# Targeted Modulation of Fibrin Formation and Stability with Intravenous Hemostatic Nanotechnologies

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**Statement of Purpose:** Severe hemorrhage associated with trauma, surgery, and other coagulopathies can be life-threatening and requires rapid hemostatic management[1]. For bleeding that cannot be easily managed externally with topical hemostats, intravenous hemostatic resuscitation is needed. The clinical gold standard for this is the transfusion of blood products, but due to donor-dependency, specialized storage requirements, high risk of contamination and short shelf-life, blood product usage face significant challenges. Consequently, synthetic biomaterials-based I.V.-administrable technologies (nanoparticles, polymers etc.) that can provide specific hemostatic functions while allowing donor-independent manufacturing, scale-up, and on-demand in-hospital and pre-hospital availability, have emerged as a clinically promising area of bleeding management[1]. To this end, here we report on two unique synthetic hemostatic technologies: (i) injury-**t**argeted **T**hrombin-loaded **L**ipid **N**anoparticles (**t-TLNPs**) and (ii) Fibrin-crosslinking pullulan derivative (**FibrinX-Pull**), that render injury site-specific *fibrin generation* and *fibrin stabilization* respectively. Since coagulopathic impairment of hemostasis is majorly associated with compromised fibrin kinetics and stability, we hypothesized that intravenous hemostatic technologies that enhance fibrin formation and stability in an injury site-specific manner can significantly reduce bleeding. Here we present our studies on testing this hypothesis using these technologies as stand-alone as well as in combination.

**Methods:** The **t-TLNP** design involves conjugating von Willebrand Factor (vWF)-binding and collagen-binding CBP peptides (VBP, CBP) to Distearoyl phosphatidyl-ethanolamine (DSPE) via polyethylene glycol (PEG) spacers and combining the resultant DSPE-PEG-peptide conjugates at controlled ratios with Distearoyl phosphatidylcholine (DSPC) and Cholesterol to manufacture liposomal nanoparticles (LNPs) using thin film rehydration and extrusion technique. Aqueous-dissolved thrombin was loaded in these LNPs during the lipid film hydration step. Resultant t-TLNPs, once I.V.-administered, can target to the injury site via simultaneous anchorage to vWF and collagen exposed at the site, and release thrombin locally via diffusion as well as phospholipase-induced LNP degradation. The thrombin converts fibrinogen to fibrin locally for rapid hemostatic action. The **FibrinX-Pull** design is adapted from studies by *Chan et. al.* for fibrin-binding PolySTAT polymer[2]. Briefly, Pullulan was TEMPO-oxidized to generate carboxyl (-COOH) group at C6 position, which was then conjugated to a fibrin-binding peptide (FBP) via carbodiimide chemistry. Both t-TLNPs and FibrinX-Pull were evaluated in coagulopathic settings simulated in human whole blood and plasma (e.g. platelet depletion or dysfunction, anticoagulation, tPA-induced fibrinolysis etc.), using overall hemostatic potential (OHP) assay and rotational thromboelastometry (ROTEM) to characterize fibrin and temporal clot characteristics. Fibrin kinetics in these settings were also studied using BioFlux microfluidics. Subsequently the effect of t-TLNPs and FibrinX-Pull as stand-alone and combination, were evaluated *in vivo* in mouse and rat models of bleeding.

**Results:** Our OHP studies demonstrated that t-TLNPs can rescue fibrin generation kinetics when endogenous fibrin formation is compromised due to platelet and coagulation defects. Corresponding ROTEM studies corroborated this ability of t-TLNPs to improve clotting kinetics. OHP and ROTEM studies also demonstrated that FibrinX-Pull significantly improves (and maintains) fibrin clot stability under a tPA-induced lytic environment even when the endogenous fibrin crosslinking molecule, coagulation factor FXIIIa, is inhibited. The fibrin generation ability of t-TLNPs and fibrin stabilization (under lysis) by FibrinX-Pull were also confirmed in BioFlux microfluidics by real-time imaging of AlexaFluor-647-labeled fibrin. In these studies, the fibrin was additionally quantified by D-Dimer ELISA assay to confirm the effects of t-TLNPs and FibrinX-Pull. Significant enhancement of clot characteristics (acceleration of clotting time, enhancement of clot amplitude, reduction of clot lysis) was observed in ROTEM and BioFlux, when t-TLNPs and FibrinX-Pull were combined. The *in vivo* studies showed that treatment with tTLNPs (in animals with clot formation defects) or with FibrinX-Pull (in animals with clot lysis induction) or both (in animals with acute trauma coagulopathy), can significantly improve hemostasis. Some representative results from our studies are shown in **Figure 1**.

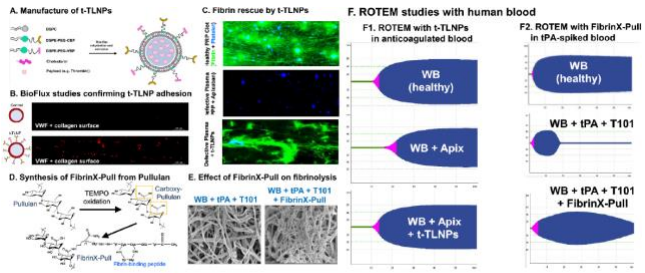


**Figure 1.** Representative results: **A.** t-TLNP manufacture; **B.** Red fluorescent t-TLNPs significantly adhere to 'vWF+collagen' surface under flow; **C.** t-TLNPs rescue green fibrin in 'platelet depleted + anticoagulated' plasma; **D.** FibrinX-Pull synthesis; **E.** FibrinX-Pull maintains fibrin morphology under lysis; **F.** ROTEM data showing that t-TLNPs rescue clotting time in anticoagulated blood and FibrinX-Pull maintain clot stability in tPA-spiked blood.

**Conclusion:** Our studies altogether demonstrate that targeted enhancement of fibrin kinetics and stability using I.V. administration of t-TLNPs and FibrinX-Pull can provide significant hemostatic benefit in treating bleeding.

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**References:**

**[1]** Hickman DA et al. *Adv. Mat.*, 2018; 30(4).
**[2]** Chan LW et al. *Sci Trans Med*, 2015, 7(277).