NOVEL SYNTHESIS OF POLYMERIC NANOPARTICLES FOR DRUG DELIVERY APPLICATIONS USING MICROFLUIDIC RAPID MIXING

P.Valencia[1], P. Basto[1], F. Gu[1], L. Zhang[1], C. Cannizzaro[1], R. Langer[1], O. Farokhzad[2] and R. Karnik[1]

[1]Massachusetts Institute of Technology,USA, [2]Brigham and Women's Hospital, Harvard Medical School, USA

ABSTRACT

We report the use of microfluidic rapid mixing using hydrodynamic flow focusing to control the self-assembly of polymeric nanoparticles (NPs) that can be used for drug delivery. PLGA-PEG polymeric NPs and PLGA-lipid hybrid NPs were synthesized through nanoprecipitation—a process that involves dilution of a block copolymer from a solvent to an anti-solvent resulting in the precipitation of NPs. We demonstrated that by controlling mixing of precursors with anti-solvent (i.e. water), the particle size could be tuned and more homogeneous NPs could be synthesized. This work is the first implementation of nanoprecipitation on a microfluidic platform.

KEYWORDS: Nanoparticles, Nanoprecipitation, Hydrodynamic Flow Focusing

INTRODUCTION

The development of smart targeted NPs that can deliver drugs at a sustained rate directly to specific cells may provide better efficacy and lower toxicity for treating many diseases. For these applications, control of the NP properties such as size and polydispersity is of utmost importance for the particle's end therapeutic effects. The ability of microfluidics to rapidly mix reagents, provide homogeneous reaction environments, continuously vary reaction conditions, and add reagents at precise time intervals during reaction progression makes it an attractive technology for nanocarrier synthesis applications [1].

NPs made from PLGA-PEG diblock copolymer, a model biomaterial for drug delivery, are typically prepared by nanoprecipitation by the drop-wise addition of polymer/drug solution into water with slow mixing [2]. Others have reported the use flow focusing for synthesis of polymeric particles but only at the micron scale through the formation of emulsions. Concurrently, PLGA-lipid hybrid NPs are attractive because they combine the long circulation half-life and ease of surface modification of lipids with the high loading and sustained drug release of polymers. The current method to synthesize lipid-polymer complexes requires at least two steps to formulate and results in NPs with sizes > 150nm [3]. Our work differs from the others aforementioned in that we make PLGA-PEG and PLGA-lipid particles in the sub-100 nanometer scale in a single step process through self-assembly, using rapid mixing to control particle characteristics.

978-0-9798064-1-4/μTAS2008/$20©2008CBMS

THEORY

PLGA-PEG NPs are synthesized in a microfluidic channel by rapidly mixing solvent with anti-solvent solutions using hydrodynamic flow focusing [4]. In flow focusing, the fluid stream to be mixed flows along the central channel and is squeezed by the two adjacent streams flowing at higher flow rates (Figure 1a). The narrow width of the focused stream enables rapid mixing through diffusion. The mixing time can be tuned by controlling the flow ratio of the solvent to the anti-solvent streams. In the case of PLGA-lipid NPs, passive mixing structures, called Tesla structures [4] (Figure 2a), are used in junction with flow focusing to ensure the complete homogenization of the precursors dissolved in different fluid streams (i.e. Lipid in water and PLGA in acetonitrile).

EXPERIMENTAL

PDMS microfluidic devices with cross-secitonal area of 20×60μm were used to synthesize the NPs. $PLGA_{15K}$-$PEG_{3.5K}$ was dissolved in acetonitrile (50 mg/mL) and mixed with water (anti-solvent) at fixed flow rates using syringe pumps. Flow ratio of acetonitrile to water stream was varied from 0.1 to 0.01 with a total flow rate of 10μL/min. PLGA-Lipid NPs were formed by mixing $PLGA_{15K}$ in acetonitrile (1mg/ml) with an aqueous solution of the lipid lecithin and the lipid-polymer conjugate DSPE-PEG (lecithin: DPSE-PEG, 9:1). Lipid solution concentration was varied from 10^2 to $5x10^{-3}$ μg/ml at a constant flow rate of 50μl/min.

RESULTS AND DISCUSSION

Figure 1b and 1c shows the polymer stream being focused by two water streams and a TEM image of the resulting NPs, respectively. Figure 1d shows the effect of flow ratio of acetonitrile to water on PLGA-PEG NP size. As flow ratio is increased mixing time decreases as well as particle size. These results agree with the idea that self-assembly of block copolymers into NPs by nanoprecipitation yields smaller particles as mixing time is decreased [5] . In addition, the NP size distribution of the PLGA-PEG NPs formed in the microchannel was narrower and more homogeneous that the one of bulk synthesis (figure 3). This microfludic platform allows for tuning the NP size by changing the mixing time in a controlled manner.

