure the required amount of PLGA to achieve a 10% (w/v) concentration based on the total solution volume. Transfer the PLGA into a clean, dry container, and add the appropriate amount of ethyl acetate. Stir gently and apply mild heat if necessary to fully dissolve the PLGA. Once dissolved, dilute the 10% PLGA solution with ethyl acetate to prepare 1% and 3% PLGA solutions.

All liquids were filtered before use.

2.3 3D Flow Focusing Chips

The Dolomite 3D Flow Focusing chips, available in various junction sizes, are included in both PLGA application packs. The PLGA Particles 10-60 µm Application Pack (Part No. 3201094) features 3D flow focusing chips with 30 µm and 65 µm junctions, while the PLGA Particles 40-140 µm Application Pack (Part No. 3201095) includes chips with 100 µm and 170 µm junctions.

The 3D Flow Focusing Droplet Chip (shown as in Figure 5) is designed to efficiently create droplets while minimizing fouling of the channel walls post-junction. This is achieved through a unique pore structure at the outlet side of the droplet-forming junction, which generates a 3D sheath flow in the pinch-off region. This flow pattern prevents PLGA fouling on the junction surface, enabling extended operation without the risk of blockage. The design is particularly effective for handling fluids prone to fouling, ensuring reliable and consistent droplet production over long durations.

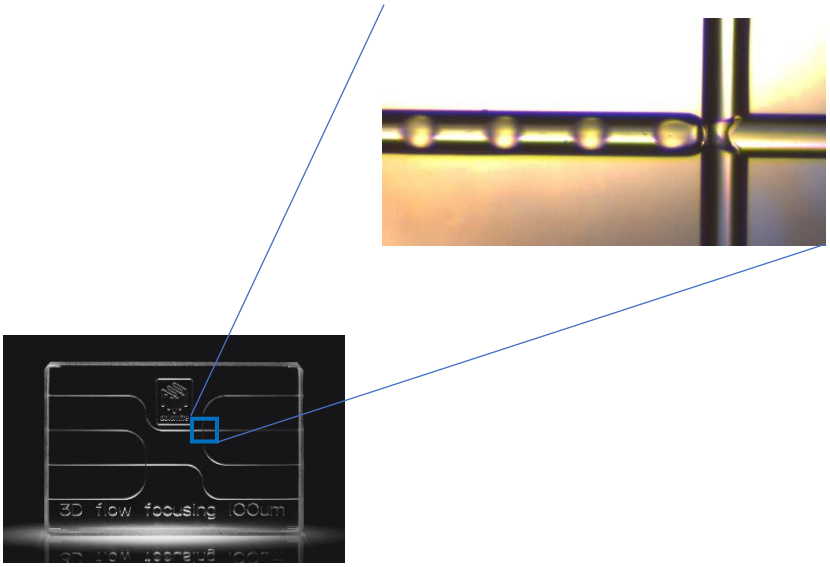

Figure 5 Dolomite 3D Flow Focusing 100 µm Chip (left), X-junction of the 3D pore chip forming droplets (right).

2.4 Priming Method

Priming begins with in-line valve for droplet phase closed and valve for wash phase open. In pressure control mode, ethyl acetate droplets are established in the carrier fluid to prevent backflow, jetting, or chaotic flow, which could cause blockages. This step purges gases from the fluid pathways and conditions the chip surface with surfactant, ensuring chemical compatibility with all wetted parts. Priming is best performed at a relatively high pressure (2-3 bar).

Once droplet production is stable, switch valve for droplet phase open and valve for wash phase closed, keeping the droplet pump pressure the same as the priming pump. This change introduces the droplet fluid to the chip. After a few minutes, the polymer mix reaches the chip, initiating polymer particle production. Both pumps are then switched to flow control mode.

To shut down, the process is reversed: switch the pumps to pressure control mode, revert the valves to the priming fluid flow, and cleanse the system for 10-15 minutes.

Notes:

- Temperature control is important in pressure control mode to maintain a constant flow rate, especially during long runs.
- Flow control mode allows for small pressure adjustments to maintain flow rate but cannot compensate for significant blockages.

