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Facile assembly and loading of theranostic polymersomes via multi-impingement flash nanoprecipitation

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ABSTRACT

Flash nanoprecipitation (FNP) has proven to be a powerful tool for the rapid and scalable assembly of solid-core nanoparticles from block copolymers. The process can be performed using a simple confined impingement jets mixer and provides an efficient and reproducible method of loading micelles with hydrophobic drugs. To date, FNP has not been applied for the fabrication of complex or vesicular nanoarchitectures capable of encapsulating hydrophilic molecules or bioactive protein therapeutics. Here, we present FNP as a single customizable method for the assembly of bicontinuous nanospheres, filomicelles and vesicular, multilamellar and tubular polymersomes from poly(ethylene glycol)-*bl*-poly(propylene sulfide) block copolymers. Multiple impingements of polymersomes assembled via FNP were shown to decrease vesicle diameter and polydispersity, allowing gram-scale fabrication of monodisperse polymersomes within minutes. Furthermore, we demonstrate that FNP supports the simultaneous loading of both hydrophobic and hydrophilic molecules respectively into the polymersome membrane and aqueous lumen, and encapsulated enzymes were found to be released and remain active following vesicle lysis. As an example application, theranostic polymersomes were generated via FNP that were dual loaded with the immunosuppressant rapamycin and a fluorescent dye to link targeted immune cells with the elicited immunomodulation of T cells. By expanding the capabilities of FNP, we present a rapid, scalable and reproducible method of nanofabrication for a wide range of nanoarchitectures that are typically challenging to assemble and load with therapeutics for controlled delivery and theranostic strategies.

1. Introduction

Nanocarriers present a versatile method of controlled delivery for bioactive molecules that may otherwise be too hydrophobic or susceptible to degradation for therapeutic applications. A key parameter of nanocarrier design is the nanoarchitecture, which strongly influences in vivo transport, biodistribution, and cellular uptake [1–3]. The ability to tailor nanocarrier architecture has resulted in numerous advancements in targeted delivery, providing enhanced circulation time, membrane permeation and the simultaneous loading of multiple molecules that differ in water solubility [2,4,5]. The self-assembly of block-copolymers allows the formation of diverse soft nanoarchitectures, but presents several engineering challenges, namely: loading efficiency, scalability, repeatability and ease of fabrication. Flash nanoprecipitation (FNP) is a fabrication technique capable of addressing the majority of these issues, but has so far only been applied for the formation of solid-core nanoparticles and their loading with hydrophobic drugs [6,7].

Protocols for FNP employ multi-stream mixers in which an organic solution of solubilized hydrophobic drug and an amphiphilic block copolymer dissolved in a water-miscible common solvent are impinged upon an aqueous solution briefly under turbulent conditions and subsequently introduced into an aqueous reservoir (Fig. 1A) [8,9]. The supersaturated conditions generated by the turbulent mixing induce precipitation and nucleation of the hydrophobic solute and coprecipitation of the block copolymer for stabilization of monodisperse nanoparticles with hydrophobic drug cores [6,7,10]. Mixing occurs over millisecond timescales and is followed by transfer to a reservoir of aqueous nonsolvent to strip away solvent still associating with the aggregated drug and block copolymer. Modifications of this protocol has resulted in the rapid, scalable formation of a variety of nanocolloids with hydrophobic cores containing therapeutics and imaging agents with low water solubility [11–13], and only rarely for hydrophilic and

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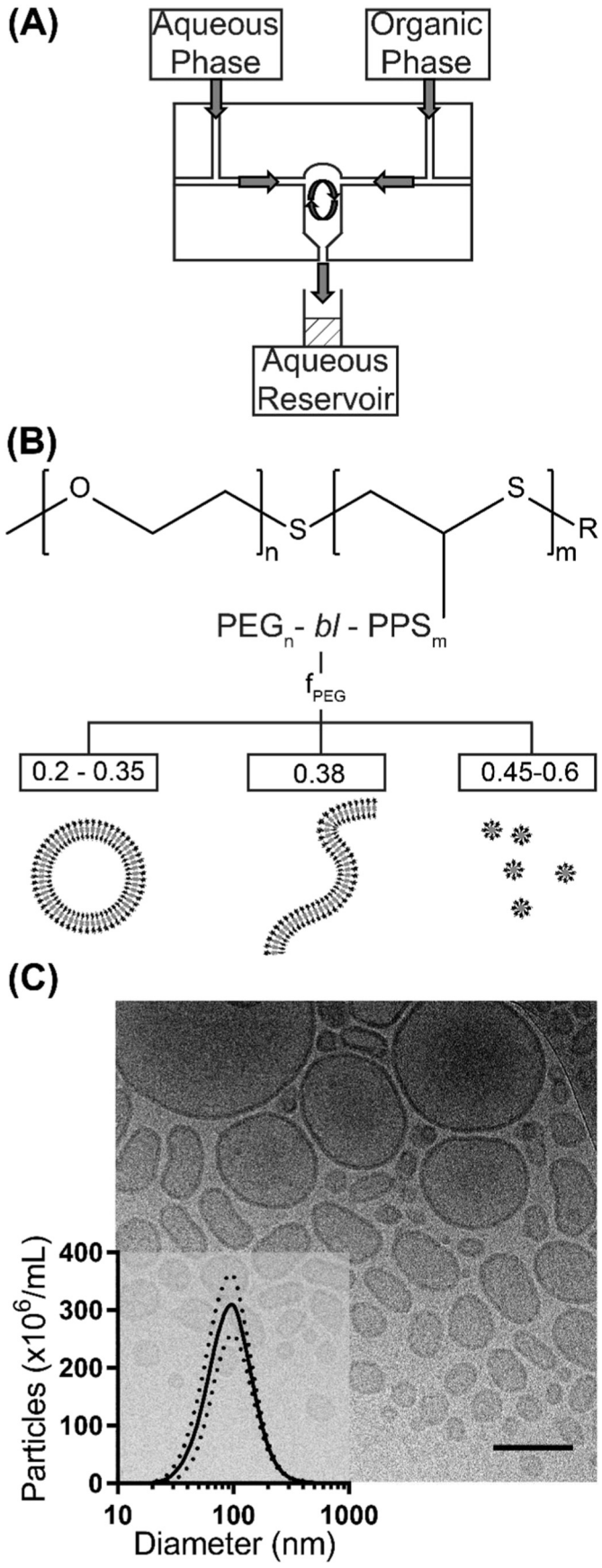

