O4-2	**Microfluidic Synthesis of Polymeric Nanoparticles for Targeted Cancer Therapy**

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INTRODUCTION ANS OBJECTIVES

The ability of microfluidics to rapidly mix reagent; provide homogeneous reaction environments; continuously vary reaction conditions; and add reagents at precise time intervals during reaction progression, has made it an attractive technology for a myriad of applications (DeMello, 2006). Over the past decade, microfluidic devices have enabled screening of a variety of reaction conditions by systematically varying flow rates, temperature, and reactant concentrations in order to optimize the quality of the resulting products using very small amounts of reagents. In parallel, there has been an increasing interest in the development of novel nanoparticle and microparticle technologies for drug delivery, imaging, bioanalysis, photonics and optoelectronic applications. The convergence of microfluidic and particle technologies has shown considerable promise allowing for the development of inorganic nanoparticles and microparticles, and in some cases with narrow size distribution or distinct shapes, addressing an important challenge for their maximal exploitation. Relatively little has been done to harness the benefits of microfluidics for the synthesis of organic nanoparticles. This is particularly important since the synthesis of biodegradable polymeric nanoparticles by bulk mixing and nanoprecipitation of drugs and biodegradable polymeric precursors typically lacks control over the mixing processes, which may compromise the properties of the resulting nanoparticles. Rapid and tunable mixing in microfluidics may allow for better control over the process of nanoprecipitation, and also enable screening of various formulation conditions on a single platform by varying parameters such as flow rates, precursor composition, and mixing time.

Our groups and others have previously used the Poly(lactide-co-glycolide)-b-poly(ethyleneglycol) (PLGA-PEG) block copolymers as a model biodegradable and biocompatible biomaterial to synthesize nanoparticles by nanoprecipitation for a variety of biomedical applications (Gref 1994). The PLGA component of the PLGA-PEG nanoparticles provides a biodegradable and biocompatible matrix for encapsulation and controlled release of drugs, while the PEG component provides "stealth" properties for immune evasion and long circulation half-life in blood. Nanoprecipitation offers the advantages of simple and gentle formulation under ambient conditions without the use of chemical additives or harsh formulation processes. However, typical synthesis of PLGA-PEG nanoparticles by nanoprecipitation involves drop-wise addition of polymer-organic solvent solution into a larger quantity of water, resulting in slow and uncontrolled mixing (Gu 2008). Nanoprecipitation through rapid and controlled mixing may enable the formation of more homogeneous PLGA-PEG nanoparticles and provide better control of nanoparticle properties such as size, surface characteristics, and drug loading (Karnik 2008). Here we demonstrate that rapid and tunable microfluidic mixing may be used to synthesize drug-encapsulated biodegradable polymeric PLGA-PEG nanoparticles with defined size, lower polydispersity, and higher drug loading.

MATERIALS AND METHODS

Microfluidic devices were fabricated with poly(dimethylsiloxane) (PDMS) using a standard micromolding process. The mixing channel was 20 μm wide, 60 μm high and 1 cm long. The 500 μL syringe was mounted on a syringe pump (SP220I, World Precision Instruments) while the 25 μL syringe was mounted of another syringe pump (PHD 22/2000, Harvard Apparatus) to control flow through the device. Water flow rate was maintained at 10 μL/min, while the solvent flow rate was varied between 0.3 μL/min to 1 μL/min. Pulsing of the flow was minimized by introducing small air bubbles into the syringes. Flow without significant pulsing could be achieved up to a solvent flow rate of 0.3 μL/min. Water flow rates higher than 10 μL/min caused significant distortion of the PDMS channel, while solvent flow rates higher than 1 μL/min resulted in insufficient length of the mixing channel causing uncontrolled mixing to occur in the outlet region.

Nanoparticles were prepared starting with 50 mg/mL solutions of PLGA111K-PEG3.4K and PLGA222K polymers in acetonitrile. For experiments in which the aqueous to polymer stream flow ratio was varied, deionised water comprised the aqueous stream, while 50 mg/mL PLGA111K -PEG3.4K polymer solution in acetonitrile comprised the polymer stream. The resulting outlet nanoparticle stream was collected in disposable cuvettes (Eppendorf) or glass vials and used for further analysis. This prevented slight (less than 5 %) deviations of the measured size, presumably due to different viscosity of the solvent or slight swelling of the nanoparticles.

RESULTS AND DISCUSSION

We synthesized PLGA-PEG nanoparticles in a microfluidic channel by rapidly mixing polymer-acetonitrile solutions and water using hydrodynamic flow focusing in a controlled nanoprecipitation process. In hydrodynamic flow focusing, the fluid stream to be mixed flows along the central channel meeting two adjacent streams flowing at higher flow rates. We further varied the mixing time for solvent exchange by changing the flow ratio of water and acetonitrile streams from 10 µL/min : 1 µL/min to 10 µL/min : 0.3 µL/min, resulting in mixing time τmix ranging from approximately 0.4 ms to 0.04 ms. We observed that as the mixing time was decreased, the nanoparticle size decreased from about 29 nm to 23 nm for 50 mg/mL polymer concentration, and from about 26 nm to 20 nm for 20 mg/mL polymer concentrations, respectively, which was smaller than the 30 – 35 nm size obtained by bulk nanoprecipitation. Furthermore, nanoparticle size distributions obtained using dynamic light scattering indicated an increase in the homogeneity of the nanoparticles as the rate of mixing was increased (Figure 1).