2.5 PLGA Solidification

Once droplet formation reaches stability, transition the output tubing from the droplet generation setup into a collection vial. Ensure that the collection vial has been preloaded with an adequate amount of Aqua-Phase, serving as the collection phase, and a precleaned stirring bar. Mount the collection vial onto a stirrer plate and set the stirring speed to 250 rpm. This setup enables continuous stirring within the collection vial, maintaining homogeneity within the Aqua-Phase and facilitating further processing or analysis of the generated droplets.

As the droplets initially formed a thin layer on top of the Aqua-Phase, they gradually sank to the bottom while the PLGA particles solidified. To ensure homogeneity and facilitate further processing, add an appropriate amount of Aqua-Phase into the collection vial at the end of the experiment. Maintain stirring for 2 hours to promote thorough mixing and dissolution of the formed PLGA microbeads within the Aqua-Phase. After the mixing period, leave the vial open overnight to allow for evaporation of any residual solvents and to promote air exchange, aiding in the stabilization of the solution.

2.6 Solvent Flow Rate Calibration

The flow rate on the Dolomite Flow Control Center (FCC) software was calibrated against standard aqueous or organic phases. Despite ethyl acetate not being included in the standards, its flow rate would be adjusted based on a calibration curve.

The ethyl acetate flow was initiated and run for a predetermined duration. The volume of ethyl acetate passing through the system was collected in an Eppendorf tube. Before and after volume collection, the weight of the Eppendorf tube was recorded to calculate the actual flow rate. This process was repeated several times to ensure accuracy, with flow rate settings adjusted as necessary based on the obtained actual flow rates.

3 Results and Discussion

3.1 PLGA Droplet Formation

In the 3D flow focusing chips, PLGA droplets are generated through a highly controlled microfluidic process using the Dolomite Mitos System. This system integrates three fluid streams: the PLGA solution in ethyl acetate as the droplet phase, Aqua-Phase as the carrier phase, and pure ethyl acetate as the wash phase.

Within the microfluidic chip, the PLGA solution is injected into the central channel, while the carrier fluid flows through the adjacent side channels. At a narrow junction in the chip, these fluids converge, with the carrier fluid exerting precise shear forces on the PLGA solution, breaking it into droplets. This flow-focusing mechanism efficiently produces monodisperse PLGA droplets, with their size carefully controlled by adjusting the flow rates of both the PLGA solution and the carrier fluid, as well as by the chip's geometry.

Our microfluidic setup, showcased here with the Dolomite Mitos System using two of the four 3D Flow Focusing Chips, includes the 100 µm (A) chip from the PLGA Particles 40-140 µm Application Pack (Part number: 3201095) and the 65 µm (B) chip from the PLGA Particles 10-60 µm Application Pack (Part number: 3201094). These chips enabled the formation of uniformly sized PLGA droplets in Aqua-Phase, as demonstrated in Figure 6. The images highlight the precise formation and consistent collection of these monodisperse droplets, with scale bars representing 200 µm. The precise control of shear forces and solvent extraction was crucial for achieving reproducibility and scalability in particle fabrication throughout our experiments.

