PRECISION NANOSYSTEMS

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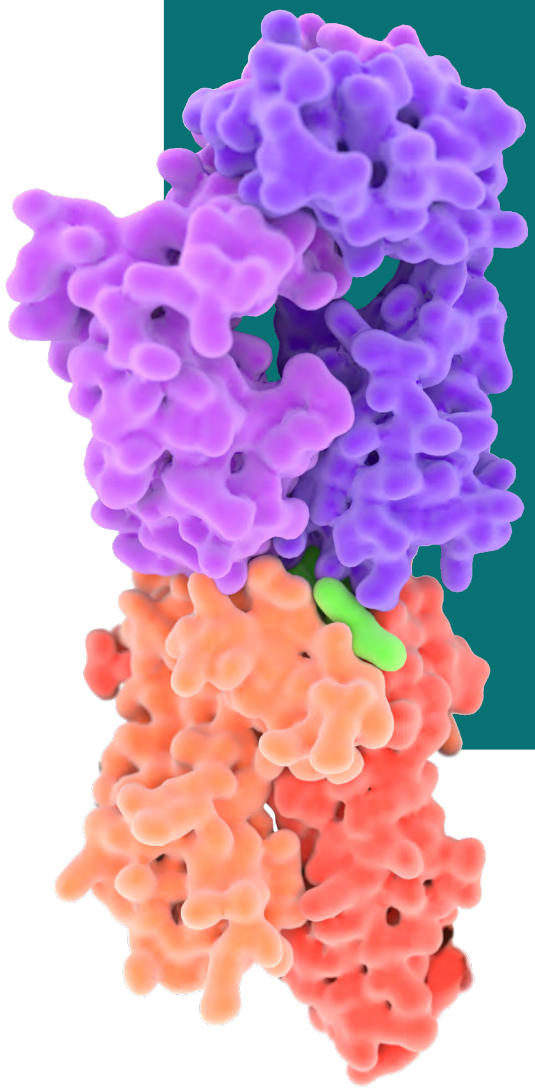
Protein Delivery Systems



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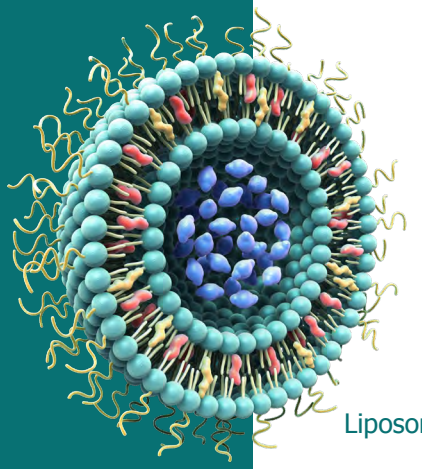
Introduction

Proteins and peptides represent the second generation of therapeutic molecules. Being biological molecules, they have the ability to interact with other biological molecules to treat diseases such as cancer, autoimmunity, infectious diseases and more with improved specificity and targeting. Delivery technologies have not kept pace with the discovery of new protein drugs so there remain many challenges in formulating therapeutic proteins.

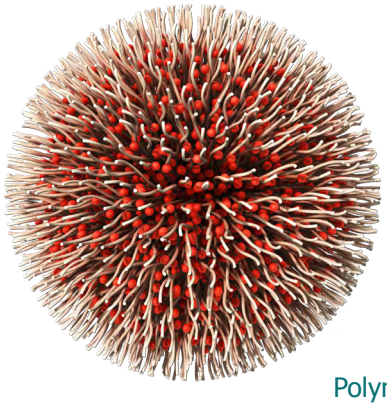
Researchers are encapsulating proteins and peptides into nanoparticles to:

- Improve solubility
- Control aggregation and concentration
- Control release
- Reformulate for different routes of administration

Proteins can be encapsulated into several types of nanoparticles including:



Liposomes



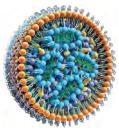
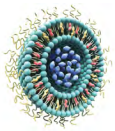
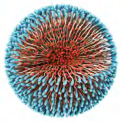
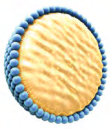
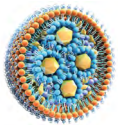
Polymer Nanoparticles and Micelles

This collection highlights applications of NanoAssemblr technology for formulating proteins into lipid and polymer-based nanoparticles. Several examples from peer-reviewed literature illustrate how soluble proteins, transmembrane proteins and viral envelope proteins have been incorporated into nanoparticles. Additionally, application notes with original results illustrate how liposomes and polymer nanoparticle formulations can be optimized in general. These illustrate the multitude of applications of NanoAssemblr technology for developing and optimizing delivery systems for proteins and peptides.

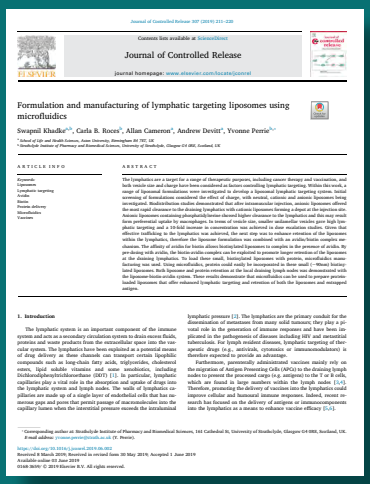
Precision NanoSystems' solutions are trusted by world-leading biopharma and academic researchers to drive development of diverse nanomedicines

Vaccines | Cell Therapy & Regenerative Medicine | Immuno-Oncology | Targeted Therapeutics | Small Molecule Delivery | RNA & DNA Therapeutics | CRISPR & Gene Editing

Versatile Applications

Particle Type		Active Ingredient				Example Application	Carrier Materials
Nucleic acid Lipid Nanoparticles (LNP)		Nucleic Acids	Peptides and Proteins			• Rare genetic diseases • mRNA protein replacement • mRNA vaccines • Gene and cell therapy	• Ionizable lipids • Phospholipids • Cholesterol • PEG-Lipids
Liposomes				Small Molecules	Imaging Contrast Agents	• Vaccine adjuvants • Antimicrobials • Cancer chemotherapy • Diabetes combination therapy	• Phospholipids • Cholesterol • PEG-Lipids
Polymer NPs						• Cancer chemotherapy • Targeted protein delivery • Controlled release/ biodistribution • Immuno-oncology	• Poly-lactides (ex: PLGA) • Block copolymers (ex: PEG-b-PLGA) • Polysaccharides (ex: chitosan, cellulose)
Emulsions						• Cancer chemotherapy • Drug formulation • Controlled release/ biodistribution	• Triolein/POPC • Oil/Surfactant
Organic/ Inorganic NPs						• Theranostics • Imaging	• Lipids • Noble metal NPs • Rare Earth Metals • III-V semiconductors

Featured Protein-loaded Nanoparticle Publications



Journal of Controlled Release

Formulation and manufacturing of lymphatic targeting liposomes using microfluidics

Swapnil Khadke, Carla B. Roces, Allan Cameron, Andrew Devitt, Yvonne Perrie

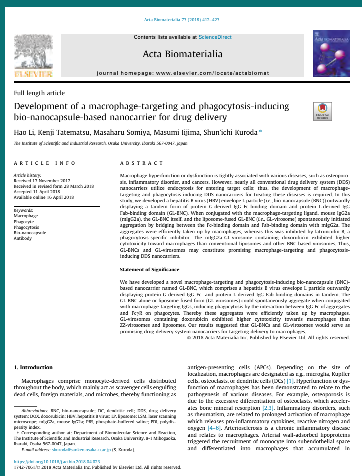
Journal of Controlled Release, 2019

Formulation And Manufacturing Of Lymphatic Targeting Liposomes Using Microfluidics

Khadke S, Roces C, Cameron A, Devitt A, Perrie Y. Journal of Controlled Release 2019; 307: 211-220.

Summary

- The lymphatic system provides a secondary circulation conduit to transport drugs for different diseases so targeted drug delivery towards the lymphatic system is desirable to attain higher therapeutic concentration while minimizing off-target organ toxicities.
- In this work, Dr. Yvonne Perrie's group utilized nanocarriers to target lymphatics, systematically exploring how physico-chemical characteristics and surface targeting of liposomes could impact preferential biodistribution and retention within the lymphatic system.
- They found both surface charge and size affected accumulation in lymph nodes following intramuscular injection, with sub-120 nm anionic liposomes containing PS lipid out-performing both cationic liposomes of the same size and larger (900nm) anionic liposomes.
- Protein incorporation was also explored to enhance retention at the lymph nodes: mice were pre-administered with avidin followed by injecting biotinylated liposomes, and following drainage to lymph nodes, strong affinity between biotin and avidin crosslinks the liposomes leading to higher retention than control non-biotinylated liposomes or treatments without avidin.
- This active targeting mechanism also improved accumulation within the secondary lymph nodes
- NanoAssemblr technology was used to control and reproducibly make liposomes at the optimal size of ~ 100nm with incorporated biotin.
- Overall, the study explains the effect of liposome design parameters on enhancing lymphatic accumulation identifying key properties leading to improved therapeutic dose at lymph nodes where cancer has been metastasized, with minimal non-specific organ toxicity.


Acta Biomaterialia 73 (2018) 412–423

Acta Biomaterialia

Full length article

Development of a macrophage-targeting and phagocytosis-inducing bio-nanocapsule-based nanocarrier for drug delivery

Hao Li, Kenji Tatematsu, Masaharu Somiya, Masumi Iijima, Shun'ichi Kuroda

Acta Biomaterialia, 2018

Development of a Macrophage-Targeting and Phagocytosis-Inducing Bio-nanocapsule-based Nanocarrier for Drug Delivery

Li H, Tatematsu K, Somiya M, Iijima M, Kuroda S. Acta Biomaterialia 2018; 73: 412-423.

