Polymersome formation via Flash Nanoprecipitation (FNP) induces immunological activity to improve cancer immunotherapy

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Introduction

Objective: To utilize Flash Nanoprecipitation (FNP) as a production method for pH-responsive, polymeric nanoparticles (NPs) – polymersomes – to reproducibly formulate improved, large quantities of polymersomes towards clinical usage

- Utilizing immunotherapeutics, treatments that induce and/or enhance immunological responses, for cancer shows greater potential than conventional treatments
- Cancer immunotherapy utilizes innate immune system to eradicate cancer – more targeted, fewer side effects, decreased risk of metastasis or resurgence
  - Treatments such as Immune Checkpoint Blockade (ICB) largely ineffective in clinical treatments (killer T-cells aren't present in 'cold' tumors)
- Stimulator of interferon genes (STING) pathway utilizes cyclic dinucleotides (CDNs) to turn 'cold' tumors into 'hot' tumors via cytokine release
  - Free CDN efficacy is limited by barriers in drug delivery:
- pH-responsive, polymeric nanoparticle (polymersome) mediated drug delivery can be used for improved cytosolic access and cell-targeted delivery
  - Current polymersome production method (Direct Hydration) is not possible for clinical translation

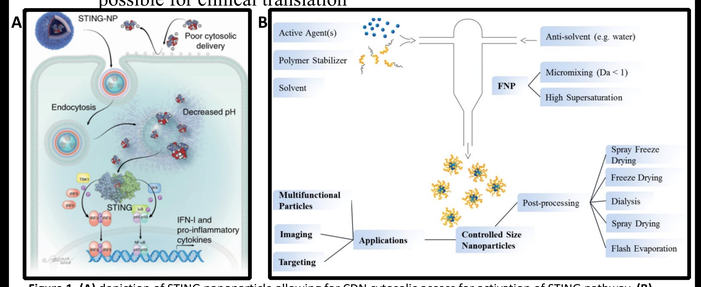

Figure 1. (A) depiction of STING nanoparticle allowing for CDN cytosolic access for activation of STING pathway. **(B)** depiction of the FNP platform using confined impingement jet (CIJ) for supersaturation of particles.

Methods

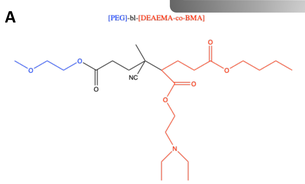

Figure 2. (A) depiction of [PEG]-bl-[DEAEMA-co-BMA] di-block polymer used. 3 polymers were created: a 2kDa PEG block molecular weight was used, while the second block's molecular weight was varied (approximately 6kDa, 12kDa, and 20kDa). **(B)** Model showing FNP impingement protocol. The nanoparticles and drugs were impinged simultaneously, and the solution was recollected in equal volumes in two syringes 4 times. On the 5th impingement, volume was measured and impinged into a stirring aqueous reservoir with twice the volume