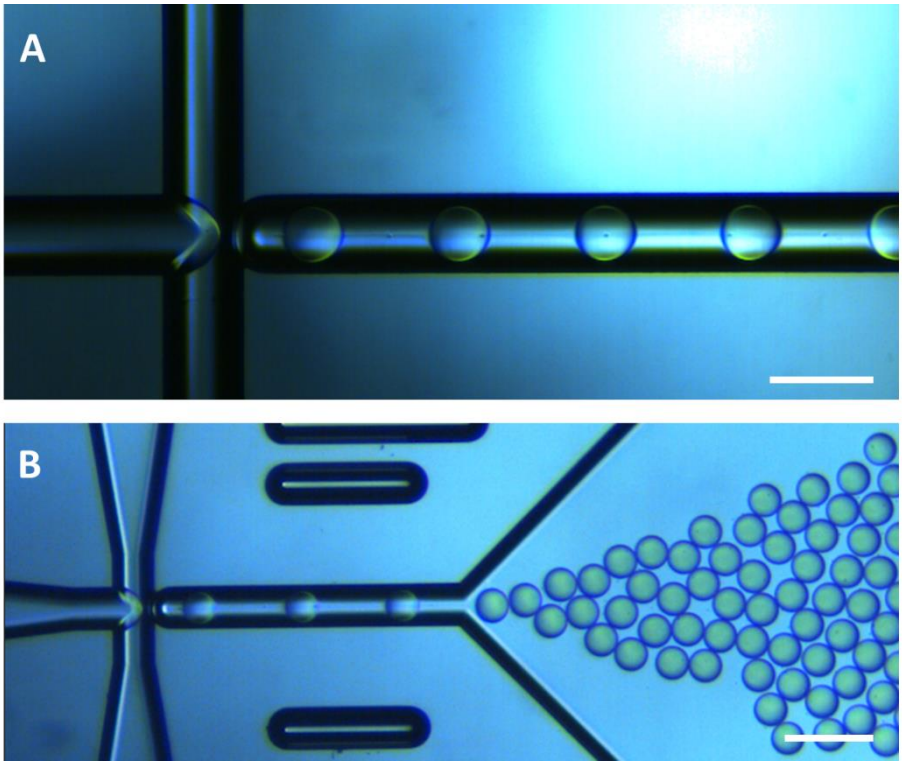

Figure 6. 100 µm (A) and 65 µm (B) 3D Flow Focusing Chips producing monodisperse PLGA droplets in Aqua-Phase as carrier phase. Scale bar = 200 µm

3.2 PLGA Particle Formation

Ethyl acetate was chosen as the solvent for this process because of its moderate solubility in water and compatibility with the microfluidic system. Although other solvents such as dichloromethane (DCM) are commonly used in PLGA particle synthesis due to their faster evaporation rates, ethyl acetate offers a safer and less toxic alternative. Furthermore, the

Dolomite Mitos System is compatible with a range of solvents, including DCM, allowing for flexibility depending on the specific requirements of the experiment.

After droplet formation, ethyl acetate begins to diffuse into the surrounding aqueous phase, given its ~8% (v/v) solubility in water at room temperature. This diffusion depletes the droplets of solvent, gradually increasing the PLGA concentration until supersaturation occurs, leading to the precipitation of PLGA particles. Solvent removal is a continuous process, driven by the diffusive movement of ethyl acetate from the droplet, across the droplet/carrier fluid interface, and into the carrier fluid. As long as sufficient fresh water surrounds each droplet, this diffusion continues, gradually reducing the droplet volume and resulting in visible shrinkage.

The conceptual molecular schematic in Figure 7 illustrates this solvent removal process. Initially, PLGA droplets are rich in ethyl acetate (left). As ethyl acetate diffuses out into the aqueous phase (center), the droplets shrink in size, eventually leaving behind solidified PLGA particles suspended in the aqueous fluid (right).

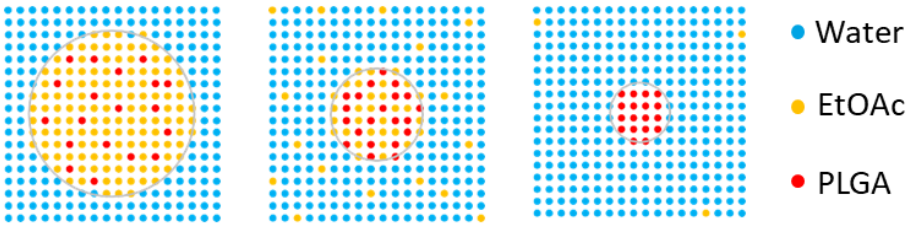

Figure 7. Conceptual molecular schematic of solvent removal. Left: On-chip at the point of droplet formation, Center: ethyl acetate loss from droplet as it travels downstream in tubing and in collection vessel. Right: Final PLGA solid particle in aqueous fluid.

The droplets formed on-chip are collected in a 1.5 mL Eppendorf tube pre-filled with 100 µL of carrier fluid (Aqua-Phase). Due to the lower density of ethyl acetate compared to water, a layer of PLGA in ethyl acetate initially floats on the aqueous phase. As the solvent is gradually extracted and evaporated, the PLGA solidifies, causing the beads to sink to the bottom of the tube.

After solvent evaporation, the PLGA beads were washed and resuspended in Molecular Biology grade water for imaging. Microscope images of the resulting PLGA beads are shown in Figure 8. Panels A-D illustrate the uniformity of particle formation across different samples, with scale bars representing 25 µm. These images highlight the precise formation and consistent size distribution achieved through careful control of the microfluidic process.

