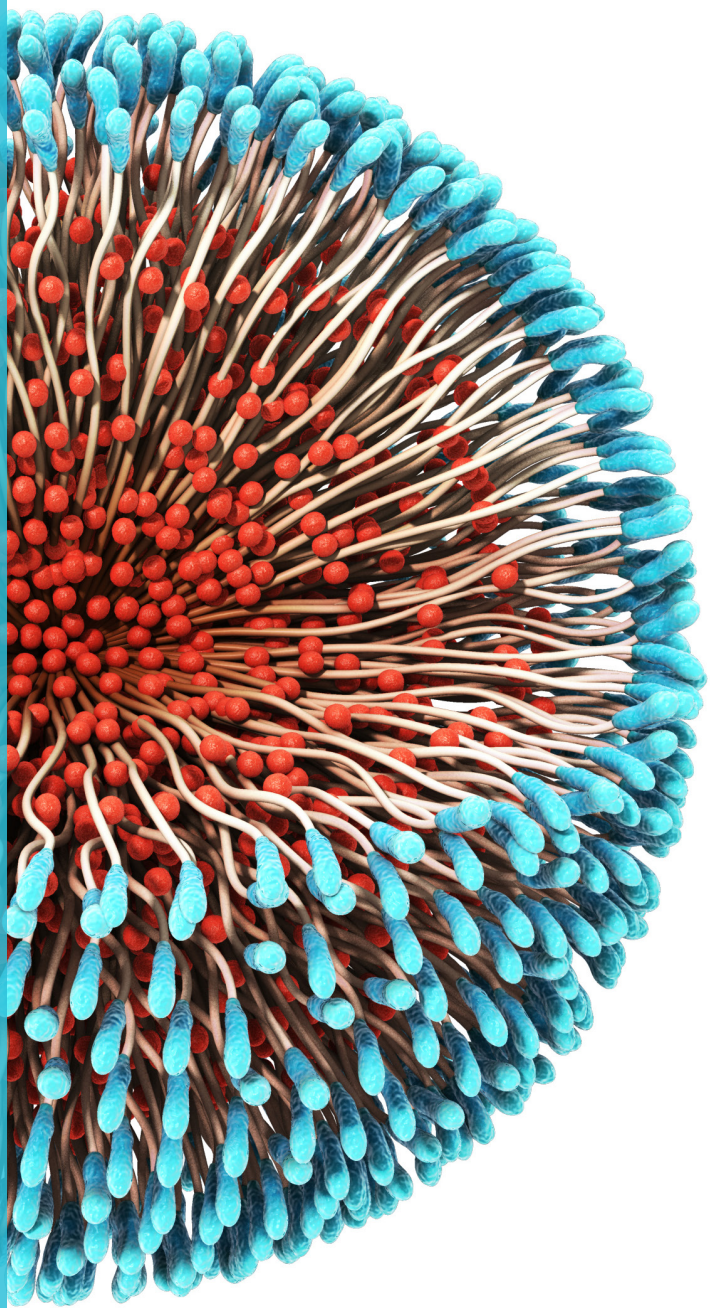
PRECISION NANOSYSTEMS

Create Transformative Medicines™

PLGA 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

Document ID: plgareproducibility-AN-0918

Precision NanoSystems Inc., Vancouver, BC, Canada

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.

Result

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.

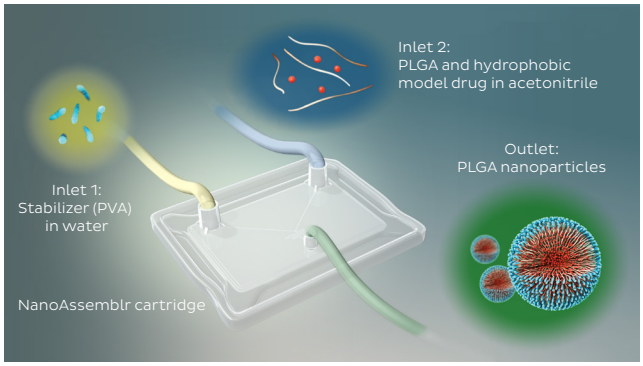


Figure 1. *Illustration showing the manufacture of PLGA nanoparticles using the NanoAssemblr Benchtop.*