Summary

- Macrophage dysfunction is associated with various diseases such as osteoporosis, inflammatory disorder, and cancer so development of nanoparticles to specifically deliver drugs to macrophages could have great therapeutic potential
- Researchers at Osaka University led by Dr. Shun'ichi Kuroda demonstrate a novel virosomes - lipsomes containing viral envelope proteins - that are designed to aggregate in the presence of IgG which triggers phagocytosis by macrophages
- The Kuroda group used NanoAssemblr technolgy to make the liposomes which they later fuse with viral envelope extracts and load with doxirubicin (Dox)
- They found the virosomes containind Dox exhibited higher cytotoxicity toward macrophage cell lines than liposomes containing Dox
- These findings indicate phagocytosis-inducing virosomes could be ideal candidates for active targeting toward mononuclear phagocytic system for diverse applications


COMMUNICATION

ADVANCED MATERIALS

Design and Development of Biomimetic Nanovesicles Using a Microfluidic Approach

Roberto Molinaro,* Michael Evangelopoulos, Jessica R. Hoffman, Claudia Corbo, Francesca Taraballi, Jonathan O. Martinez, Kelly A. Hartman, Donato Cosco, Giosue' Costa, Isabella Romeo, Michael Sherman, Donatella Paolino, Stefano Alcaro, and Ennio Tasciotti*

DOI: 10.1002/adma.201702749

Advanced Materials, 2018

Design and Development of Biomimetic Nanovesicles Using a Microfluidic Approach

Molinaro R, Evangelopoulos M, Hoffman J, Corbo C, Taraballi F, Martinez J et al. Advanced Materials 2018; 30: 1702749.

Summary

- Biomimetic and biohybrid nanoparticles consisting of liposomes with membrane-bound proteins are potentially advantageous in drug delivery applications, for instance by mimicking leukocytes, nanoparticles take on their innate biological features including long circulation and selective targeting to biological compartments, but clinical translation of these technologies is hindered by challenges in manufacturing and scale-up production
- Dr. Ennio Tasciotti's group at Houston Methodist Research Institutue have leveraged NanoAssemblr technology to make and load liposomes with membrane proteins isolated from human leukotcytes incorporated within the bilayer in a single, continuous-flow step
- Their process involved injecting membrane protein extracts in aqueous suspension in one inlet of the NanoAssemblr mixer and lipids dissolved in ethanol in the other inlet, where rapid mixing triggers self-assembly of liposomes incorporating the membrane protein

- Compared to conventional methods, NanoAssemblr technology simplified particle optimization in terms of size, homogeneity, zeta-potential and protein incorporation into the bilayer, with 90% of membrane proteins initially added being associated with he final formulation compared to 63% for conventional thin film methods
- The NanoAssemblr Platform offers a robust and scalable path towards clinical development and manufacturing of biomimetic nanovesicles that are advantageous for a spectrum of drug delivery applications

Vol. 41, No. 6 *Biol. Pharm. Bull.* **41**, 899–907 (2018) 899

Regular Article

The Use of an Efficient Microfluidic Mixing System for Generating Stabilized Polymeric Nanoparticles for Controlled Drug Release

Yoshinori Morikawa, Tatsuaki Tagami, Akihiro Hoshikawa, and Tetsuya Ozeki*

Drug Delivery and Nano Pharmaceutics, Graduate School of Pharmaceutical Sciences, Nagoya City University; 3–1 Tanabe-dori, Mizuho-ku, Nagoya 467–8603, Japan.

Received December 25, 2017; accepted April 1, 2018

Microfluidics is a promising system for efficiently optimizing the experimental conditions for preparing nanomedicines, such as self-assembled nanoparticles. Poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles are promising drug carriers allowing sustained drug release. Here, we encapsulated the model drug curcumin, which has many pharmacological activities, into PLGA nanoparticles and investigated the effects of experimental conditions on the resulting PLGA nanoparticles using a microfluidics system with a staggered herringbone structure that can stir solutions through chaotic advection. The total flow rate and flow rate ratio of the solutions in the microfluidics system affected the diameters, polydispersity index, and encapsulation efficiency of the resulting PLGA nanoparticles and produced small, homogenous PLGA nanoparticles. The incorporation of polyethylene glycol (PEG)–PLGA into the PLGA nanoparticles reduced the particle size and improved the encapsulation efficiency. Initial burst release from the PLGA nanoparticles was prevented by the incorporation of PEG2000–PLGA. Curcumin-loaded PEGylated PLGA nanoparticles showed cytotoxicity similar to that of other formulations. This microfluidics system allows high throughput and is scalable for the efficient preparation of PLGA nanoparticles and PEGylated PLGA nanoparticles. Our results will be useful for developing novel PLGA-based polymer nanoparticles by using the microfluidics.

Key words curcumin; drug delivery; microfluidic system; nanoparticle; poly(lactic-*co*-glycolic acid) (PLGA); sustainable release

International Journal of Pharmaceutics, 2019

Rapid and scale-independent microfluidic manufacture of liposomes entrapping protein incorporating in-line purification and at-line size monitoring

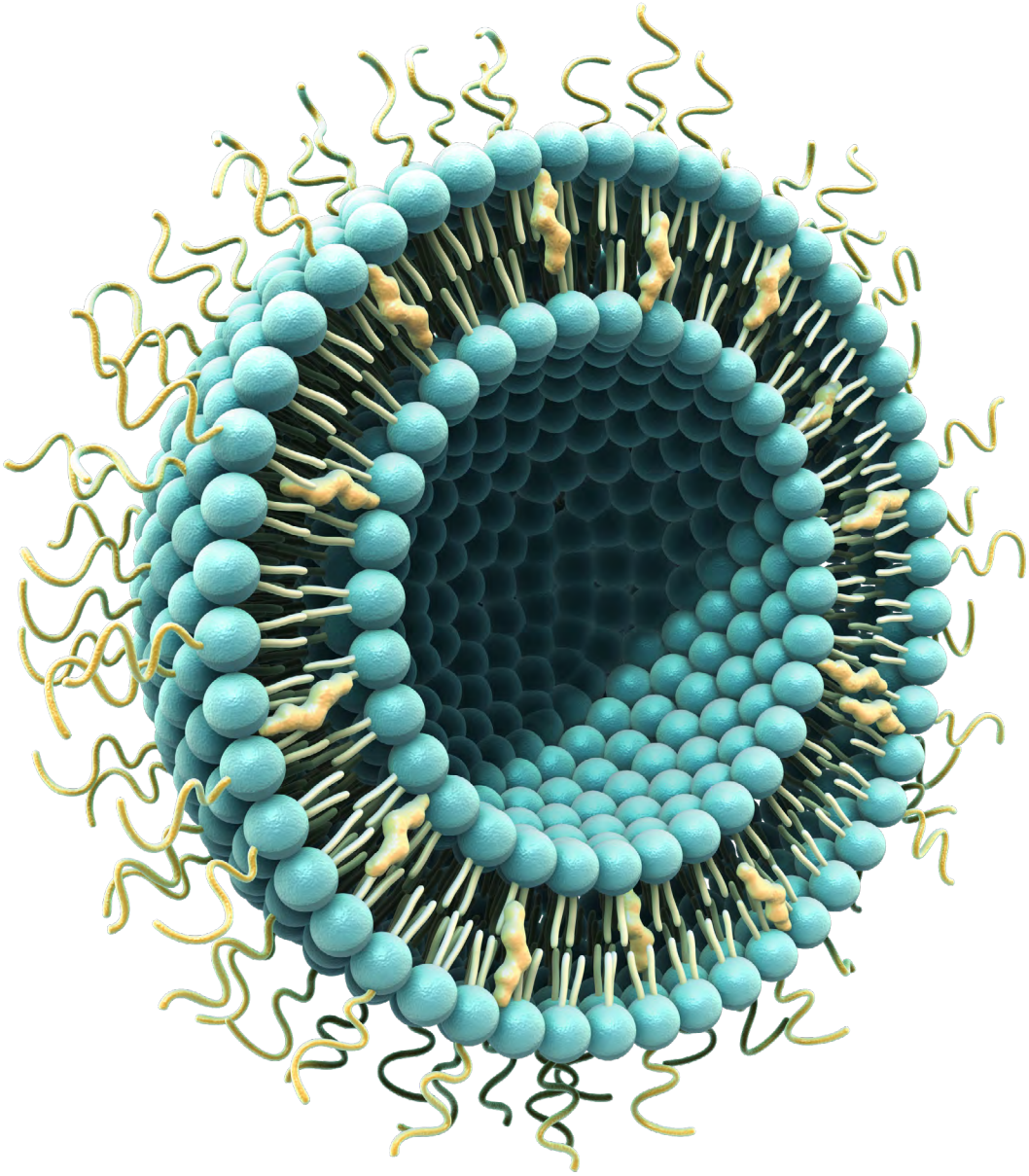
Forbes N, Hussain M, Briuglia M, Edwards D, Horst J, Szita N et al. International Journal of Pharmaceutics 2019; 556: 68-81.

Summary

- Dr. Yvonne Perrie's lab at Strathclyde University, - part of Precision Nanosystems' Nanomedicine Innovation Network - have demonstrated a robust, optimized and scalable method for the production of protein loaded liposomes
- They demonstrated effective production of size controlled, homogenous, high load liposomes in a scale independent manner using the NanoAssemblr Benchtop and Blaze instruments
- This is the first time that a simple and scale-independent method to manufacture, purify and monitor the production of liposomes encapsulating proteins has been developed and used
- This process has potential to offer a reliable and quick method to translate production of protein loaded liposomes from the lab bench to a manufacturing process for nanomedicines

Liposomes

Using the NanoAssemblr® Benchtop instrument process parameters to reproducibly tune size



Andrew Brown, Anitha Thomas, Mina Ordobadi, Shell Ip, Gesine Heuck, Euan Ramsay.

Abstract

Liposomes are used as drug carriers to deliver a variety of therapeutic molecules such as small molecules, proteins, and nucleic acids. Liposome size and homogeneity are crucial factors that affect efficacy of liposomal drugs. Hence, a liposome production method that streamlines the optimization of these characteristics will expedite formulation development. Here we demonstrated the utility of the NanoAssemblr® microfluidic technology for rapidly and reproducibly generating homogeneous liposome populations with the ability to fine-tune liposome size through computer-controlled parameters. Bench-scale batches of liposomes were produced at sizes ranging from 40 nm to 120 nm with exceptional uniformity (polydispersity index, PDI as low as 0.05). Additionally, the robustness of the NanoAssemblr® process was demonstrated by comparing the size and PDI of 6 independent formulations conducted by different instrument operators. These data demonstrate how the technology can be used to expedite development of liposome formulations.