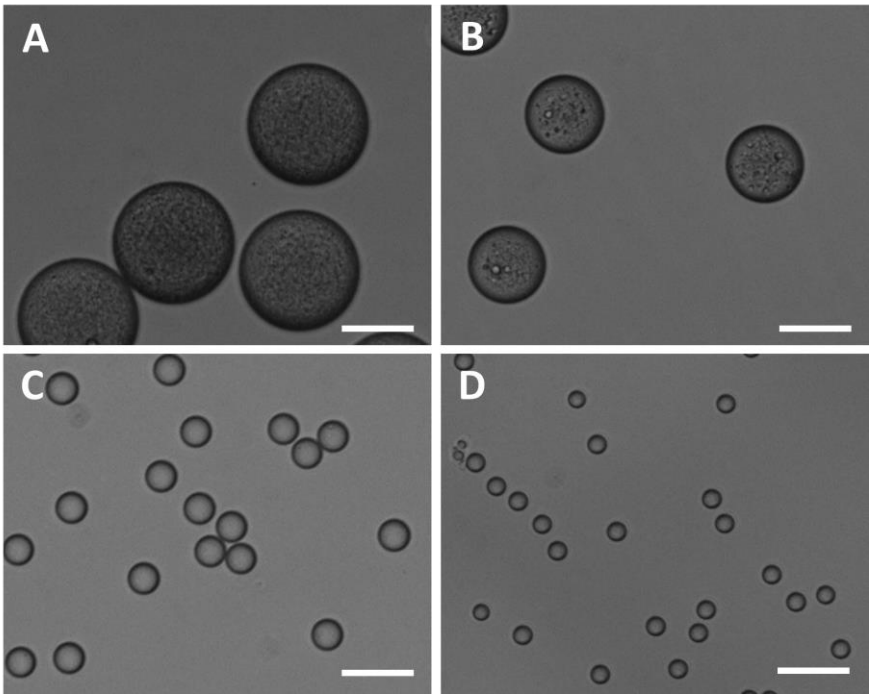

Figure 8 Microscope images of PLGA particles at various concentrations post solvent evaporation and washing, displaying monodispersed PLGA particles suspended in molecular biology grade water. Scale bar = 25 µm.

3.3 Importance of Chip Junction Size in PLGA Droplets and Particles

Selecting the appropriate chip junction size is critical for controlling both droplet and particle sizes during PLGA particle synthesis. The junction size of the 3D Flow Focusing Chips directly influences the size of the droplets formed, which in turn determines the final particle size after solvent evaporation. Therefore, careful selection of the chip junction size is essential to achieve the desired droplet and particle characteristics tailored to specific experimental needs.

Figures 9 clearly demonstrate the relationship between the junction size of different 3D Flow Focusing Chips and the resulting droplet and particle sizes, combining data from PLGA concentrations of 1%, 3%, and 10% in ethyl acetate. As expected, larger junction sizes produce larger droplets, while smaller junction sizes result in smaller droplets. Specifically, the 170 µm chip generates droplets ranging from approximately 89 µm to 180 µm, the 100 µm chip produces droplets between 40 µm and 105 µm, the 65 µm chip forms droplets in the range of 10 µm to 50 µm, and the 30 µm chip creates the smallest droplets, ranging from about 6 µm to 25 µm.

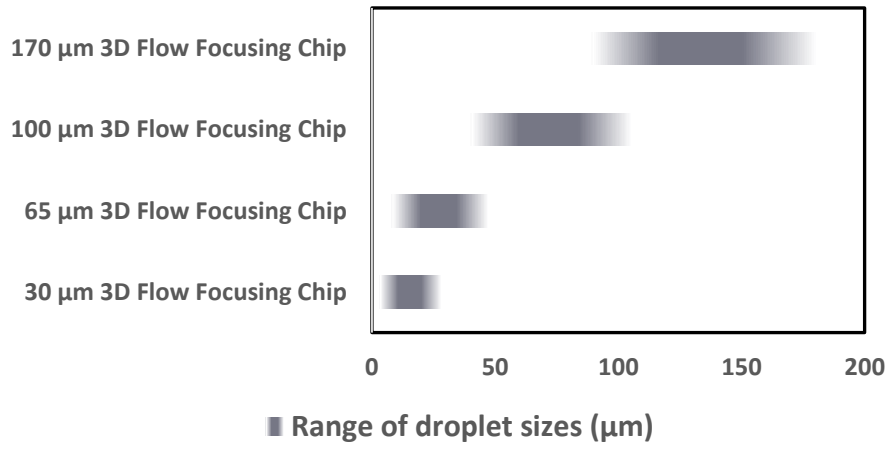

Figure 9 Range of droplet size produced using chips in different junction sizes.

