High Throughput Continuous Synthesis of Monodisperse 10 to 40 µm PLGA Microparticles Using Flow Focusing

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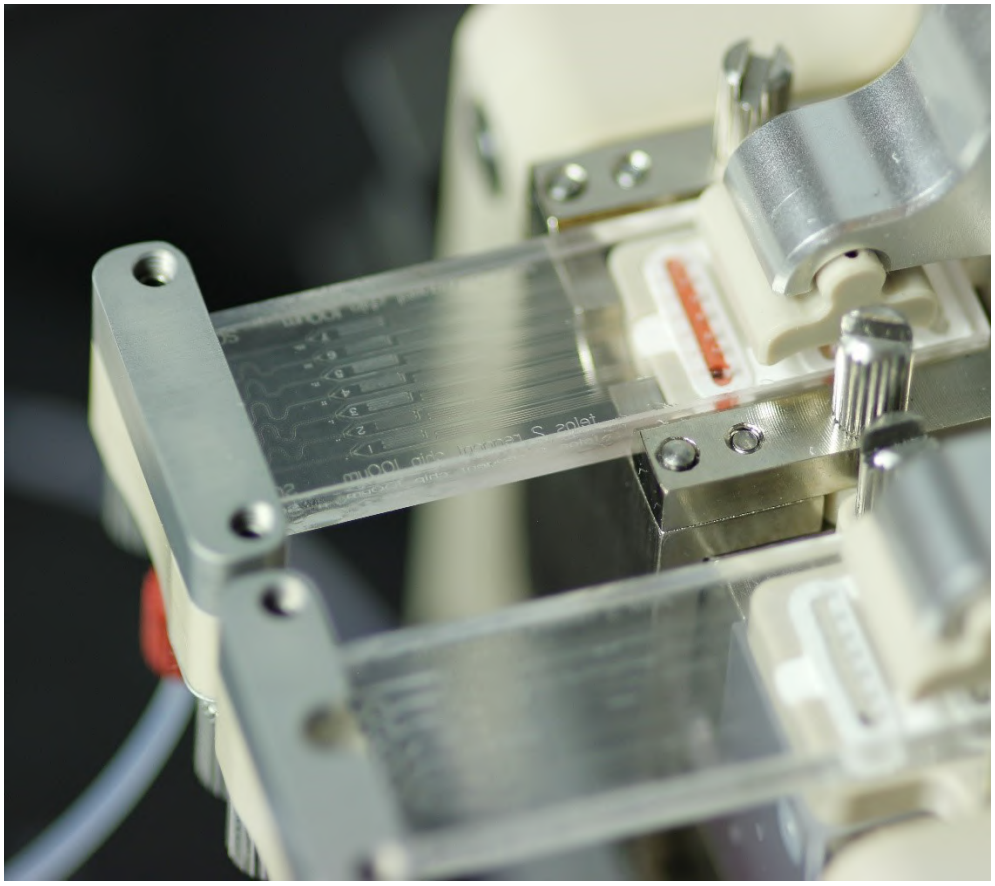

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1 Introduction

This short document describes the procedure for fabrication of highly monodisperse Poly (lactic-co-glycolic acid) (PLGA) beads with sizes ranging from 10 to 40 µm using Dolomite's high throughput microfluidic Telos® system.

The Telos system allows scaling up PLGA microparticles production using the same chip geometry (3D flow focussing droplet chip 100 µm hydrophilic), reagents and method described in the previous Dolomite application note

"Continuous Synthesis of Monodisperse PLGA Particles using Droplets"

https://www.dolomite-microfluidics.com/applications/polymer-microparticle-synthesis/

2 Experimental Setup

Figure 1 and Figure 2 show the Telos high throughput setup typically used for PLGA microparticles:

