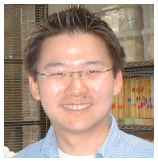
Development of Chemotherapeutic Nanoparticles for Targeted Cancer Therapy



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INTRODUCTION

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 J., 2004 ; deMello A. J., 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 (deMello A. J., 2006 ; Yen B. K., 2003). 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 (Yen B. K., 2005 ; Wagner J., 2005 ; Shestopalov I., 2004 ; Krishnadasan S., 2007 ; Edel J. B., 2002 ; Chan E. M., 2005) and microparticles (Xu S. ; 2005), 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 (Quintanar-Guerrero D., 1998) 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(ethylene glycol) (PLGA-PEG) block copolymers as a model biodegradable and biocompatible biomaterial to synthesize nanoparticles by nanoprecipitation for a variety of biomedical applications (Zhang L., 2007a ; Zhang L., 2007b ; Gref R., 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. 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. 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.

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EXPERIMENTAL METHODS

Device fabrication and experimental setup : Microfluidic devices were fabricated with poly(dimethylsiloxane) (PDMS) using a standard micromolding process. To make the master molds, 4” silicon wafers were spin-coated with SU-8 50 photocurable epoxy (Microchem, Inc.) to a thickness of 60 µm. Baking, lithography, and development procedures were followed as per the recommendations of the manufacturer to obtain a negative relief of the microchannels on the wafer. The wafers were annealed at 150 ºC to eliminate surface cracks in SU-8. The resulting mold was then treated with hexamethyldisilane by placing a few drops of the chemical in an evacuated desiccator along with the wafers and leaving it overnight. The PDMS component was then bonded to a 1” ☒ 2” glass slide using air plasma. The resulting device had one inlet each for water and solvent streams, and one outlet. The water stream was split into two in order to achieve two water streams at the flow-focusing junction. 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.

Preparation of nanoparticles : Nanoparticles were prepared starting with 50 mg/mL solutions of $PLGA_{111K}$-$PEG_{3.4K}$ and $PLGA_{222K}$ 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 $PLGA_{111K}$ -$PEG_{3.4K}$ polymer solution in acetonitrile comprised the polymer stream. Syringes and tubing were rinsed with deionized water for the aqueous streams and with acetonitrile for the polymer stream before loading. After starting the syringe pumps, enough volume (typically 10 µL) was allowed to flow through to ensure rinsing of the outlet tubing (dead volume c.a. 1 µL) and steady operation of the device. Fluid flow in the mixing channel was monitored throughout the nanoprecipitation process. 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.

Particle sizing : Dynamic Light Scattering was used to obtain the hydrodynamic particle diameter. Particle sizing was performed using dynamic light scattering with Zetasizer Nano ZS (Malvern Instruments Ltd., U.K.). For each measurement, c.a. 60 µL or more volume of the sample was loaded in a disposable low-volume cuvette. Three measurements were performed on each sample. The Z-average size and distribution fits were obtained using Dispersion Technology Software (Malvern Instruments Ltd. U.K.). We observed that the presence of acetonitrile changed the nanoparticle size by less than 3 % when water-acetonitrile mixtures containing up to 5 % acetonitrile were further diluted in water. All measurements were performed at acetonitrile concentrations of less than 6 % acetonitrile to ensure that any observed variation in particle size was not due to the solvent. Size distributions and Z-average sizes were obtained by averaging over three measurements.

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. At the low Reynolds numbers, the central stream is squeezed into a narrow stream between the two adjacent streams. The narrow width of the focused stream then enables rapid mixing through diffusion.

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).

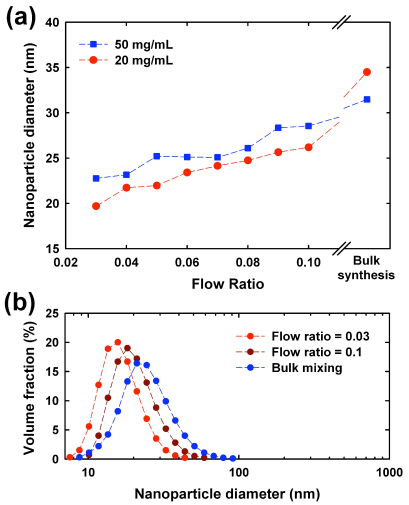

Figure 1. Effect of flow ratio on nanoparticle size. (a) Size of nanoparticles obtained by nanoprecipitation using hydrodynamic flow focusing of PLGA15K-PEG3.4K decreased as the flow ratio was decreased and rate of mixing was increased. Nanoparticles thus obtained were smaller in size than those obtained 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. However, nanoprecipitation of mixtures of PLGA and PLGA-PEG by bulk synthesis results in large nanoparticles[12-14], and we were interested in exploring whether rapid mixing improved this size distribution. 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). Dynamic light scattering revealed a bimodal distribution of large nanoparticles with a broad size distribution (50 – 300 nm) in addition to the 30 nm nanoparticles. In contrast, the nanoparticle size increased from 24 nm to only 34 nm for on-chip nanoprecipitation for the same increase in PLGA content. 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.

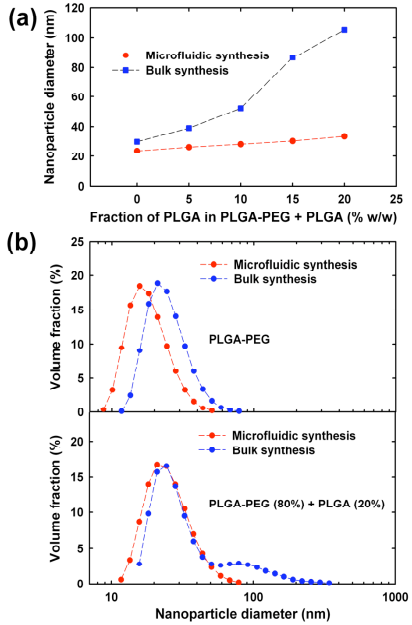

Figure 2. Effect of polymer composition on nanoprecipitation. (a) Size of nanoparticles prepared by bulk nanoprecipitation of PLGA15K-PEG3.4K increased dramatically as increasing amounts of free PLGA100K were added to the precursor solution. However, the size of nanoparticles prepared by hydrodynamic flow focusing remained relatively unchanged upon addition of PLGA100K (Total polymer concentration 50 mg/mL). Comparison of size distributions of nanoparticles in the absence (b) and presence (c) of 20 % w/w PLGA100K reveal PLGA100K induced aggregation of nanoparticles form a tail of larger nanoparticles under conditions slow mixing. However, this aggregation did not occur under conditions of rapid mixing in a microfluidic device, preserving the homogeneity and size of the resulting nanoparticles.

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