This trend continues with particle sizes, where chips with larger junction sizes produce a broader range of larger particles, and smaller junction sizes yield smaller particles with a narrower size distribution, shown in Figure 10. For instance, the 170 µm chip produces particles ranging from 20 µm to 75 µm, the 100 µm chip generates particles between 5 µm and 39 µm, the 65 µm chip produces particles in the range of 2 µm to 19 µm, and the 30 µm chip yields the smallest particles, between 1 µm and 7 µm. Additionally, the concentration of PLGA in ethyl acetate also impacts particle size, contributing to the variation in particle sizes corresponding to the size of the generated droplets.

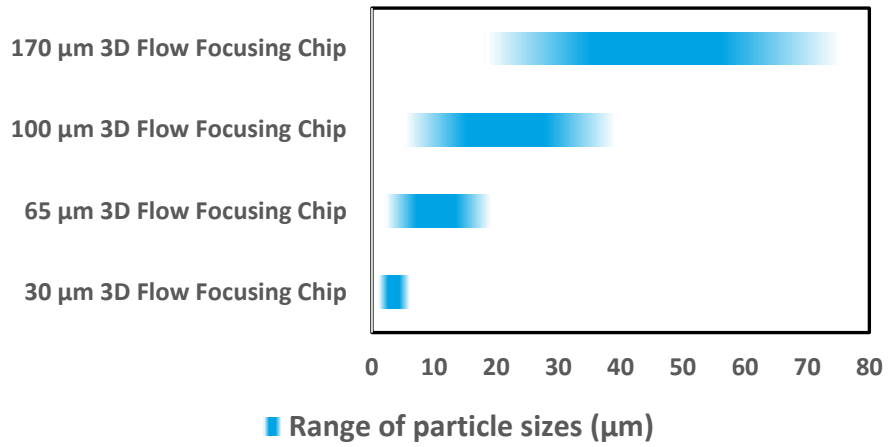

Figure 10 Range of particle size produced using chips in different junction sizes.

3.4 Impact of Flow Rate on Droplet Size

The flow rate is an essential factor in determining droplet size during the formation of PLGA droplets in the carrier phase, which in this case is Aqua-Phase. To illustrate this effect, we used a 1% PLGA solution in ethyl acetate with a 65 µm 3D Flow Focusing Chip, as shown in the graph below (Figure 11).

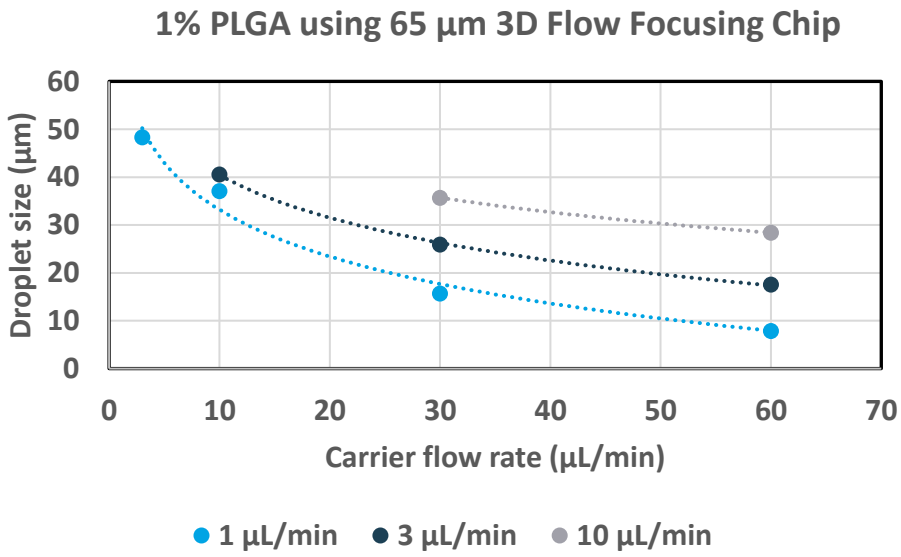

Figure 11 1% PLGA in 65 um 3D Flow Focusing Chip.