Introduction

Traditional methods of liposome formulation include sonication and extrusion. These methods are laborious and offer limited control over size and polydispersity of liposomes, both of which can affect liposome circulation time, drug retention or its ability to penetrate different tissues1 . Prior research has demonstrated the utility of the NanoAssemblr® Benchtop instrument for formulating unilamellar liposomes2 for remote loading of chemotherapeutics3, in situ loading with hydrophobic and hydrophilic small molecules 4,5, nucleic acids6, and vaccine adjuvants7. The NanoAssemblr platform works through controlled nanoprecipitation of liposomes (Figure 1). When lipid molecules dissolved in a low-polarity organic solvents are mixed with water or buffer, the resulting change in polarity triggers the spontaneous self-assembly of the lipids into unilamellar vesicles. The NanoAssemblr platform combines microfluidic mixing with independent computer controlled injection of both fluids to achieve control over precipitation conditions that directly influence liposome size. This method also ensures consistency among liposomes within any given batch, from batch to batch, and from operator to operator. With a reproducible process, effects of altering the formulation can be isolated (see Related Materials, below, for additional resources). Additionally, the NanoAssemblr platform offers a straightforward path towards clinical development by streamlining scale-up through continuous flow and multiple parallel mixers. Here, we demonstrated that the NanoAssemblr® Benchtop consistently formulates homogenous liposomes by comparing the size and polydispersity (PDI) of 6 independent formulations. Next, we demonstrated how precise computer control over injection speed and the ratio between aqueous and organic phases is used to finely tune liposome size and minimize heterogeneity.

Figure 1

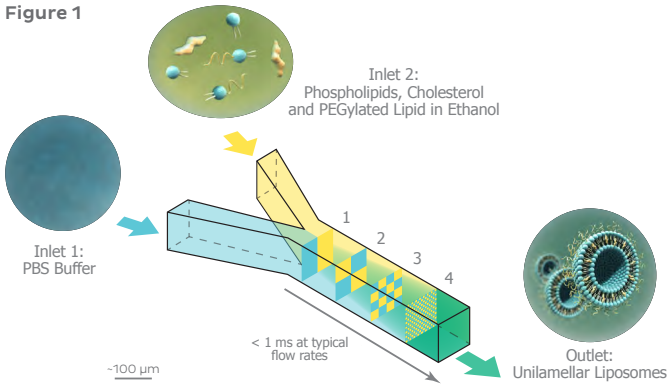


Figure 1. PBS buffer is injected into the left inlet while an organic solvent with dissolved lipids is injected into the right inlet of the NanoAssemblr Benchtop microfluidic cartridge. *Following controlled mixing in microfluidic channels, unilamellar liposomes are spontaneously formed. Computer controlled injection allows specification of the total flow rate and the flow rate ratio of aqueous to organic solvents to control liposome size.*

1. Two fluids (Buffer and ethanol) entering the channel under laminar flow do not mix.
2. Microscopic features in the channels are engineered to cause the fluid streams to mingle in a controlled, reproducible, and non-turbulent way.
3. Intermingling of the fluids increases as they continue through the mixer.
4. Fluids emerge from the mixer completely mixed. The process takes < 1 ms; faster than molecules can assemble into particles.

Result

LIPOSOME BATCH REPRODUCIBILITY USING NANOASSEMBLR BENCHTOP

To assess batch-to-batch variation of liposome formulations prepared using the NanoAssemblr Benchtop Instrument, multiple batches of liposomes with the same lipid composition were formulated under identical instrument parameters by independent operators. This led to comparable liposome sizes and PDIs demonstrating consistency across batches and operators **(Figure 2)**.

TUNING LIPOSOME SIZE BY ALTERING FLOW RATE RATIO AND TOTAL FLOW RATE RATIO

The aqueous: organic Flow Rate Ratio (FRR) and Total Flow Rate (TFR) are the primary process parameters that impact nanoparticle characteristics. FRR is the volumetric ratio of the organic and aqueous phases being mixed through the microfluidic cartridge. TFR is the total speed in mL/min at which both fluid streams are being pumped through the two separate inlets of the microfluidic cartridge.

Figure 3 indicates the effect of FRR on liposome size. At a constant liposome composition, particle size decreased from 90 to 50 nm as the FRR increased from 1:1 to 3:1. At very high FRRs, size plateaus as liposomes form the smallest thermodynamically possible structures defined as their "limit" size[2].

The effect of TFR on liposome size can be observed in Figure 4. Liposome size decreased from 100 to about 50 nm when the TFR was increased from 1 to 12 mL/min. The FRR, lipid composition, and total lipid concentration were kept constant throughout the experiment.

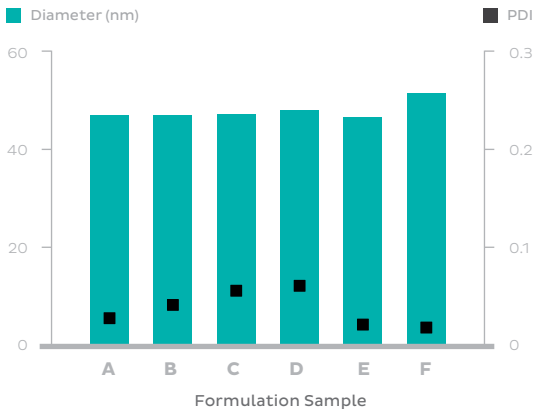
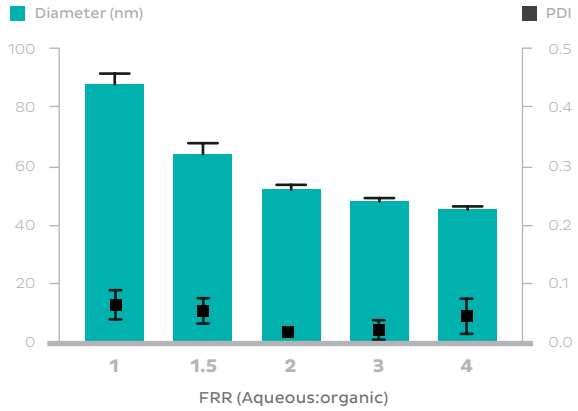


Figure 2. Liposome size and polydispersity were consistent across batches prepared by different operators. *Six independent batches of liposomes, with identical lipid composition and NanoAssemblr process parameters were generated by different operators. Liposomes generated had a size range of 46-54 nm and consistently low polydispersity indices (PDI).*

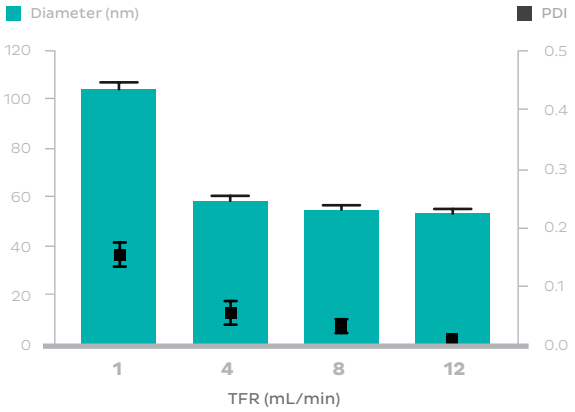
Composition	POPC/Chol/DSPE-PEG_{2000} (52:45:3 mol%)
Lipid concentration in organic solvent	10 mg/mL
Organic solvent	Ethanol
Aqueous solvent	PBS pH 7.4
Total Flow Rate (TFR)	12 mL/min
Flow Rate Ratio (FRR)	5:1

Figure 3. Liposome size tuning by Flow Rate Ratio. *Higher aqueous:organic Flow Rate Ratio (FRR) reduces liposome size asymptotically. Samples were prepared in triplicate. Measurements represent the mean and error bars represent standard deviation.*



Composition	POPC/Chol/DSPE-PEG$_{2000}$ (52:45:3 mol%)
Lipid concentration in organic solvent	10 mg/mL
Organic solvent	Ethanol
Aqueous solvent	PBS pH 7.4
Total Flow Rate (TFR)	12 mL/min
Flow Rate Ratio (FRR)	n:1, n as indicated

Figure 4. Liposome size tuning by Total Flow Rate. *Increasing Total Flow Rate (TFR) increases mixing speed and reduces liposome size. Samples were prepared in triplicate. Measurements represent the mean and error bars represent standard deviation.*



Composition	POPC/Chol/DSPE-PEG$_{2000}$ (52:45:3 mol%)
Lipid concentration in organic solvent	10 mg/mL
Organic solvent	Ethanol
Aqueous solvent	PBS pH 7.4
Total Flow Rate (TFR)	As indicated (mL/min)

Discussion

Liposome size and polydispersity are crucial factors affecting efficacy. Hence, size control and size uniformity within a batch and from batch-to-batch are crucial factors to optimize in liposomal drug formulation development. The NanoAssemblr process is highly reproducible, as shown in **Figure 2**, where multiple independent operators produced liposome batches with comparable sizes and PDIs. This is achieved through computer control of parameters that affect liposome size. Understanding the factors that affect liposome size provides insight into factors that affect uniformity of size and reproducibility. During nanoprecipitation, lipid molecules start off fully solubilized in a water-miscible organic solvent. When this solution is mixed with water, the polarity of the resulting solution increases, which causes the lipid molecules to self-assemble into unilamellar liposomes. Given a fixed lipid composition, two factors that affect the resulting liposome size are the magnitude of polarity change and the speed of mixing. On the NanoAssemblr platform, the former is controlled by the Flow Rate Ratio (FRR) while the latter is controlled by the Total Flow Rate (TFR). **Figure 3** illustrates that increasing the aqueous-to-organic FRR decreases the size of the resulting liposomes asymptotically. This is because increasing the relative amount of the aqueous phase increases the magnitude of the polarity change upon mixing, which increases the driving force for self-assembly of lipids into liposomes. Additionally faster mixing, achieved by increasing TFR through the NanoAssemblr microfluidic mixer, leads to smaller liposomes, as illustrated in **Figure 4**. This behavior is understood by considering the rate of polarity change compared to the rate of self-assembly. If the rate of polarity change exceeds the rate of self-assembly, lipid molecules experience an immediate super-saturation in the new solvent environment. Rates of diffusion and reorganization then limit the local supply of lipid molecules that can come together into a single liposome. This leads to smaller liposomes in greater abundance. From this understanding, the asymptotic behavior observed in both **Figures 3** and **4** can also be understood. As the driving force and rate of mixing are increased, they meet the limits of how small a particle can be, given the volume and packing of the constituent molecules.

