Modular Nanoparticle Scaffolds for Tunable Drug Delivery

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Statement of Purpose: Traumatic brain injuries (TBI) account for about 30% of all injury deaths.[1] TBIs are also responsible for various disabilities and could cause dramatic changes to a person's personality. Neuronal cell death can accompany such an injury, therefore neurogenesis and progenitor cell differentiation into neurons could be a way to mitigate the effects of TBIs. Basic fibroblast growth factor (FGF-2), a heparin binding protein, is known to cross the blood brain barrier and can regulate neurogenesis.[2] On the other hand, retinoic acid (RA) is a small molecular weight drug that could induce neuronal differentiation of progenitor/stem cells.[3] To this end, we have devised a scaffold that has the potential to carry RA/FGF-2 for tunable delivery. Specifically, we designed a nanoparticle (NP) system composed of cationic poly-L-lysine (PLL) and anionic and modified heparin (Hep). Uniquely, we synthesized and incorporated heparin-methyltetrazine (Hep-mTz) to layered NPs for improving their inclusion into thiol-norbornene hydrogels. The incorporation of mTz on NPs also allows subsequent bio-orthogonal labeling of NPs with other norbornene-tagged bioactive cues for targeted delivery.

Methods: NPs were synthesized via sonicating PLL and Hep for 5 min (Fig. 1A, first step sonication conditions: 10% amplitude, 20kHz. Bronson Digital Sonifier). The weight ratio of Hep to PLL was systematically varied to obtain NPs with different surface charges. NP size and zeta potential (ζ) were measured via dynamic light scattering (DLS). To improve the quality of the NPs, a two-step NP synthesis method was developed. Briefly, core NPs were synthesized using a 0.7 Hep/PLL weight ratio (0.7x). Additional Hep and/or Hep-mTz were added to the core NP solution, followed by secondary sonication. Hep-mTz was synthesized by reacting Hep with methyltetrazine-PEG-amine via standard carbodiimide chemistry. Hep-mTz was modularly added during the second step of NP preparation. For hydrogel crosslinking, desired amounts of 8-arm poly(ethylene glycol)-norbornene (PEG8NB) and NPs were mixed and incubated at 37°C for 20 hrs, followed by addition of dithiothreitol (DTT) and LAP. The precursor mixtures were exposed to 365nm light (5 mW/cm^2) for 2 min for gel crosslinking and NP incorporation. Gel moduli were characterized by oscillatory rheometry.

Results: We designed a unique two-step sonication protocol to improve the tunability and quality of the polyelectrolyte NPs (Fig. 1A). Zeta potential results showed that NP surface charge could be readily tuned by controlling [Hep]/[PLL] ratio (Fig. 1B). The sonication conditions developed in this protocol yielded NPs that were highly stable (i.e., ζ ~ ± 50 mV) regardless of surface charge or size. From the DLS results, we decided to use [Hep]/[PLL] ratios of 0.7x and 1.3x for the subsequent tests. These NPs all had similar size distribution (~120nm, Fig. 1C) and the NPs were stable for 1 month (not shown), which is ideal for extending the shelf-life of therapeutics. With the core NP already formed after the first step sonication, a layer of Hep and/or Hep-mTz could be added to the NP surface rather than distributing throughout the entire NP. Addition of Hep-mTz during the second sonication step increased NP size (from ~157 to ~175 nm. [PLL] =1.54 mg/mL), but had little to no effect on ζ, implying similar stability. Modular tuning of Hep/Hep-mTz in the second sonication step yielded modular and tunable incorporation of 'clickable' motif (i.e., mTz) to NP surface. We tested if mTz incorporation could improve NP retention within the thiol-norbornene hydrogels (Fig. 1D). An increase in shear moduli of the photopolymerized thiol-norbornene hydrogels (Fig. 1D) alongside an increase in retention of NPs suggested that the Hep-mTz functionalized NPs were covalently 'clicked' into the gel network.

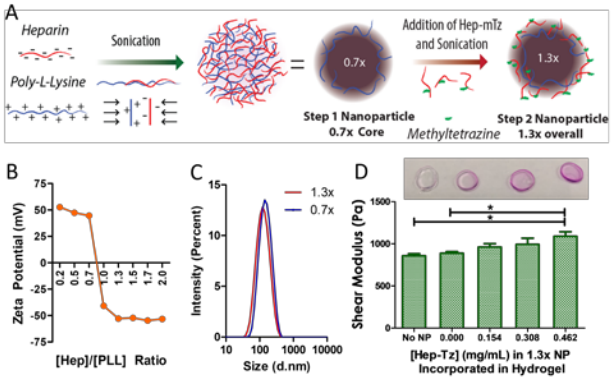

Figure 1. (A) Schematic of two-step sonication protocol for NP synthesis. Hep and/or Hep-mTz was added immediately before the second sonication step. (B) Zeta potential of NPs with varying [Hep]/[PLL] ratios at a constant [PLL] of 0.25 mg/mL. (C) Representative size distribution data. (D) Image and shear moduli of 3wt% PEG8NB-DTT hydrogels incorporated with modular NPs. NP-loaded hydrogels were swelled in DMMB for visualization of NP incorporation.

Conclusions: We have designed a two-step sonication and modular NP formation scheme that did not compromise the quality (i.e. size, PDI, and stability) of the NP produced, while providing a tunable surface charge. The use of Hep-mTz provided additional "clickable' motif on NP surface for further surface modification (e.g., targeting ligand, PEGylation, etc.) through orthogonal norbornene-tetrazine click reaction. Overall, this NP synthesis protocol provided a modular and tunable scaffolding for future dual drug delivery for disease treatment. Current work is focused on loading and controlled delivery of FGF-2/RA.

References: [1] Taylor et al. Surveillance Summaries March 17, 2017 66(9);1–16. [2] Wagner *et al*. Journal of Neuroscience July 15, 1999, 19(14):6006-6016. [3] Maia *et al*. ACS Nano, 2011, 5 (1), pp 97–106