The graph depicts the relationship between carrier flow rate and droplet size at three different PLGA flow rates: 1 µL/min, 3 µL/min, and 10 µL/min. As the carrier flow rate increases, the droplet size decreases consistently across all three PLGA flow rates. This trend can be attributed

to the increased shear forces exerted by the carrier fluid at higher flow rates, which more effectively pinches off smaller droplets from the PLGA solution.

For example, at a PLGA flow rate of 1 µL/min (represented by light blue), the droplet size decreases from approximately 50 µm to 10 µm as the carrier flow rate increases from 3 µL/min to 60 µL/min. A similar trend is observed for PLGA flow rates of 3 µL/min (dark blue) and 10 µL/min (grey).

This data highlights the critical importance of optimizing flow rates to achieve the desired droplet size, which directly impacts the final particle size. By carefully adjusting the flow rates, precise control over droplet formation can be achieved, allowing for the fine-tuning of particle characteristics in various PLGA applications.

3.5 Influence of PLGA Concentration on Particle Size

The concentration of PLGA in the organic solvent, such as ethyl acetate used in this application note, plays a crucial role in determining particle size, providing an effective method for fine-tuning PLGA particle dimensions. The graph in Figure 12 clearly illustrates a positive correlation between droplet size and the resulting particle size across three PLGA concentrations - 1%, 3%, and 10% in ethyl acetate cross all 3D Flow Focusing Chips sizes applied in this application note including 170um, 100um, 65um and 30um. As droplet size increases, the size of the resulting particles also increases, a trend that is consistent across all PLGA concentrations.

However, the slope of this relationship varies significantly with PLGA concentration. At higher concentrations, such as 10% (represented by the blue data points), the resulting particles are noticeably larger for a given droplet size compared to those formed at lower concentrations like 1% (grey data points). This indicates that higher PLGA concentrations lead to larger particle sizes, likely due to the greater amount of polymer available for particle formation as the solvent evaporates. The 3% concentration (black data points) exhibits an intermediate trend, with particle sizes falling between those of the 1% and 10% PLGA solutions.

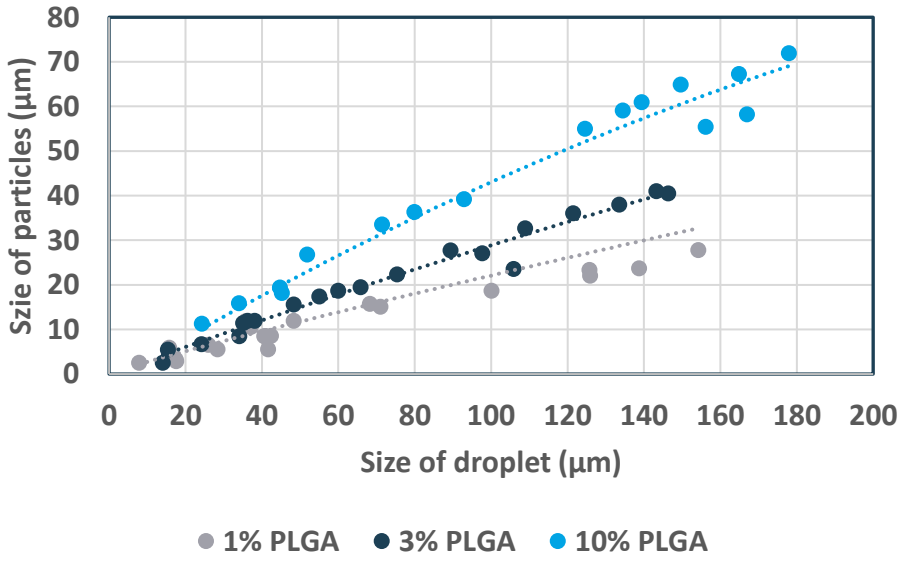

Figure 12 Size of droplets and particle size using 1%, 3% and 10% PLGA (w/v) in ethyl acetate.