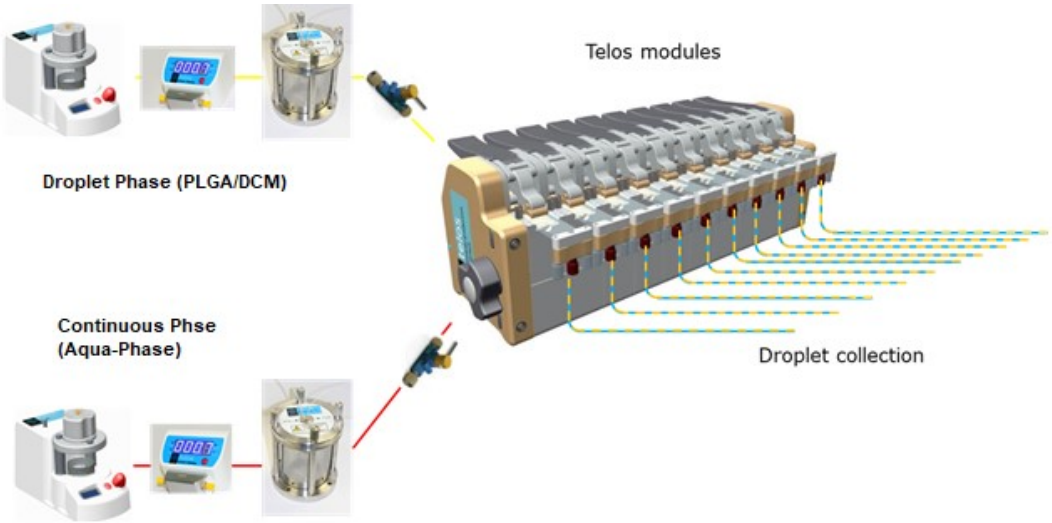

Figure 1Telos System Setup

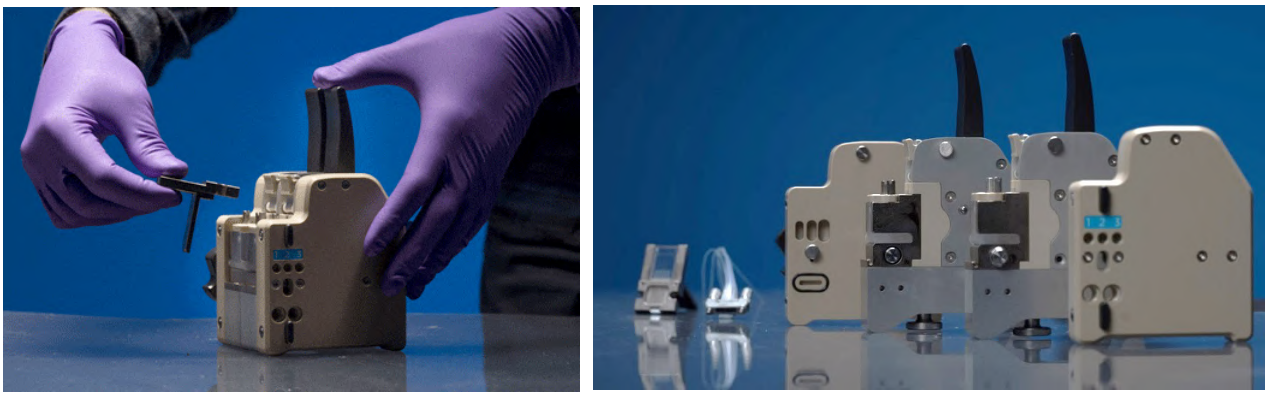

Figure 2 Telos Manifolds Assembled (left) and Disassembled (right)

The two fluids, PLGA in DCM and Aqua-Phase (Dolomite aqueous stabilizer for PLGA particle production), are kept within two different 400 mL reservoirs that are pressure controlled by means of pressure driven pumps. The p-pumps are accurate pressure regulators: the pressure within each reservoir is adjusted to meet the desired flow rates. For this reason, each pump is coupled with a flow sensor (0.2 - 5 ml/min) and plugged to a gas supply (helium) to prevent outgassing of the PLGA/DCM solution. Outgassing must be avoided as it leads to uncontrolled precipitation of polymer in the system which creates flow instabilities (and blockages in some extreme cases). Using Helium solves the problem because Helium does not dissolve in the fluids. The fluids are delivered to the Telos module using 0.8 mm ID, 1.6 mm OD FEP tubing.