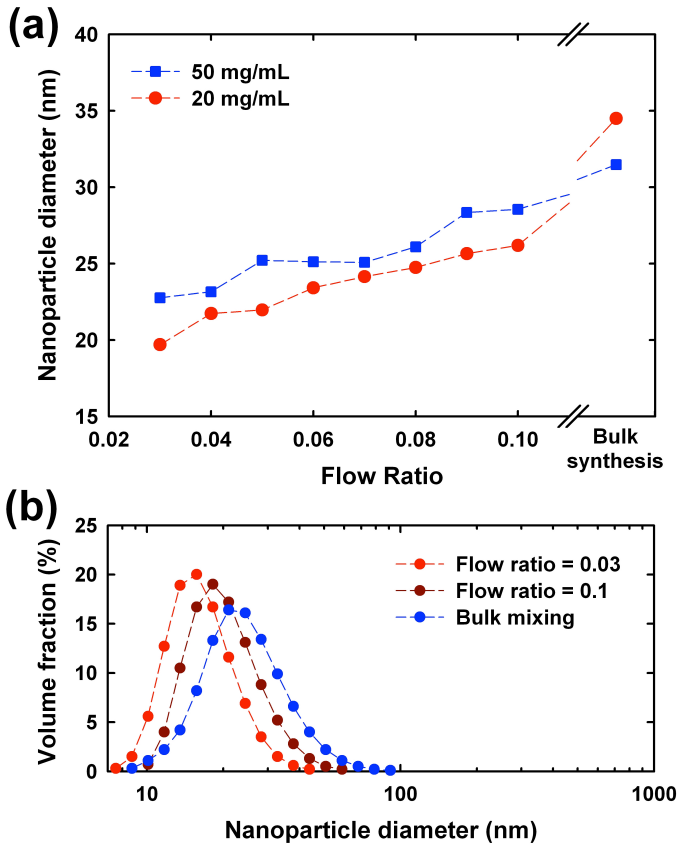

Figure 1. Effect of flow ratio on nanoparticle size.

Figure 1(a) Size of nanoparticles obtained by nanoprecipitation using hydrodynamic flow focusing of PLGA15K-PEG3.4K decreased as the flow ratio was decreased and rate of mixing was increased. Nanoparticles thus obtained were smaller in size than those obtained by bulk synthesis. (b) The homogeneity of nanoparticles obtained by nanoprecipitation of 20 mg/mL PLGA15K-PEG3.4K increased as the rate of mixing increased, corresponding to smaller flow ratios. Size measurements were obtained using dynamic light scattering.

We also examined the effect of polymer composition on nanoprecipitation by adding different amounts of PLGA polymer to the PLGA-PEG diblock polymer. We hypothesized that addition of PLGA to PLGA-PEG nanoparticles may enable control of nanoparticle composition and properties, which may be used to control drug encapsulation and release. Precursor composition was varied by addition of PLGA100K to PLGA15K-PEG3.4K, and the sizes of nanoparticles formed by nanoprecipitation in bulk and by hydrodynamic flow focusing were compared. Addition of PLGA had a large effect on bulk nanoprecipitation and the Z-average nanoparticle diameter increased from 30 nm to 105 nm as the PLGA content was increased to 20 % w/w (Figure 2). Interestingly, size distribution by dynamic light scattering showed a unimodal nanoparticle size distribution with absence of larger aggregates for nanoparticles prepared by hydrodynamic flow focusing. These observations indicate that addition of PLGA decreased the barrier to nanoparticle aggregation and favored the formation of larger nanoparticles under conditions of slow mixing. However, rapid mixing by hydrodynamic flow focusing prevented nanoparticle aggregation resulting in smaller and more homogeneous nanoparticles. These observations indicate that polymer composition may play a significant role in determining the sensitivity of nanoprecipitation to the rate of mixing.

CONCLUSIONS

Nanoprecipitation of polymers and drugs dissolved in organic solvents with non-solvents. We used rapid and tunable mixing through hydrodynamic flow focusing in microfluidic channels to control nanoprecipitation of diblock copolymers as a model polymeric biomaterial for drug delivery. We demonstrate that by varying 1) flow rates, 2) polymer composition, and 3) polymer concentration we can control the size, improve polydispersity, and enhance drug loading of the resulting nanoparticles. This work suggests that microfluidics may find applications for the development and optimization of polymeric nanoparticles in the newly emerging field of nanomedicine.

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