3.6 Conditions for PLGA Particle Max Throughput

Many factors influence the throughput of PLGA particle production, including chip size, flow rates, and PLGA concentration. The table below provides the suggested conditions for each 3D Flow Focusing chip to achieve maximum throughput while producing specific particle sizes. These parameters have been optimized for throughput, but can be further adjusted to meet specific particle size requirements or experimental needs, ensuring flexibility in tailoring the conditions for different applications.

Table 1 Conditions for maximizing PLGA bead production

PLGA concentration [%]	3D Flow Focusing Chip	PLGA Droplet Size (µm)	PLGA Beads Size (µm)	Production per Hour (mg)	Droplet Phase Flow Rate (µL/min)	Carrier Phase Flow Rate (µL/min)
3	65 µm	36	**12**	18	10	30
10	100 µm	73	**25**	60	10	30
10	170 µm	167	**58**	60	10	30

3.7 SEM Analysis of PLGA Particles

Scanning Electron Microscopy (SEM) was utilized to provide detailed visualization of the PLGA particle morphology, confirming the uniformity and consistency of particle sizes across different batches (Figure 13). The SEM images reveal that the particles are highly monodisperse, with smooth surfaces and consistent size distribution, which is critical for applications requiring precise particle characteristics.

The relationship between chip size, flow conditions, polymer concentration, and the resultant particle size was systematically analyzed, demonstrating the system's capability to tailor particle specifications for specific therapeutic needs. The uniformity observed in the SEM images reflects the effectiveness of the Dolomite Mitos System in producing high-quality PLGA particles, highlighting its potential for scalable and reproducible particle production in pharmaceutical applications.

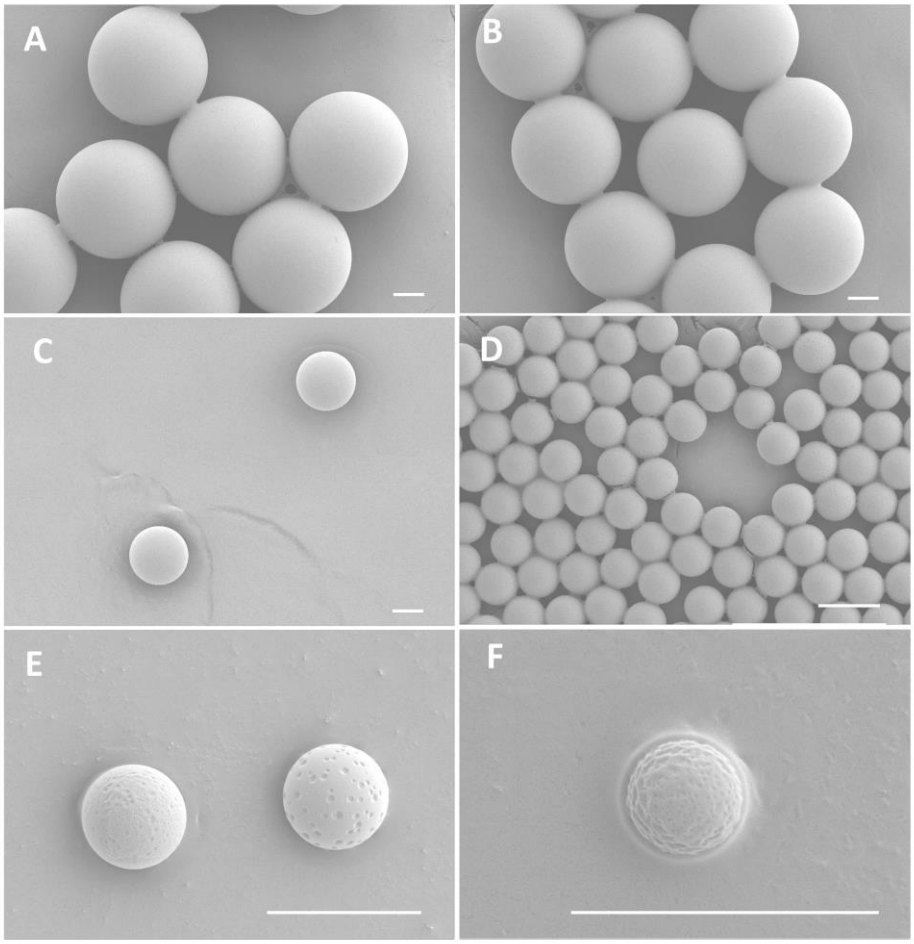

Figure 13. Scanning Electron Microscopy (SEM) images of PLGA particles produced using the Dolomite Mitos System, shown uniformly sized particles at different magnifications. Scale bar = 10 µm.