With this understanding of how the solvent environment affects liposome size, it is clear that precise control over the mixing ratio and the mixing rate is necessary to achieve uniform liposome size. Within a given batch, the homogeneity of the population of liposomes is measured by the polydispersity index, where values below 0.2 are preferred for in vivo applications. Homogeneity of the solvent environment throughout the volume of the batch is required in order to obtain a homogeneous population of liposomes. PDIs achieved on the NanoAssemblr Benchtop were below 0.2, with many formulations below 0.1, which is difficult to achieve with conventional methods. In the microfluidic channels, the organic and aqueous phases are spatially confined, which results in laminar, as opposed to turbulent flow. Laminar mixing is gentle and highly consistent. This ensures that, under continuous flow and at steady state, each unit volume flowing through the mixer is experiencing nearly identical mixing conditions. Furthermore, the "Autoswitch" feature of the NanoAssemblr Benchtop automatically separates microliter volumes from the head and tail of the process in order to isolate the particles produced under steady state. To achieve batch-to-batch consistency the conditions of liposome precipitation must be highly reproducible. In addition to laminar flow mixing, precise computer-controlled injection of the organic and aqueous phases ensures consistent flow rates and mixing ratios between batches, and between independent instrument operators. Additionally, computer controlled injection allows more precise control over TFR and FRR and thus enables rational optimization of these two parameters.

Conclusion

We established that the NanoAssemblr Benchtop can rapidly tune the size of liposomes by adjusting instrument factors (TFR and FRR) independent of liposome composition. This is a convenient and powerful means of optimizing size and PDI of liposomal drug formulations. Additionally, it was confirmed that liposomes formulated by NanoAssemblr Benchtop maintained exceptional batch-to-batch consistency and minimal population heterogeneity. Taken together, these results demonstrate how the NanoAssemblr platform facilitates rapid optimization of liposomal drug formulations. Furthermore, the process offers a straightforward path to scaling production to clinically relevant quantities by increasing lipid concentration and implementing continuous flow and multiple parallel microfluidic mixers.

Materials & Methods

Liposomes were composed of cholesterol, POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine), and DSPE-PEG_{2000} (1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000]). Lipids were dissolved in ethanol as the organic solvent and Calcium- (Ca^{2+}) and magnesium- (Mg^{2+}) free PBS buffer at neutral pH was used as the aqueous phase. The organic and aqueous phases were rapidly mixed using the NanoAssemblr Benchtop microfluidic instrument at aqueous to organic Flow Rate Ratios between 1:1 and 4:1 and Total Flow Rates between 2 mL/min and 12 mL/min. Formulations were then dialyzed against PBS to remove ethanol. At Flow Rate Ratios 1 and 1.5, formulations were diluted down to 10% ethanol immediately following the mixing process and before dialysis, since high amounts of ethanol can destabilize liposomes. Particle size and integrity were measured before and after dialysis using a Dynamic Light Scattering technique Particle size and integrity was then investigated using Dynamic Light Scattering (Zetasizer, Malvern Instruments, UK). Where indicated, formulations were prepared in triplicate and size and polydispersity index (PDI) are represented as the mean of 3 samples, and error bars represent standard deviation (SD).

Related Material

precisionnanosystems.com/liposomes

Visit our liposome page regularly for the latest information, Application Notes, webinars, and other helpful resources.

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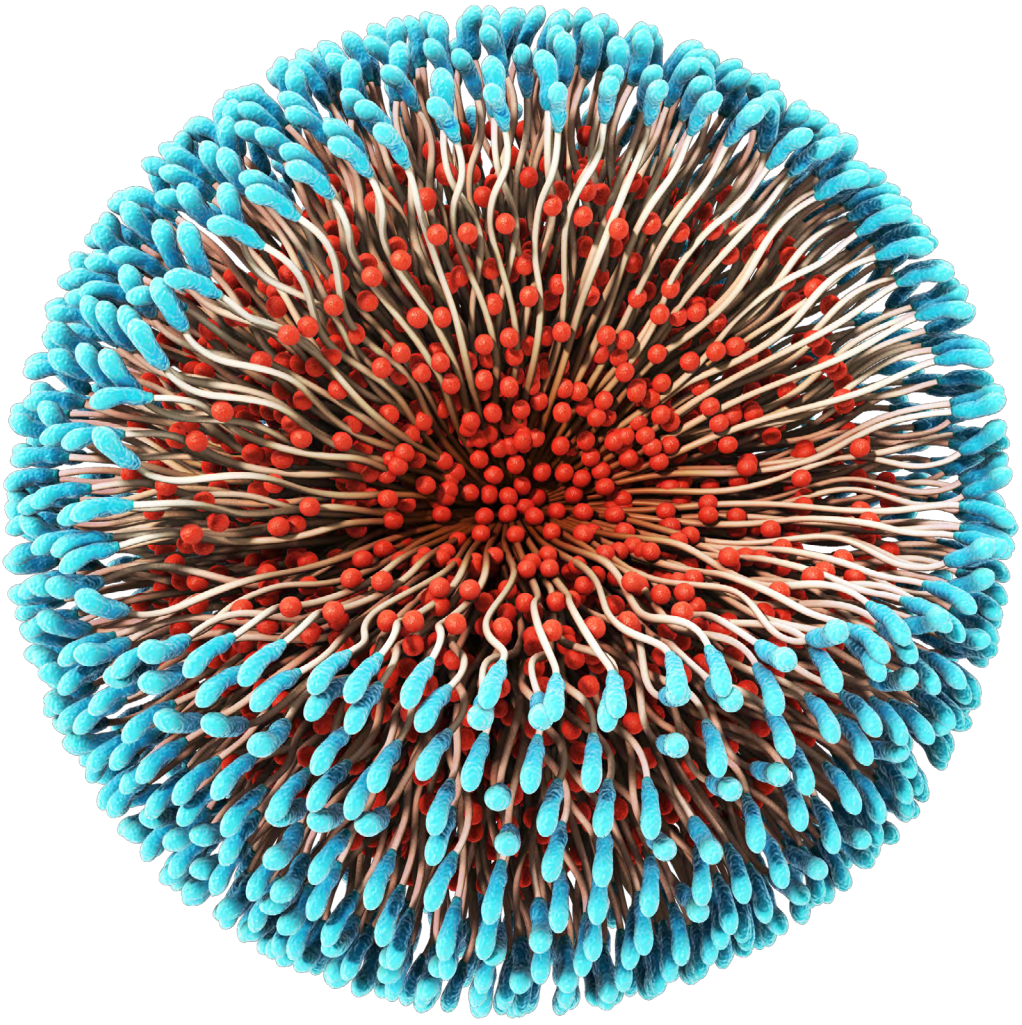
PGLA Nano-particles

Reproducible Production of Sub-100 nm PLGA Nanoparticles using the NanoAssemblr® Microfluidic Platform



S.M. Garg, A. Thomas, G. Heuck, A. Armstead, J. Singh, T.J. Leaver, A.W. Wild, S. Ip, R.J. Taylor, E.C. Ramsay

Abstract

Polymeric nanoparticles of biodegradable and biocompatible polymers such as poly(lactic-co-glycolic acid) (PLGA) are emerging as a promising tool for drug delivery applications. However, there are several challenges which exist in translating these technologies from the bench to clinic. Prominently, current nanoparticle manufacturing methods lack batch-to-batch reproducibility, and are unable to generate PLGA nanoparticles below 100 nm in size with narrow particle size distributions. This leads to inconsistencies in nanoparticle quality that hinder the clinical success of the formulation. Here, we present a novel method for the manufacture of polymeric nanoparticles using a microfluidic technology that addresses these key manufacturing concerns.

Introduction

As the field of nanomedicine continues to expand and the number of polymeric nanoparticle based drugs under development increases, the role of the manufacturing process becomes a critical factor to the drug's success. The specific processes used to produce nanoparticles can affect the size, drug encapsulation efficiency and drug release properties. Batch-to-batch consistency across all these characteristics is important, so a high level of manufacturing reproducibility is required. For polymer-based nanoparticles such as poly (lactide-co-glycolide) or poly (lactic-co-glycolic acid) (PLGA), two key challenges for conventional manufacturing are; the ability to obtain particle sizes below 100 nm, and to maintain batch-to-batch consistency.

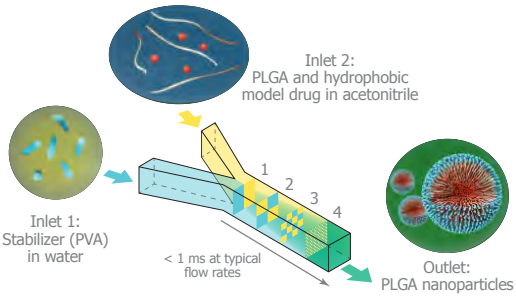
Nanoparticle size plays a critical role in tissue penetration, biodistribution, drug release kinetics and drug efficacy.[1] Literature reports suggest that larger size particles tend to be cleared rapidly from the body whereas smaller size particles below 100 nm exhibit reduced clearance and have a greater ability to reach their intended target.[2, 3] Nanoparticles below 100 nm also exhibit higher uptake into cells when compared to nanoparticles with sizes > 100 nm of the same composition.[4] Conventional manufacturing methods such as Emulsion Solvent Diffusion (ESD), Emulsion Solvent Evaporation (ESE), and nanoprecipitation can produce PLGA nanoparticles in the range of 100 - 1000 nm, however, these methods lack the precise control to specifically tune the size of the nanoparticles.[5] Barring a few reports, most conventional methods of manufacturing PLGA nanoparticles are unable to achieve stable sizes below 100 nm.[6]

In addition to nanoparticle size itself, size uniformity is another key factor associated with the manufacturing process. Narrow size distributions (low polydispersity) lead to more consistent results amongst batches, which is important for downstream clinical success. Most current methods for manufacturing PLGA nanoparticles operate under heterogeneous mixing environments, which leads to inconsistency amongst batches and typically yields broad size distributions (high polydispersity). These nanoparticle batches require additional purification and processing steps, which lowers the overall yield and contributes to product loss during manufacturing. For PLGA nanoparticles in particular, lack of uniformity and batch-to-batch size variability can cause differences in drug efficacy, which highlights the need for a highly reproducible manufacturing process.[5]

Microfluidic methods for manufacturing nanoparticles provide precise control over nanoparticle characteristics such as nanoparticle size and size distribution by providing a rapid and controlled mixing environment at the nanolitre scale.[7] The NanoAssemblr™ platform (Precision NanoSystems Inc.) is an automated microfluidic system, which, in addition to precise control, removes user variability leading to a high degree of reproducibility between batches. Here, the authors present the NanoAssemblr platform for manufacturing PLGA nanoparticles, which utilizes a homogenous mixing environment to achieve high batch-to-batch reproducibility, sub-100 nm nanoparticle size, and low polydispersity in particle size.