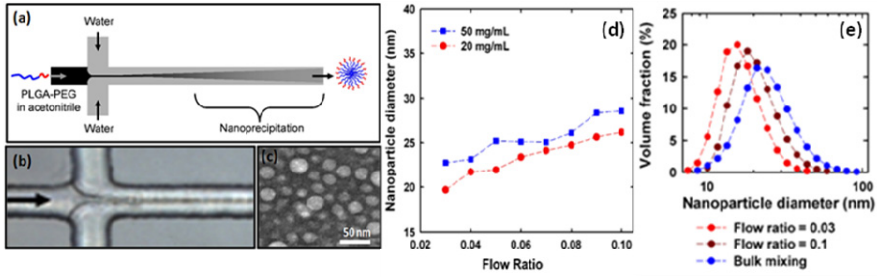

Figure 1. (a) Schematic of synthesis of NPs using hydrodynamic flow focusing. (b) Micrograph of device in operation. (c) TEM image of NPs synthesized. (d) and (e) Effect of flow ratio on NP size and size distribution, respectively.

Figure 2b shows a micrograph of the device used to form PLGA-lipid NPs. Aqueous to organic flow ratio was fixed at 10. Figure 2c shows the effect of lipid concentration in the aqueous stream on the NP size. These NPs are rapidly formed in a one-step process. We find at high lipid concentration, liposomes are the preferred self-assembled structure. At lower lipid concentrations, lipid-polymer NPs at the sub 100nm scale are formed. At the lowest lipid concentrations PLGA NPs are formed stabilized by small amounts of lipid and lipid-PEG. There are experiments being carried by our group to further confirm the structure of these resulting NPs.

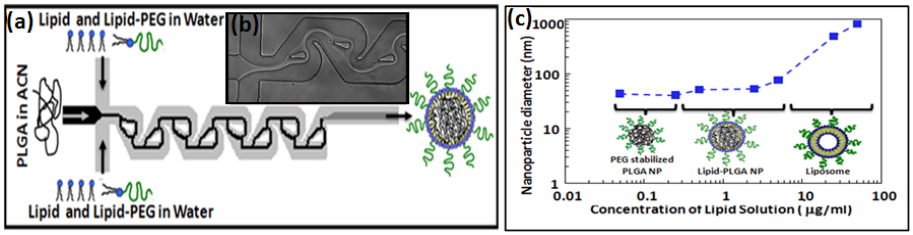

Figure 2. (a) Schematic of synthesis of PLGA-lipid NPs using flow focusing together with passive mixing. (b) Insert: Micrograph of the 20x60 μm device in operation. (c) Change of particle size as a function of lipid concentration in solution.

CONCLUSIONS

Our experiments demonstrate that microfluidic synthesis of polymeric NPs with rapid mixing allows for tuning of NP size and distribution through control of flow rates. These results lay the foundations of a microfluidic platform for controlled synthesis of NPs that may result in improved performance in drug delivery.

ACKNOWLEDGEMENTS

This research was supported by the Prostate Cancer Foundation (RL and OCF) and by NIH grants CA119349 (RL and OCF) and EB003647 (OCF)

REFERENCES

[1] I. Shestopalov, J. D. Tice, J. D and R. F. Ismagilov, Multi-step synthesis of particles performed on millisecond time scale in a microfluidic droplet-based mixer. *Lab on a Chip,* 4, (4), pp. 316-321, (2004).

[2] O. C. Farokhzad, J. J Cheng, B. A. Teply, I. Sherifi, S. Jon, P. W. Kantoff, J. P Richie, R. Langer, Targeted nanoparticle-aptamer bioconjugates for cancer chemotherapy in vivo, *PNAS,* 103, (16), pp. 6315-6320 (2006)

[3] S. Sengupta, D. Eavarone, I. Capila, G. Zhao, N. Watson, T. Kiziltepe, R. Sasisekharan, Temporal targeting of tumour cells and neovasculature with a nanoscale delivery system, *Nature*, 436, pp. 568-572 (2005).

[4] N.T. Nguyen, Z. G. Wu ZG, Micromixers – a review, *J Micromech Microeng*, 605 15(2) pp. R1–R16 (2005)

[5] B. K. Johnson, R. K. Prud'homme, Mechanism for rapid self-assembly of block copolymer nanoparticles, *Physical Review Letters*, 91, 11 pp. 1-4 (2003).