3.8 Flow Rate Calibration for Ethyl Acetate

Calibration curves provide a means to calculate the actual flow rates of non-standard fluids based on the set flow rate of common standard fluids. The calibration chart below in Figure 14 is utilized to ascertain the actual flow rates of ethyl acetate at reference (set flow rate) readings obtained from the flow sensor, which is calibrated using Hexadecane as the working fluid on the FCC (Flow Control Centre). This calibration process enables accurate flow rate adjustments for non-standard fluids, such as ethyl acetate. The selection of hexadecane as the reference fluid is critical, as its viscosity closely matches that of ethyl acetate, resulting in similar flow behavior. This similarity allows for precise flow rate adjustments and accurate measurements of ethyl acetate, ensuring reliable control within experimental setups.

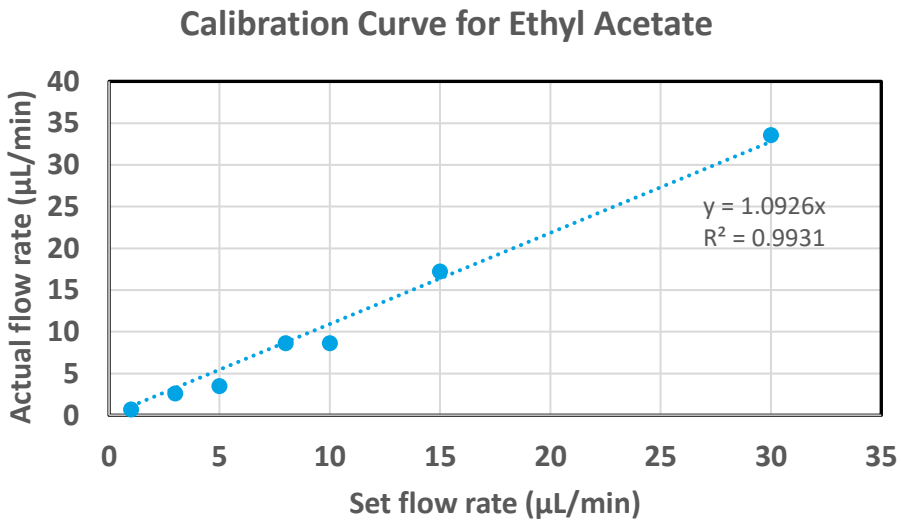

Figure 14. Calibration curve for ethyl acetate flow rate.

4 Conclusions

This application note demonstrates the significant advantages of using the Dolomite Mitos System, combined with the PLGA application packs, for the synthesis of PLGA microparticles, a critical component in advanced biomedical applications such as drug delivery, vaccine delivery, gene therapy, and tissue engineering. The microfluidic technology integrated into this system offers unparalleled precision in controlling particle size and distribution, addressing the limitations of traditional bulk synthesis methods, which often fail to achieve the required uniformity and consistency.

The ability to fine-tune particle size through adjustments in flow rates, phase ratios, and chip geometries ensures that PLGA microparticles meet the stringent requirements necessary for predictable pharmacokinetics and pharmacodynamics. This precision is essential for the efficacy and safety of therapeutic interventions. The Dolomite Mitos System's advanced features, such as precise flow control, temperature regulation, and automated operations, contribute to its robustness and scalability, making it an ideal platform for reproducible and efficient particle generation.

Furthermore, the inclusion of 3D Flow Focusing Chips and the use of Dolomite's Aqua-Phase as the continuous phase enhance the stability and consistency of the particle generation process, reducing surface fouling and extending chip lifetime. These features underscore the system's capability to produce high-quality, monodisperse PLGA particles tailored to specific biomedical applications.

In summary, the Dolomite Mitos System provides a powerful solution for the precise and scalable production of PLGA microparticles, enabling researchers to achieve consistent and reliable results. This technology is poised to significantly advance the development of next-generation micromedicines, offering improved therapeutic outcomes through enhanced control over drug delivery and other biomedical technologies.