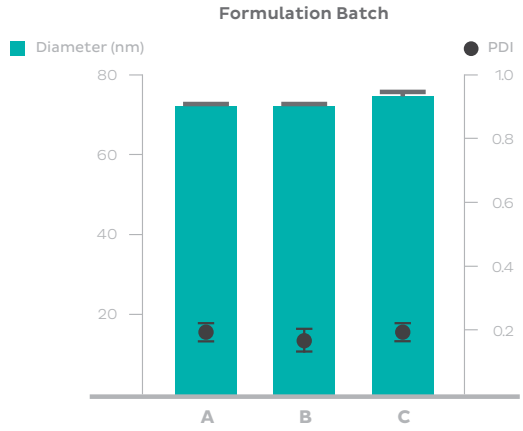
PLGA Nanoparticles were manufactured on the NanoAssemblr® Benchtop as illustrated in **Figure 1**. The platform provided a rapid and controlled mixing environment which favored the formation of PLGA nanoparticles at reproducible sizes below 100 nm when manufactured at different times by different users (**Figure 2**). As seen in the figure, three batches of PLGA nanoparticles prepared by different users exhibit a size of ~75 nm which is not significantly different between each batch ($P > 0.05$). Similarly, the Polydispersity (PDI) was not significantly different between batches ($P > 0.05$) and was at or below 0.2, indicating a narrow size distribution for PLGA nanoparticles.

Figure 1. *Illustration showing the manufacture of PLGA nanoparticles using the NanoAssemblr Benchtop.*



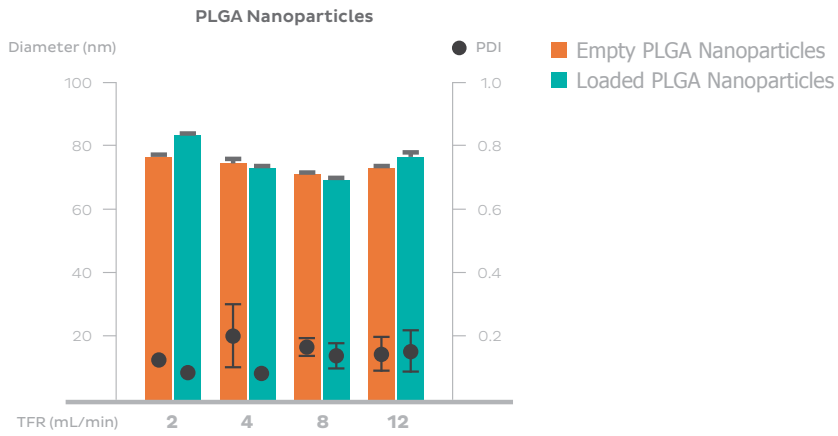
1. Two fluids (Buffer and ethanol) entering the channel under laminar flow do not mix.
2. Microscopic features in the channels are engineered to cause the fluid streams to mingle in a controlled, reproducible, and non-turbulent way.
3. Intermingling of the fluids increases as they continue through the mixer.
4. Fluids emerge from the mixer completely mixed. The process takes < 1 ms; faster than molecules can assemble into particles.

Figure 2. *PLGA nanoparticles of size ~75 nm and PDI ~0.2 were reproducibly manufactured using the NanoAssemblr Benchtop by three different users. Each bar/plot represents the mean ± SD for 3 independent size measurements on the same sample. *denotes significant difference in size between different users (P < 0.05), #denotes significant difference in PDI between different users (P < 0.05) (One way ANOVA followed by Tukey's post-hoc test, P < 0.05).*



Additionally, the NanoAssemblr platform was assessed for its utility in generating drug-loaded PLGA nanoparticles below 100 nm, using Coumarin-6 as a model hydrophobic small-molecule drug. As demonstrated in Figure 3, the NanoAssemblr Benchtop produced PLGA nanoparticles of similar size, at or below 100 nm at different flow rates, regardless of the presence of Coumarin-6, indicating no change in size post-encapsulation of Coumarin-6 in the nanoparticles. The PDI of the nanoparticles was between 0.1-0.2 indicating a narrow size distribution for drug-loaded PLGA nanoparticles.

*Figure 3. Effect of Coumarin-6 encapsulation on the size and PDI of PLGA nanoparticles at polymer concentrations of 5 mg/mL prepared using the NanoAssemblr Benchtop and purified using dialysis. Each bar/plot represents the mean ± SD for 3 independent size measurements on three independent samples (n = 3). *denotes significant difference in size between empty and loaded PLGA nanoparticles (P < 0.05), #denotes significant difference in PDI between empty and loaded PLGA nanoparticles (P < 0.05) (Students' t-test, P < 0.05).*



Discussion

Research on synthetic biodegradable polymers such as PLGA has recently gained momentum in drug delivery applications due to their biocompatibility and biodegradability. While there are a number of methods that are currently used for the manufacture of PLGA nanoparticles for drug delivery, challenges remain such as achieving sub-100 nm sizes, and consistent particle characteristics from batch-to-batch. Here, we described the production of PLGA nanoparticles using a novel microfluidic manufacturing solution, the NanoAssemblr Benchtop, which addresses these key issues. PLGA was selected as the model polymeric system, as it is one of the most attractive polymers for nanoparticle-based drug delivery applications due to its current approval by the United States Food and Drug Administration (US FDA) and European Medical Agency (EMA) for parenteral drug delivery systems.8

The results presented here demonstrate the ability of the NanoAssemblr Benchtop instrument to produce PLGA nanoparticles in a highly reproducible manner, achieving sizes below 100 nm that are difficult to obtain using conventional manufacturing methods. The smaller size of 100 nm increases the applications of PLGA nanoparticles for use in drug delivery applications that favor small size particles. As shown in **Figure 2**, the size of the PLGA nanoparticles remain consistent across batches, highlighting the excellent reproducibility of the PLGA nanoparticle formulations developed using the NanoAssemblr platform. We also demonstrated that sub-100 nm nanoparticles are achievable in both the 'empty' state (PLGA only) or when loaded with a model hydrophobic drug such as Coumarin-6, shown in **Figure 3**. Together, these data demonstrate that microfluidic manufacturing of polymer-based nanoparticles using the NanoAssemblr platform is an attractive alternative to conventional manufacturing methods.

Conclusion

The NanoAssemblr Benchtop can reproducibly achieve sub-100 nm size PLGA nanoparticles, thereby increasing their applications in drug delivery and also eliminating concerns regarding batch-to-batch variability in particle characteristics. In conclusion, the NanoAssemblr platform is an important tool for the efficient manufacture of PLGA nanoparticles to enable the encapsulation and delivery of hydrophobic small-molecule therapeutics.

Materials & Methods

MATERIALS

PLGA ester-terminated (lactide to glycolide ratio 50:50, molecular weight 45,000 - 55,000) was obtained from Akina, Inc (West Lafayette, IN). Poly(vinyl alcohol) (PVA) (molecular weight ~31,000, 87-89 mol% hydrolyzed) and Coumarin-6 were purchased from Sigma-Aldrich (St Louis, MO). All other materials were reagent grade.

MICROFLUIDIC MANUFACTURE OF PLGA NANOPARTICLES

PLGA nanoparticles were prepared using a microfluidic mixer with the NanoAssemblr Benchtop instrument (Precision NanoSystems Inc., Vancouver, BC) by mixing appropriate volumes of PLGA solutions in acetonitrile with aqueous solutions containing PVA. Briefly, PLGA was dissolved in acetonitrile at a concentration of 5 mg/mL, whereas, PVA was dissolved in deionized water at a concentration of 2 % w/v. For synthesis of PLGA nanoparticles, the PLGA solution in acetonitrile was injected through one inlet of the

microfluidic mixer whereas, the 2% w/v PVA solution was injected through the other inlet of the microfluidic mixer. The total formulation volume, aqueous:organic flow rate ratio (FRR), and the total flow rate (TFR) was set at 2 mL, 1:1, and 8 mL/min, respectively. The PLGA nanoparticles were collected in the sample collection tube by discarding an initial and final waste volume of 0.25 and 0.05 mL, respectively. The sample collected was then dialyzed against 1L deionized water using a dialysis bag (MWCO - 10,000) for 12 h replacing the dialysis medium twice in the first 4 h. For testing the reproducibility between batches, three independent experiments were carried out by three different users by following the above mentioned method.

For encapsulation of Coumarin-6, the drug was dissolved in a stock solution of PLGA in acetonitrile such that the concentration of PLGA is 5 mg/mL and the drug/polymer w/w ratio is 1/400. The Coumarin-6 - PLGA solution in acetonitrile was then injected through one inlet of the microfluidic mixer whereas, the 2% w/v PVA solution was injected through the other inlet of the microfluidic mixer to form nanoparticles using the same parameters as listed above at different flow rates from 2 - 12 mL/min. The samples were dialyzed as mentioned above, followed by centrifugation at 8000 × g for 5 minutes to remove and/or precipitate the free drug.

CHARACTERIZATION OF PLGA NANOPARTICLES.

PLGA nanoparticles prepared using the NanoAssemblr Benchtop were characterized for their size (Z-Average size) and polydispersity index (PDI) by dynamic light scattering using a Malvern Zetasizer 3000 (Malvern, UK) by diluting 50 µL of the prepared nanoparticle formulation with 300 µL of deionized water and measured at 25 ºC.