Results

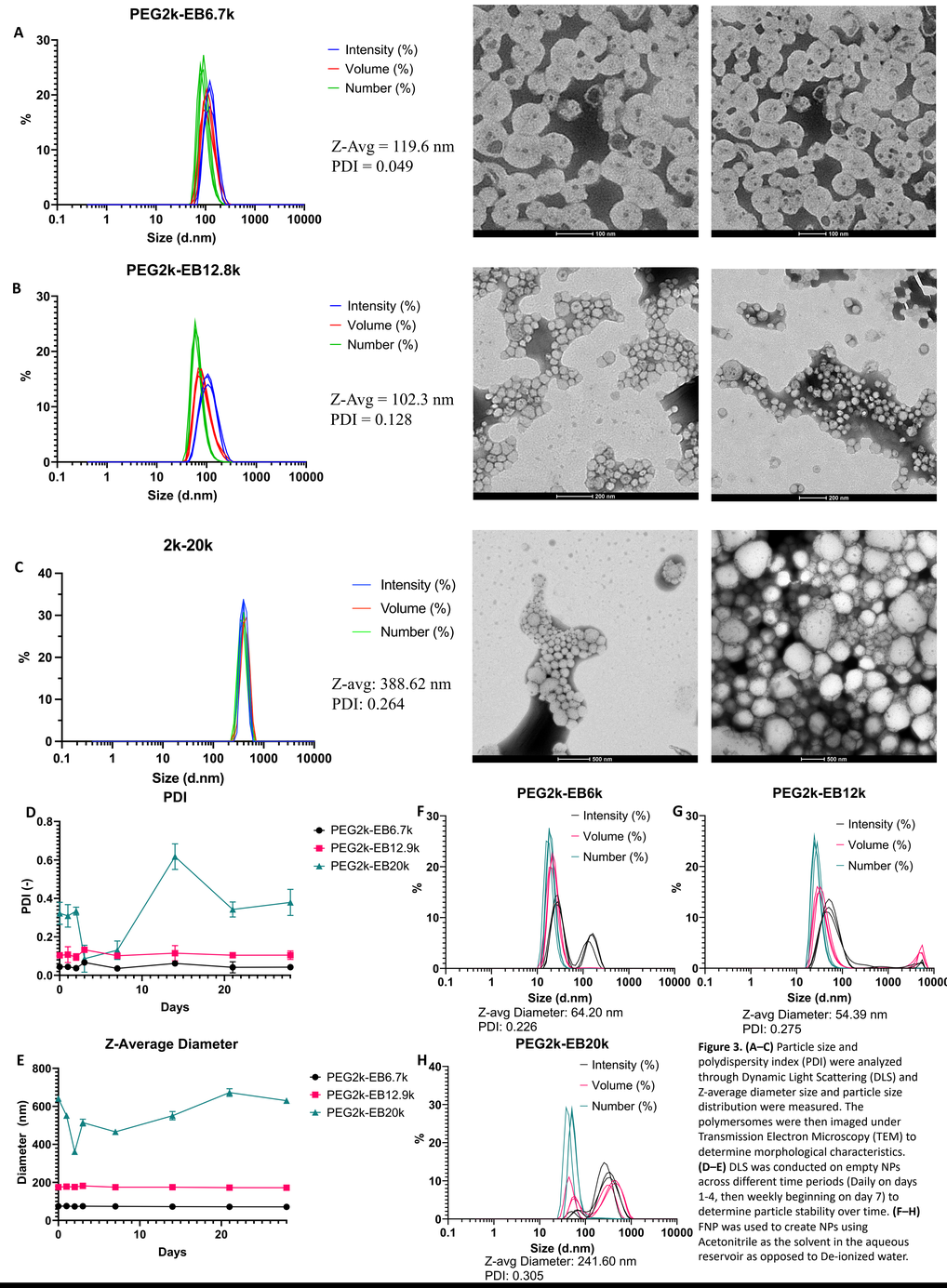

Figure 3. (A–C) Particle size and polydispersity index (PDI) were analyzed through Dynamic Light Scattering (DLS) and Z-average diameter size and particle size distribution were measured. The polymersomes were then imaged under Transmission Electron Microscopy (TEM) to determine morphological characteristics. **(D–E)** DLS was conducted on empty NPs across different time periods (Daily on days 1-4, then weekly beginning on day 7) to determine particle stability over time. **(F–H)** FNP was used to create NPs using Acetonitrile as the solvent in the aqueous reservoir as opposed to De-ionized water.

Results

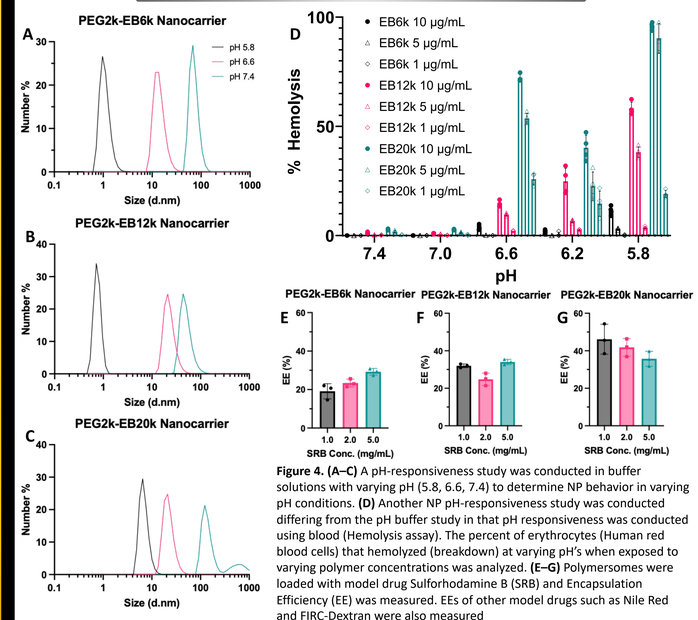

Figure 4. (A–C) A pH-responsiveness study was conducted in buffer solutions with varying pH (5.8, 6.6, 7.4) to determine NP behavior in varying pH conditions. **(D)** Another NP pH-responsiveness study was conducted differing from the pH buffer study in that pH responsiveness was conducted using blood (Hemolysis assay). The percent of erythrocytes (Human red blood cells) that hemolyzed (breakdown) at varying pH's when exposed to varying polymer concentrations was analyzed. **(E–G)** Polymersomes were loaded with model drug Sulforhodamine B (SRB) and Encapsulation Efficiency (EE) was measured. EEs of other model drugs such as Nile Red and FIRC-Dextran were also measured

Future Directions

- Utilizing Cryogenic Electron Microscopy (CryoEM) for more accurate imaging of morphological and size characteristics
- Using a Cytotoxicity assay to ensure polymersomes are not inherently toxic to cells
- Performing a HEK-G8G8 assay utilizing the cystolically dispersed Galectin-8 protein and luciferase to quantify the polymersomes entering the cytosol (inside the cell)
- Loading polymersomes with adjuvant drugs such as MPLA or cGAMP (TLR4 and STING agonists, respectively) *in vitro* to activate various immunological pathways
- Utilizing various antisolvents in polymersome FNP formulations to increase PEG solubility to improve encapsulation efficiencies
- Conducting *in vivo* mouse studies
- Studying the synergism between utilizing multiple adjuvant drugs, and thus, multiple immunological pathways to activate the innate immune system for more enhanced immunological activity

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References

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[2] Saad & Prud'homme, *Nano Today*, 2016.