The fluids reach the Telos manifold device which is formed by two lateral frames that hold 10 modules. Each module clamps a 7-channel junction microfluidic chip (Figure 3) for droplet production. Each junction is visible from above and below for illumination and optical access via high speed imaging. The Telos manifold equally distributes the fluids across the Telos 1 Reagent 3D Flow Focusing Chip SC 100 µm to produce monodisperse droplets of PLGA polymers in Aqua-Phase continuous phase (Figure 4). The droplets produced are collected in one common outlet by means of a linear connector 1-way which enables a single 1.6 mm OD tube to be connected to a single fluid port on a microfluidic chip.

Fast set-up time is achieved with an easy to use tool-free clamp mechanism which locates and seals the chip in place, making or breaking connections instantly. The Telos module incorporates on/off valves for excellent flow control during priming and operation. Additionally, the Telos module includes optional integrated filters and in-line valves on each tubing to be able to disconnect the module while keeping the pump reservoirs pressurized.

The two pumps and the microscope can be controlled remotely via the dedicated Flow Control Centre software (FCC) which enable quick and reliable droplet imaging and flow rate adjustment.

This application note describes the production of PLGA beads using 10 Telos modules for a total of 70 parallel channels to achieve the highest possible throughput. Fewer modules can be put together in case lower production rates are required.

More information about the Telos system can be found on Dolomite website

https://www.dolomite-microfluidics.com/microfluidic-systems/telos-high-throughput/

https://www.dolomite-microfluidics.com/contact/contact-us/

Figure 3 Telos 1 Reagent 3D Flow Focusing Chip SC 100 µm (https://www.dolomite-microfluidics.com/product/telos-1-reagent-3d-flow-focusing-sc/)

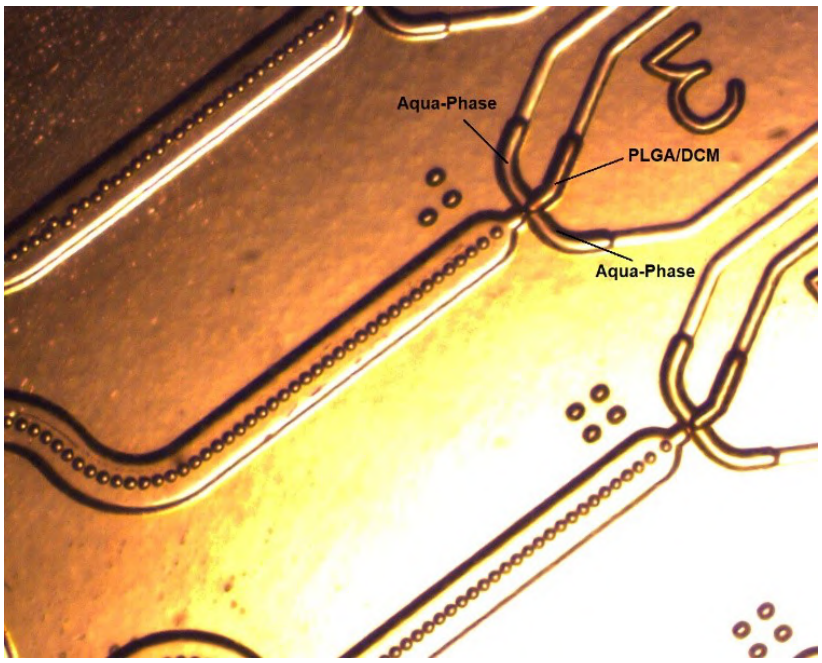

Figure 4 Image of two junctions on a Telos 2 Reagent Chip 100 µm - hydrophilic

The PLGA droplets made on chip are collected in a beaker pre-filled with a large amount of Aqua-Phase carrier fluid (at least 2-3 times as much as the droplet phase). PLGA droplets are dried down by solvent extraction and their size is measured according to the protocol described in the previous Dolomite application note "Continuous Synthesis of Monodisperse PLGA Particles using Droplets" https://www.dolomite-microfluidics.com/applications/polymer-microparticle-synthesis/