Fig. 1. Overview of polymersome formation by flash nanoprecipitation (FNP). (A) A schematic of the CIJ mixer. (B) The structure of the diblock copolymer poly(ethylene glycol)-*block*-poly(propylene sulfide), and the weight fraction (f_{PEG}) dependent nanostructures known to form using the thin film hydration method. (C) A representative cryoTEM image of polymersomes formed by FNP, scale bar = 300 nm. Inset is a size distribution of polymersomes measured by nanoparticle tracking analysis (NTA), $n = 6$. Standard deviation is represented by the dotted lines.

ionic molecules [14]. While recent focus has been placed on this process of competitive aggregation for the scalable loading of nanoparticles with hydrophobic drugs, FNP was originally applied to achieve rapid changes in solvent quality for homogenous precipitation and self-assembly of block copolymers to investigate the mechanism and kinetics of micellization [15].

While micelles can form within nanoseconds, the combined entropically and enthalpically driven transition in aggregate morphology to filomicelles, bilayer sheets, and vesicles occurs over much longer timescales [16,17]. The glass transition (Tg) of the amphiphile's hydrophobic block influences chain flexibility and as a result the timescale of aggregate shape transformations, which can range from hours for glassy high Tg polymers to milliseconds for low Tg polymers [18–20]. Thus, the rapid mixing followed by an immediate increase in water content within the reservoir during FNP effectively minimizes the chain mobility of the hydrophobic copolymer blocks to stably lock the molecular orientation of the assembly, and this is particularly effective for high Tg polymers like polystyrene [6,8,15,21,22]. It was determined that the organic solvent must be removed from the assembly quickly to prevent nanoparticle instability and ripening, which can be achieved via flash solvent evaporation or using aqueous reservoirs with large volumes to decrease the solvent concentration [23,24]. The use of a low Tg hydrophobic block in the rapid mixing context of FNP may allow access to more complex self-assembled soft nanoarchitectures such as polymer vesicles (i.e. polymersomes).

In the context of drug delivery, micelles and solid-core nanoparticles are limited to hydrophobic molecules, or must be covalently or electrostatically associated with hydrophilic molecules, generally on their surface. In contrast, some nanoarchitectures, like polymersomes, are amenable to the loading of hydrophilic molecules without chemical modification, which can be advantageous for maintaining the bioactivity of therapeutics. Structurally analogous to liposomes, polymersomes possess enhanced physical and chemical stability and have emerged as versatile drug delivery vehicles [25,26]. Polymersomes are comprised of three separate topological regions: an inner aqueous cavity, a hydrophobic membrane and an external surface that together allow simultaneous transport of both water soluble and lipophilic payloads as well as incorporation of adhesive and targeting moieties. A key advantage of the inner lumen is the ability to encapsulate and protect sensitive biologics, such as enzymes and nucleic acids. The size and shape of these nanocarriers impacts their biodistribution, systemic clearance, cellular internalization, optical properties and overall therapeutic potential [1–3,27,28]. Aggregate morphology can be specified by synthesizing block copolymers with a particular hydrophilic weight fraction (typically > 45% for polymersomes), kinetically trapping metastable intermediate structures during self-assembly or modulating the vesicles after formation often using shear forces or osmotic pressure gradients [18,19,22,29–31]. These methods have resulted in a wide range of nanostructures with unique properties and applications, including tubule polymersomes, multilamellar nested vesicles and bicontinuous nanostructures [32–35].

High throughput assembly of soft nanoarchitectures remains a challenge, as most of these morphologies can require days for formation and represent only a small fraction of the assembled aggregate population. The most commonly used methods of polymersome formation from di- or tri-block polymers are diverse variations of thin film hydration, solvent dispersion and microfluidics [22,36–38]. Of these methods, thin film hydration and microfluidics have proven to be most