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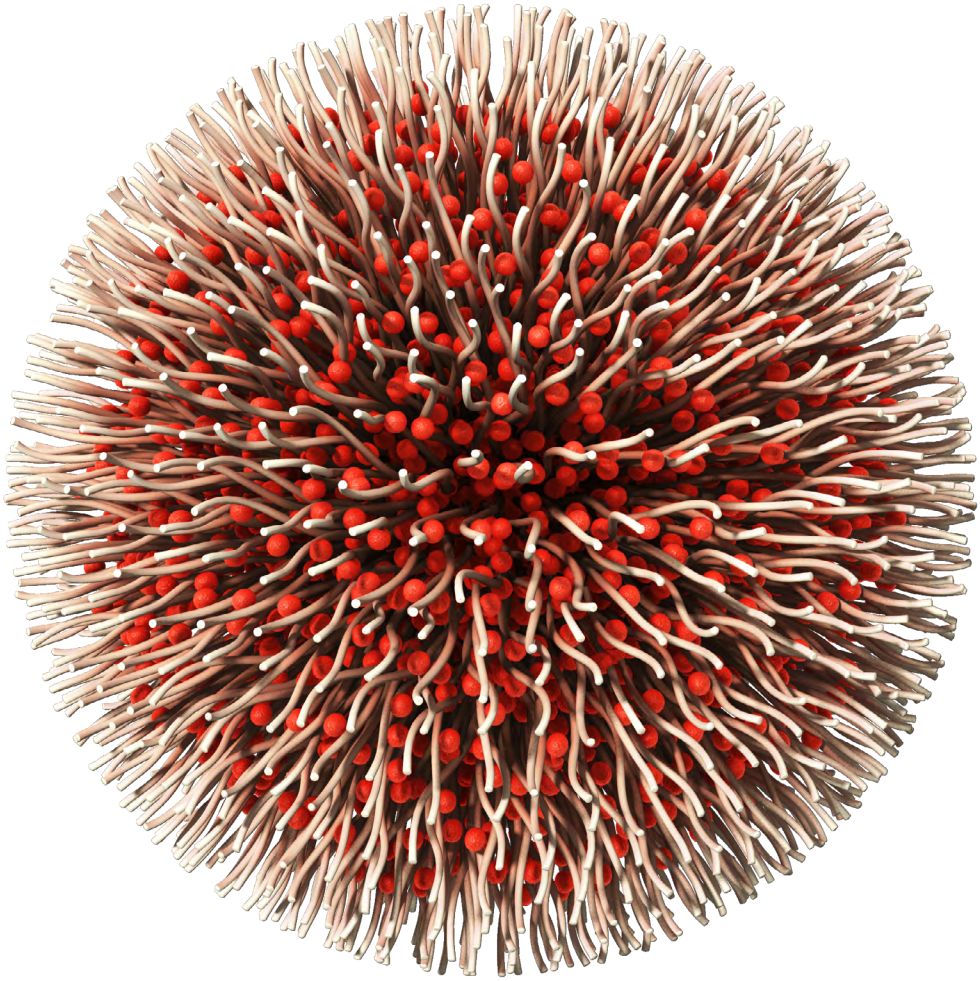
PGLA Nano-particles

Tuning Particle Size Using The NanoAssemblr® Benchtop Instrument



S.M. Garg, A. Thomas, G. Heuck, A. Armstead, J. Singh, T.J. Leaver, A.W. Wild, S. Ip, R.J. Taylor, E.C. Ramsay

Abstract

Emerging evidence from a growing number of studies about nanoparticle-based drugs indicates that the size of these drug-loaded nanoparticles (NPs) plays a critical role in drug efficacy and ultimate clinical success. It is therefore important that manufacturing processes enable precise NP size control during the production process. Current methods for producing NP drugs offer a range of achievable NP sizes but lack the ability to specifically control NP size while maintaining high drug encapsulation efficiency and low polydispersity. Here, we present a novel solution for NP production using the NanoAssemblr® Benchtop instrument, which utilizes microfluidic mixing driven by computer controlled pumps to offer a high degree of control over the solvent/antisolvent nanoprecipitation process and hence the ability to rationally optimize NP properties. Instrument and formulation parameters were systematically explored to tune NP size on the NanoAssemblr® using a representative polymeric poly(lactide-co-glycolide) (PLGA) NP system. Higher flow rates of reagents through the microfluidic mixer resulted in smaller particles, while higher aqueous-to-solvent mixing ratios increased particle size. Additionally, increasing the polymer concentration in the solvent phase led to increased particle size. Through examination of 4 parameters, particle sizes were tuned between 70 and 200 nm with PDIs < 0.2.

Introduction

With the increasing amount of research and development centered on nanoparticle (NP) based therapeutics and drug products, it is evident that NP size plays an important role in drug efficacy. NP size may impact the drug's tissue adsorption and bio-distribution,1-3 so the ability to reproducibly tune the size of drug-loaded NPs is extremely important for clinical success. Current NP production methods lack precise size control and are difficult to scale up to large clinical production batches. The NanoAssemblr microfluidic platform addresses these shortcomings by enabling users to control the environment during NP precipitation through computer controlled parameters. This leads to precise NP size control, a high degree of particle uniformity, and batch-to-batch reproducibility. Furthermore, formulations can be scaled up by increasing the quantities of materials pumped through the system or by employing multiple microfluidic mixers in parallel. These features, significantly reduce time and cost associated with developing NP-based drug candidates.4

Optimization of NP size on the NanoAssemblr platform is achieved via modulation of built-in instrument parameters that control fluid mixing within the microfluidic cartridge and/or by altering the formulation parameters. The first instrument parameter is the total flow rate (TFR), which controls the speed at which the aqueous and solvent phases are mixed together within the microfluidic cartridge. The second instrument parameter is the flow rate ratio (FRR), which controls the mixing ratio of the aqueous and solvent phases. By systematically varying one or both of these instrument parameters, one can achieve a variety of reproducible nanoparticle sizes that can be optimized for a given application.

Additionally, NP size can be tuned on the NanoAssemblr by altering formulation parameters, such as the concentration of the starting materials dissolved in the aqueous and solvent phases (i.e. increase/decrease the concentration of polymers in the solvent phase and/or changing the concentration of stabilizers in the aqueous phase). Here, we present a case study on how these different parameters can be used to tune the size of a NP composed of poly (lacticco-glycolic) acid (PLGA) core and a poly (vinyl alcohol) (PVA) coating using the NanoAssemblr microfluidic mixing platform. The tested parameters are summarized in Table 1.

Instrument Parameters	
Total flow rate (TFR) 2 - 12 mL/min	POPC/Chol/DSPE-PEG$_{2000}$ (52:45:3 mol%)
Flow rate ratio (FRR) 1:1 - 9:1 (aqueous:solvent)	10 mg/mL

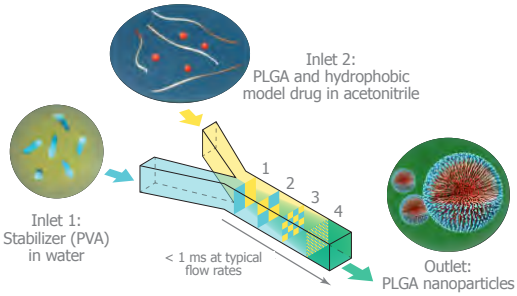
Formulation Parameters	
Polymer (PLGA) concentration 5 - 40 mg/mL	POPC/Chol/DSPE-PEG$_{2000}$ (52:45:3 mol%)
Stabilizer (PVA) concentration 0.5 - 4.0 % w/v	10 mg/mL

Table 1. *Parameters Tested on the NanoAssemblr Benchtop Instrument*

Result

PLGA NPs were manufactured on the NanoAssemblr Benchtop instrument via microfluidic mixing, illustrated in Figure 1. This approach achieves rapid and uniform mixing of the PLGA (solvent phase) with the stabilizer poly(vinyl alcohol) (PVA, aqueous phase) which drives controlled precipitation of the PLGA NP.

Figure 1. *Illustration showing the manufacture of PLGA nanoparticles using the NanoAssemblr Benchtop.*

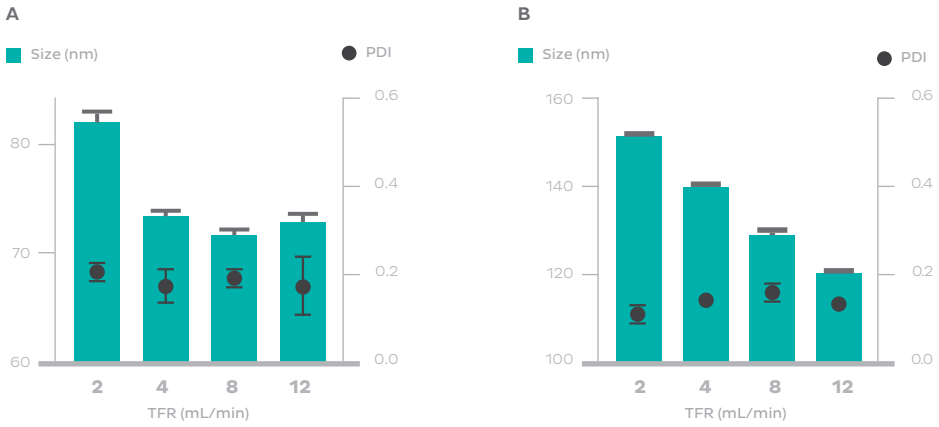


1. Two fluids (Buffer and ethanol) entering the channel under laminar flow do not mix.
2. Microscopic features in the channels are engineered to cause the fluid streams to mingle in a controlled, reproducible, and non-turbulent way.
3. Intermingling of the fluids increases as they continue through the mixer.
4. Fluids emerge from the mixer completely mixed. The process takes < 1 ms; faster than molecules can assemble into particles.

EXPLOITING INSTRUMENT PARAMETERS TO TUNE THE SIZE OF PLGA NP

The effect of total flow rate (TFR) on the size of PLGA particles using a fixed concentration of PLGA and PVA is demonstrated in **Figure 2**. Shown in **Figure 2A**, NPs produced using 5 mg/mL of PLGA showed a small decrease in size, from ~82 to ~73 nm, as TFR increased. NPs produced using a higher concentration of PLGA at 20 mg/mL (**Figure 2B**) exhibited a larger decrease in the particle size, ranging from ~150 nm down to ~120 nm, as TFR increased from 2 to 12 mL/min. The PDI remained consistent (~0.2) regardless of PLGA concentration or TFR.

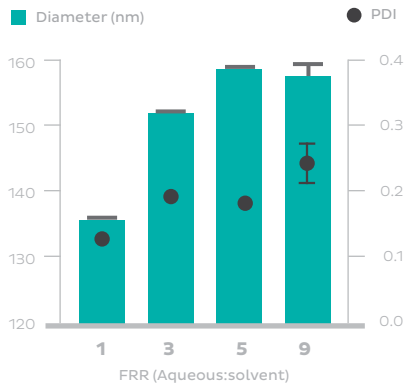
Figure 2. *Effect of total flow rate (TFR) on the size and polydispersity (PDI) of PLGA NP at polymer concentrations of (A) 5 mg/mL and (B) 20 mg/mL using the NanoAssemblr Benchtop. Each bar/ plot represents the mean ± SEM for three independent size/PDI measurements on three independent samples (n = 3).*



Polymer (PLGA) concentration	5 or 20 mg/mL
Stabilizer (PVA) concentration	2.0 % w/v
Total flow rate (TFR)	2 - 12 mL/min
Aqueous: solvent flow rate ratio (FRR)	1:1

Figure 3 demonstrates the effect of flow rate ratio (FRR) on the size and PDI of PLGA NPs. The aqueous:solvent FRR is the ratio of the two phases that are mixed with each other as they are pumped through the microfluidic device; a ratio of 3:1 indicates that 3 parts aqueous phase (PVA in water) is mixed with 1 part solvent phase (PLGA in acetonitrile). As shown in **Figure 3**, as the FRR increased from 1:1 to 9:1, the PLGA NP size increased from ~135 to ~160 nm.