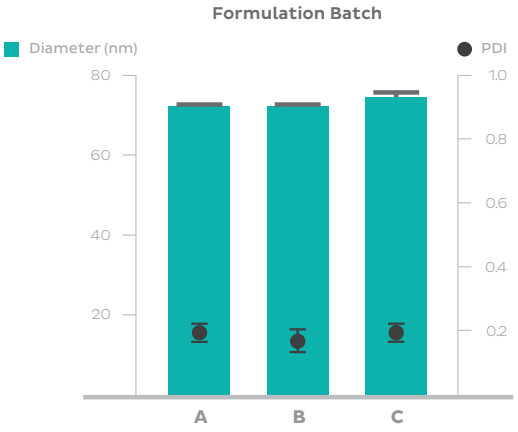


Figure 2. *PLGA nanoparticles of size ~75 nm and PDI ~0.2 were reproducibly manufactured using the NanoAssemblr Benchtop by three different users. Each bar/plot represents the mean ± SD for 3 independent size measurements on the same sample. *denotes significant difference in size between different users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

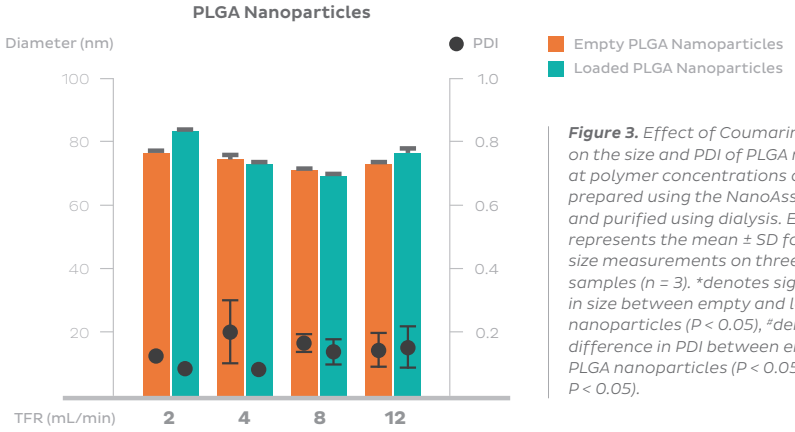


Figure 3. *Effect of Coumarin-6 encapsulation on the size and PDI of PLGA nanoparticles at polymer concentrations of 5 mg/mL prepared using the NanoAssemblr Benchtop and purified using dialysis. Each bar/plot represents the mean ± SD for 3 independent size measurements on three independent samples (n = 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.

REFERENCES

1. *Mahapatro A, Singh DK. Biodegradable nanoparticles are excellent vehicle for site directed in-vivo delivery of drugs and vaccines. J Nanobiotechnology 9 55 (2011).*
2. *Gaumet M, Vargas A, Gurny R, Delie F. Nanoparticles for drug delivery: the need for precision in reporting particle size parameters. Eur J Pharm Biopharm 69(1), 1-9 (2008).*
3. *Alexis F, Pridgen E, Molnar LK, Farokhzad OC. Factors affecting the clearance and biodistribution of polymeric nanoparticles. Mol Pharm 5(4), 505-515 (2008).*
4. *Choi JS, Cao J, Naeem M et al. Size-controlled biodegradable nanoparticles: preparation and size-dependent cellular uptake and tumor cell growth inhibition. Colloids Surf B Biointerfaces 122 545-551 (2014).*
5. *Xie H, Smith JW. Fabrication of PLGA nanoparticles with a fluidic nanoprecipitation system. J Nanobiotechnology 8 18 (2010).*
6. *Astete CE, Sabliov CM. Synthesis and characterization of PLGA nanoparticles. J Biomater Sci Polym Ed 17(3), 247-289 (2006).*
7. *Garg S, Heuck G, Ip S, Ramsay E. Microfluidics: a transformational tool for nanomedicine development and production. J Drug Target 1-15 (2016).*
8. *Lu JM, Wang X, Marin-Muller C et al. Current advances in research and clinical applications of PLGA-based nanotechnology. Expert Rev Mol Diagn 9(4), 325-341 (2009).*