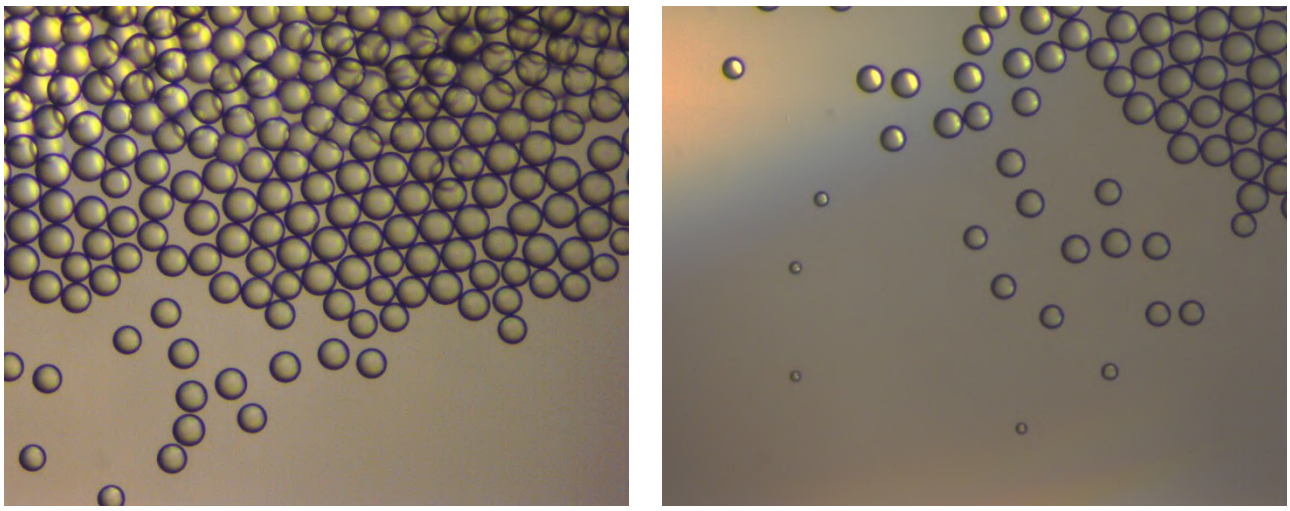

Figure 5 Example of solvent evaporation and consequent PLGA/DCM in Aqua-Phase droplet shrinkage. 100 µm PLGA/DCM 1% droplets before shrinkage (left), shrinking droplets from 100 µm to 27 µm (right). Droplets are collected on a glass slide

3 Results

Poly(D,L-lactide-co-glycolide) (PLGA) ester terminated, lactide/glycolide 75/25, Mw 76000-115000 from Sigma Aldrich is dissolved in different concentrations in dichloromethane (DCM) CHROMASOLV for HPLC (≥ 99.8 %) and used as droplet phase. Aqua-Phase is the fluid used as continuous phase to pinch PLGA/DCM droplets.

Once the system is up and running producing PLGA droplets, there are several parameters we can adjust to control particle size and production rate:

- **PLGA/DCM:Aqua-Phase ratio**

The most effective way to alter the size of the final bead is to adjust the size of the droplets forming at the chip junction. Adjusting the relative flow rates of PLGA and Aqua-Phase is the easiest way to achieve this. In general, a lower flow rate of continuous phase and a high flow rate of droplet phase will result in larger droplets. At a certain point, a larger quantity of droplet phase will be flowing than continuous phase; this is known as an inverse emulsion (slugs).

- **PLGA concentration**

Changing PLGA concentration is a convenient way of fine-tuning the bead size. Increasing the concentration of PLGA means that more PLGA will be present in each droplet. This in turn means that as the droplet dries, it will form a larger PLGA microparticle.

The total amount of PLGA present is directly proportional to the volume of the resulting PLGA microparticle. Because volume is proportional to the cube of the radius, this means that an 8x increase in PLGA concentration would be required to double the diameter of the PLGA microparticle. This method therefore has a more limited effect on bead size than e.g. changing the droplet size. In practice, highly concentrated solutions of PLGA become increasingly viscous, which can affect droplet formation.

Generally, it is considered beneficial to the performance of the microfluidic junction for the viscosities of the droplet and continuous phases to be relatively similar. Since DCM is less viscous than Aqua-Phase (which has a viscosity very similar to water), a 1-2.5 % solution of PLGA will improve the viscosity match, resulting in clean droplet formation and a high rate of droplet production. Increasingly concentrated solutions of PLGA result in solutions significantly more viscous than Aqua-Phase, and thus the droplet production rate reduces and there is an increased risk of the formation of satellite droplets (small droplets that form when the continuous phase does not pinch off the droplet cleanly). PLGA concentrations up to 7.5 % perform well, however increasing PLGA concentration beyond this may result in a deterioration of the system performance. Alternatively, reducing the concentration of PLGA can be used to produce much smaller PLGA microparticles. This approach remains effective down to very small droplet sizes, though whilst the number of particles produced will be the same, the mass yielded will be significantly smaller due to the very small amount of PLGA per droplet. A more efficient way of generating smaller microparticles is to form a larger number of smaller droplets. This can be done using a smaller chip junction.