Figure 3. *Effect of aqueous:solvent flow rate ratio (FRR) on the size and PDI of PLGA nanoparticles at polymer concentrations of 20 mg/mL using the NanoAssemblr Benchtop. Each bar/plot represents the mean ± SEM for 3 independent size/PDI measurements on three independent samples (n = 3).*

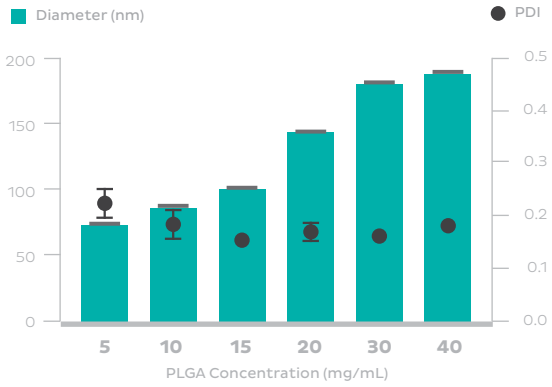


Polymer (PLGA) concentration	20 mg/mL
Stabilizer (PVA) concentration	2.0 % w/v
Total flow rate (TFR)	12 mL/min
Aqueous: solvent flow rate ratio (FRR)	1:1 - 9:1

EXPLOITING INSTRUMENT PARAMETERS TO TUNE THE SIZE OF PLGA NP

The other approach to tune the size of the NPs is to alter the formulation parameters, such as polymer concentration, shown in **Figure 4**. As the concentration of PLGA increased from 5 to 40 mg/mL, NP size also increased from ~70 to ~200 nm while maintaining a PDI around 0.2.

Figure 4. *Effect of PLGA polymer concentration on the size and PDI of PLGA nanoparticles using the NanoAssemblr Benchtop. Each bar/plot represents the mean ± SEM for 3 independent size/PDI measurements on three independent samples (n = 3).*



Polymer (PLGA) concentration	5 - 40 mg/mL
Stabilizer (PVA) concentration	2.0 % w/v
Total flow rate (TFR)	8 mL/min
Aqueous: solvent flow rate ratio (FRR)	1:1

A PLGA concentration of 5 mg/mL may be too low for certain applications which require a final NP size below 100 nm with high polymer content. To achieve high PLGA content and NP size < 100 nm, a large batch of 5 mg/mL PLGA NPs were produced and subsequently concentrated to 25 mg/mL using centrifugal filtration, shown in **Figure 5**. This approach achieves a final NP size ~90 nm at the final desired PLGA concentration of 25 mg/mL, as opposed to formulating directly at 25 mg/mL which produced NP of ~170 nm as indicated in **Figure 4**.

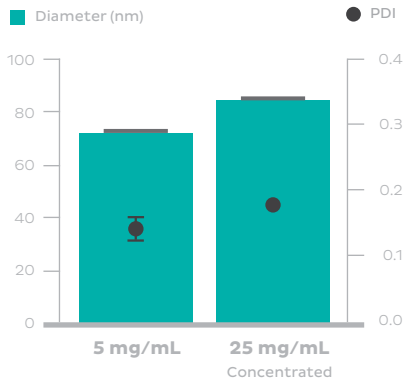


Figure 5. *Change in size of PLGA nanoparticles prepared using the NanoAssemblr Benchtop at 5 mg/mL compared to the same formulation after concentrating to 25 mg/mL. Each bar/plot represents the mean ± SEM for 3 independent size/PDI measurements on three independent samples (n = 3).*

Polymer (PLGA) concentration	5 mg/mL
Stabilizer (PVA) concentration	2.0 % w/v
Total flow rate (TFR)	8 mL/min
Aqueous: solvent flow rate ratio (FRR)	1:1

The concentration of the stabilizing agent, PVA, can also impact the size of the PLGA NP, which is highlighted in **Figure 6**. As the PVA concentration increased from 0.5 to 2% w/v, the PLGA NPs decreased in size from ~163 to ~147 nm. No further reduction in particle size was observed at PVA concentrations > 2% w/v.

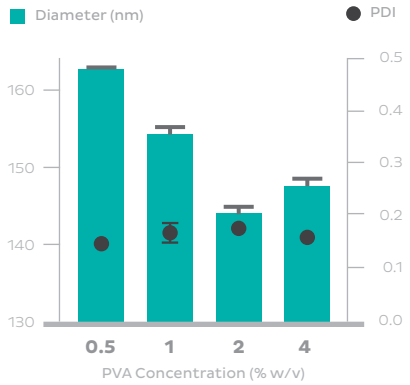


Figure 6. *Effect of PVA stabilizer concentration on the size and PDI of PLGA nanoparticles at polymer concentrations of 20 mg/mL using the NanoAssemblr Benchtop. Each bar/plot represents the mean ± SEM for 3 independent size/PDI measurements on three independent samples (n = 3).*

Polymer (PLGA) concentration	20 mg/mL
Stabilizer (PVA) concentration	0.5 - 2.0 % w/v
Total flow rate (TFR)	8 mL/min
Aqueous: solvent flow rate ratio (FRR)	1:1

Discussion

Size plays an important role in the biodistribution, tissue penetration, drug release and drug efficacy of NP-based therapeutics,[1, 2] so it is critical to maintain control over NP size during the manufacturing process. Compared to conventional methods,[5] the innovative microfluidic mixing technology employed by the NanoAssemblr platform offers exquisite control over NP size during the formulation process. This is achieved by modulating instrument parameters including the total flow rate (TFR) or flow rate ratio (FRR) and formulation parameters such as concentration. Herein, we presented examples of how these parameters can tune the size of PLGA NPs, selected as a representative biodegradable polymer that is currently approved by the FDA for drug delivery applications.[6]

The TFR in mL/min is the total combined speed at which the two fluids are being pumped into the two inlets of the microfluidic device (illustrated in **Figure 1**). As TFR increases, faster mixing time is achieved which reduces PLGA NP size, as shown in Figure 2. As mixing time becomes much faster than the precipitation time of PLGA, NP size approaches an asymptote with increasing TFR. This asymptote is believed to be the 'limit size', which is the smallest, thermodynamically stable NP size for a given system.[7] For 5 mg/mL PLGA specifically, this limit size was ~70 nm for this particular system under these conditions, shown in **Figure 2A.**

The relative amounts of aqueous and solvent phases being mixed at any given moment are dictated by the FRR. As solvent and antisolvent phases are rapidly mixed, there is a sudden shift in polarity that leads to a transient supersaturation of the molecule in the new solvent environment. This drives the precipitation of dissolved molecules into NPs. Here, larger PLGA particles were obtained with higher FRR, which contrasts with trends observed with amphiphilic molecules such as phospholipids (data not shown). The different trends can be understood through differences in the dynamics of particle assembly with these materials. With amphiphilic molecules which self-stabilize, greater changes in solvent polarity drive nucleation of particles which are rapidly stabilized by the hydrophilic portion of the molecule. With hydrophobic PLGA, rapid increase in polarity leads to rapid precipitation of the PLGA core, but surface passivation is limited by the kinetics of the assembly of the PVA corona. For the PVA, as the proportion of the aqueous phase to the organic phase increases, the magnitude of the polarity reduction experienced by the PVA upon mixing decreases. This lowers the driving force for the PVA to assemble on the surface of nascent PLGA particles. This delayed passivation favors growth of larger PLGA cores.

The effects of PLGA and PVA (stabilizer concentration) were also explored. Increasing the concentration of PLGA led to an increase in NP size (**Figure 4**), which is similar to results reported in the literature.[8] Concentrated polymer solutions are more viscous, so it is thought that this change in viscosity may decrease the speed of diffusion of the solvent phase into the aqueous phase which subsequently leads to formation of larger NPs.[8] The role of the PVA is to stabilize the particles by reducing the interfacial tension between the PLGA polymer and the aqueous phase. Changing the concentration of the stabilizer can thus have an impact on the size and PDI of PLGA nanoparticles, shown in **Figure 6**. These results are similar to that reported in literature and is due to the reduction in interfacial tension as concentration increases.[8] No further reduction in particle size was reported at PVA concentrations of 4% w/v which may be because concentrations of 2% w/v are enough to efficiently stabilize these nanoparticles.

It is important to note, that having a precisely controlled, reproducible process is necessary to isolate the effects of formulation parameters on particle size. Without such control, batch-to-batch variability could result in experimental errors that could obscure the effects of the formulation changes. In particular, the effect of PLGA concentration between 5-15 mg/mL on particle size is subtle. Batch-to-batch reproducibility afforded by the NanoAssemblr platform results in nearly negligible experimental error, which lowers the noise floor for these observations allowing changes in size to be both detected and attributed to changes in formulation.

Conclusion

These data demonstrate how NP size can be rationally tuned on the NanoAssemblr Benchtop by modulating the instrument parameters (TFR and FRR) and formulation parameters (polymer and stabilizer concentrations). Through a series of optimization experiments, the NanoAssemblr benchtop was able to formulate PLGA NPs in a range of sizes (70 - 200 nm) which is difficult to attain by most conventional methods of manufacture. Batch-to-batch reproducibility and the ease with which NP size can be tuned on this platform is promising for the field of nanomedicine. .

Materials & Methods

MATERIALS

PLGA ester-terminated (lactide to glycolide ratio 50:50, molecular weight 45,000 - 55,000) was obtained from Akina, Inc (West Lafayette, IN). Poly(vinyl alcohol) (PVA) (molecular weight ~31,000, 87-89 mol% hydrolyzed) was purchased from Sigma-Aldrich (St Louis, MO). All other materials were reagent grade.

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The NanoAssemblr® Ignite
with NxGen Technology

Precision NanoSystems is proud to introduce the next stage of evolution in nanomedicine formulation. The Ignite

embodies everything that made the NanoAssemblr Benchtop the workhorse of nanomedicine development for over 100 biopharma companies, while bringing the latest innovations developed for the cGMP system to the bench.