- **Total combined flow rate**

Increasing the flow rate of both the droplet and continuous phase in proportion with one another will increase the frequency of droplet formation. The droplet size will remain relatively similar, though the increased velocities will alter the fluid dynamics at the chip junction, which may result in some shift in droplet size. After increasing total flow rate, the droplet size can then be refined by adjusting the

overall ratio of flow rates. At a certain point, the continuous phase will be unable to pinch off droplets due to the rate of droplet phase flow resisting the pinching force. This will cause the droplet phase to jet through the junction, as a continuous cylinder of PLGA solution. Occasionally droplets will still form further down the outlet tubing, but these droplets are much more likely to be polydisperse compared to those formed within the confines of the chip junction.

- **Combinations of factors**

Each of these variables acts like a slider that can be fine-tuned to achieve your desired product. However, each variable may influence, droplet size, flow stability or production rate. It is therefore important that throughput is balanced against increasing flow instability; it may be the case that a higher stable throughput may be achieved by using a higher concentration of PLGA and targeting a smaller droplet size, or that a lower rate of production with a high PLGA concentration and smaller droplets may be more stable than trying to make overly large droplets at a low PLGA concentration.

it is very simple to adjust these variables within FCC software; adjustments to flow rates have an immediate visible effect at the droplet junction, meaning that it is possible to rapidly screen a wide range of potential combinations of variables to find the production conditions that best suit your needs. The table below shows the maximum and minimum bead size and maximum bead frequency obtained at each PLGA/DCM concentration.

Swapping to a different PLGA concentration is also simple - just switch off the P-Pump and switch out the vials, making sure to allow time for the old PLGA solution to be cleared from the tubing before collecting new samples.

Maximum Size						
PLGA concentration [%]	**Aqua-Phase Set Flow Rate [μ L/min]**	**PLGA Actual Flow Rate [μ L/min]**	**Droplets size [μ m]**	**Beads Size [μ m]**	**Frequency [kHz]**	**Production [g/h]**
0.5	1400	1687	102.5	**26**	49.9	2.15
1.0	1400	2043.3	100.4	**26.6**	64.2	2.95
2.5	2100	2832.9	106	**33.5**	75.8	6.98
3.5	1750	1871.8	98.4	**35.1**	62.4	6.62
5.0	2100	1561	87.7	**37.4**	73.6	9.45
6.0	1750	1270.5	85.1	**39.2**	65.6	9.68
7.5	1750	1270.5	79.4	**37.1**	80.6	10.1

Minimum Size						
PLGA concentration [%]	**Aqua-Phase Set Flow Rate [μ L/min]**	**PLGA Actual Flow Rate [μ L/min]**	**Droplets size [μ m]**	**Beads Size [μ m]**	**Frequency [kHz]**	**Production [g/h]**
0.5	8400	57.4	34.5	**7.5**	44.2	0.04
1.0	7700	57.4	35.3	**9**	41.2	0.07
2.5	6300	57.4	26	**13.4**	103.4	0.61
3.5	9100	63	26.7	**14**	105.7	0.71
5.0	9100	63	28.6	**18**	86.1	1.23
6.0	9100	63	33.8	**22.5**	52.2	1.46
7.5	8400	63	31.4	**22**	66	1.70

4 Conclusions

PLGA particle production was investigated using the high throughput Dolomite Telos system in combination with 7 x 10 = 70 100 µm 3D channels. Droplet size was tuned playing with different parameters such as PLGA in DCM concentrations and continuous to droplet phase flow rate ratio. PLGA beads are collected and dried using specific protocol developed by Dolomite.

The Telos PLGA system was able to produce high monodisperse PLGA beads in the 10 µm to 40 µm size at different production rate (from 5 g/h up to 11 g/h) depending on the size of the PLGA particle.