Designed with the End in Mind

Develop a robust, scalable, manufacturing process from the earliest stage enabling you to create transformative medicine at the benchtop

Designed for the future

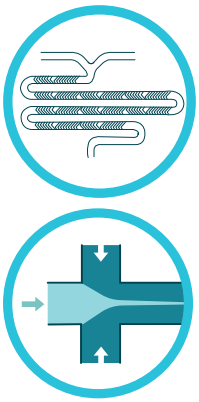
Developed for drug researchers who are targeting delivery of the drug to the disease cells/ tissues/organs enabling the creation of transformative medicines yet to be imagined

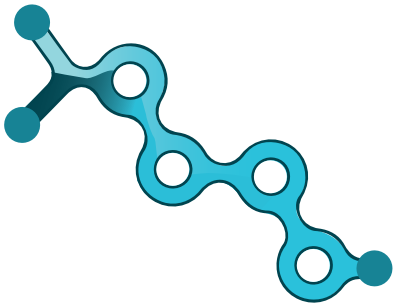
Designed to Maximize Time

Innovative, intuitive technology improves efficiency of nanomedicine development to overcome key challenges and accelerate data generation

NxGen Technology

High Energy Techniques	In-Line Macromixing	Microfluidic Approaches	NxGen Microfluidics
		- Some scaling challenges remain	+ Easy to Scale
		- Mixers are difficult to make	+ Mixers are Easy to make
- Limited applications	- Limited applications	+ Expanded Applications	+ Potential multi-mixer integration opens possibilities
- Difficult to reproduce	- Difficult to reproduce	+ Reproducible	+ Reproducible
- Harsh process conditions	+ Gentler process conditions	+ Non-turbulent process conditions	+ Non-turbulent process conditions
- Difficult to scale	+ Improved Scalability		





Freedom to Scale

The only technology that can scale a single mixer from early in-vitro screening to commercial, GMP manufacturing

Process Matters

NxGen technology provides precise, time-invariant control to ensure the best results on each run.

Empowerment

Drug developers of all backgrounds have options to bring solutions into their lab or work with out team based on their needs

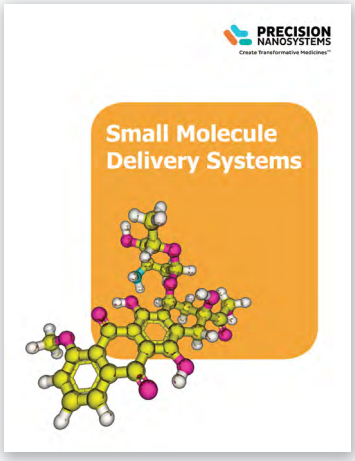
Innovation

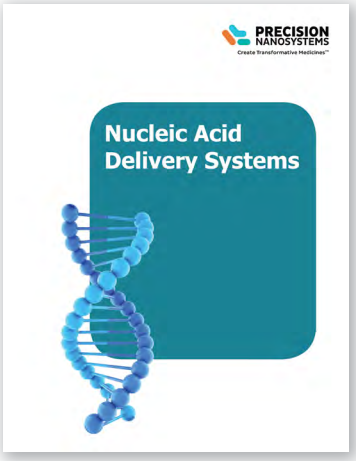
NxGen is a unique and innovative technology offering drug developers the solutions they need to bring transformative medicines to patients

The formulations described in this work were produced using the discontinued NanoAssemblr Benchtop. The NanoAssemblr Ignite can be used in place of its predecessor, the NanoAssemblr Benchtop

Other Resources

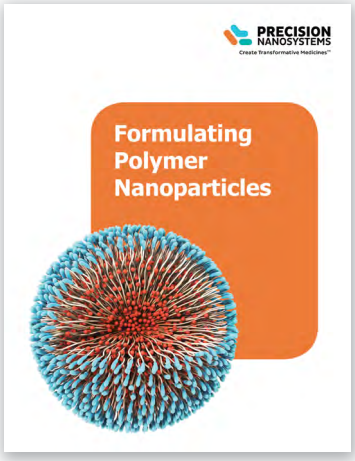
Read other application notes in this series

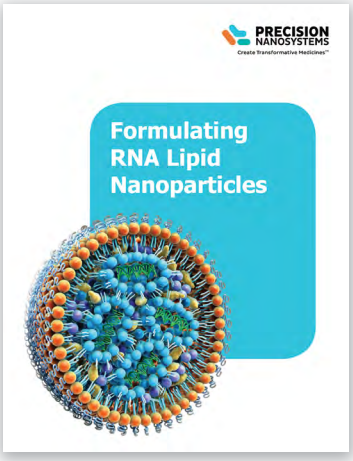


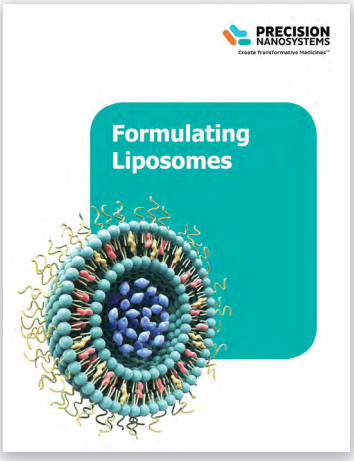


Learn about:

- The delivery of specific active ingredients with different formulation
- Or the versatile applications of different nanoparticle materials
- Exciting papers featuring NanoAssemblr technology published in peer reviewed journals
- Detailed application notes about formulating nanoparticles with the NanoAssemblr platform

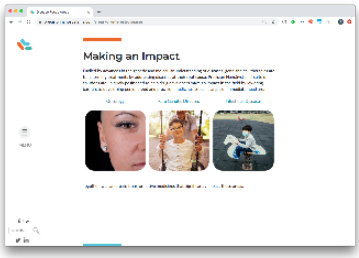






Visit us online to:

Learn about our 3 disease focus areas



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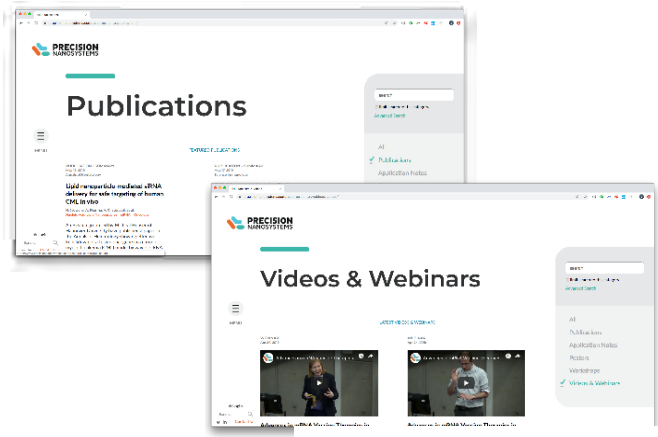


Notes

Notes

Trusted Globally

The most innovative biopharmaceutical companies and academic research institutes use the NanoAssemblr Platform to accelerate their nanomedicine programs. NanoAssemblr instruments are used in over 20 countries worldwide and supported by a global team of field application scientists.

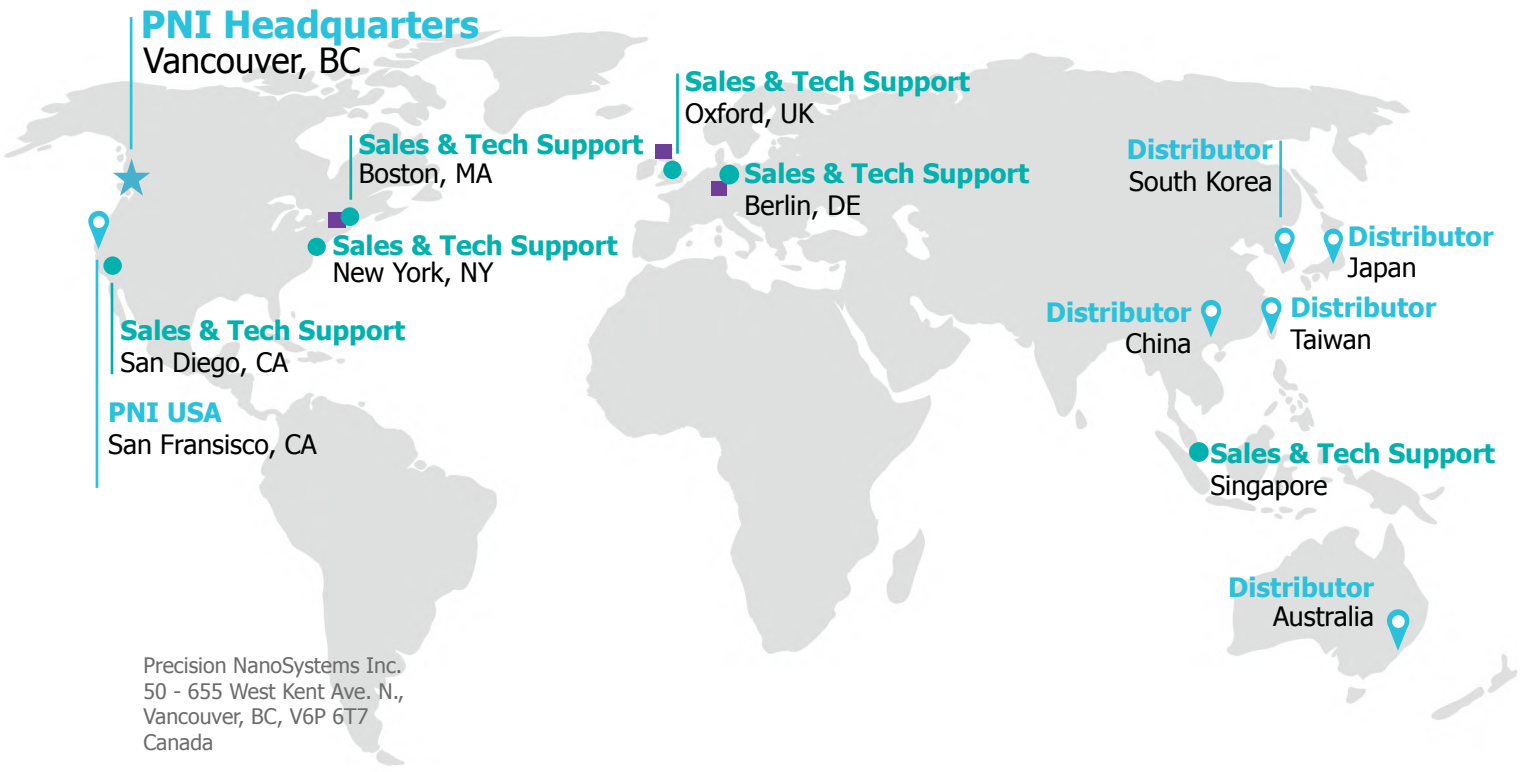
We have the expertise, flexibility and resources to provide a full range of custom formulation solutions.

Precision NanoSystems scientists apply innovative approaches to advance your drug development needs in an uncomplicated, straightforward process.

Our Locations

Nanomedicine Innovation Network
Glasgow, UK
Darmstadt, DE
Boston, MA

CMO Partners Network
Evonik
Ajinomoto bio-Pharma



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