Thrombin-triggered Shape Changing Nanogels for Development of Synthetic Platelets

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Statement of Purpose: Platelets are important to the processes of hemostasis and wound healing. Under normal circumstances, platelets circulate in the bloodstream in a rounded, inactive form. When injury occurs, platelets activate fibrin-binding surface receptors, change morphology into stellate shapes, bind to fibrin and mediate hemostasis. When activated, platelets can induce clot retraction, where platelets bind to fibrin fibers at the wound site and engage actin-myosin machinery to contract the fibers. Clot retraction increases clot density, stiffness and stability. Recent evidence also suggests that the increased clot stiffness can durotactically guide cells into the wound site and enhance subsequent wound healing.

In situations of hemorrhage, platelets can become depleted alongside coagulation factors, leading to conditions where platelet function is reduced[1]. To address the issue of platelet depletion, our group has previously developed ultra-low crosslinked (ULC) poly(N-isopropylacrylamide) (pNIPAm) nanogel based platelet-like particles (PLPs). These PLPs are able to recapitulate active platelet morphology and clot retraction[2] and they promote hemostasis and healing in animal wound models.

However, despite the promise of PLPs, they are unable to conditionally change shape, which could hinder delivery to wound sites. PLP-mediated clot retraction and healing are linked to the deformable nature and stellate morphology of the ULC nanogels. Thus, the purpose of this research is to develop a thrombin-sensitive nanogel that mimics the ability of native platelets to undergo a shape change in response to thrombin at wound sites.

Methods: To achieve thrombin-triggered nanogel shape change, particles were synthesized with a thrombin-cleavable peptide crosslinker methacrylate-Gly-dPHe-Pro-Arg-Fly-Phe-Pro-Ala-Gly-Gly-Lys-methacrylate.[3] Nanogels were synthesized by a precipitation synthesis of 85% N-isopropylmethacrylamide (NIPMAM)/10% Acrylic Acid/5% thrombin-cleavable peptide. To evaluate shape change in the presence of thrombin, nanogels were suspended in 50 U/mL and 75 U/mL thrombin. Aliquots were taken at t = 0, 2 hr, 4 hr, 24 hr, 48 hr, 96 hr, and 1 wk. Aliquots were then analyzed for dry particle morphology on AFM and hydrodynamic diameter on Nanosight.

Particles were conjugated by EDC/NHS chemistry to a Fragment E fibrin-binding antibody to create PLPs. To evaluate thrombin-triggered clot retraction, clots incorporating thrombin-degradable particles (Degradable PLPs) were allowed to polymerize for 2 hrs. An overlay of 50 µL of 100 U/mL thrombin was then added. 48 hrs later, clot structure was imaged via CryoSEM. Mean pore area was then determined using DiameterJ plugin in Image J.

Results: Unmodified particles were determined to have average dry diameters of 0.33 ± 0.05 µm and heights of 33 ± 10 nm. When exposed to 50 U/mL and 75 U/mL thrombin, population distribution markers on Nanosight show significantly decreased size starting from t = 2 hr. However, size increased after 24 hrs for 75 U/mL thrombin, potentially due to decreased crosslinks leading to increased swelling. AFM data with 50 U/mL thrombin shows no significant changes in particle diameter until t=4 hr and t-96 hr; particle height also significantly decreased starting from t=2 hr. Size increases at later time points, in combination with decreasing particle height may indicate swelling and increased deformability (Figure 1B).

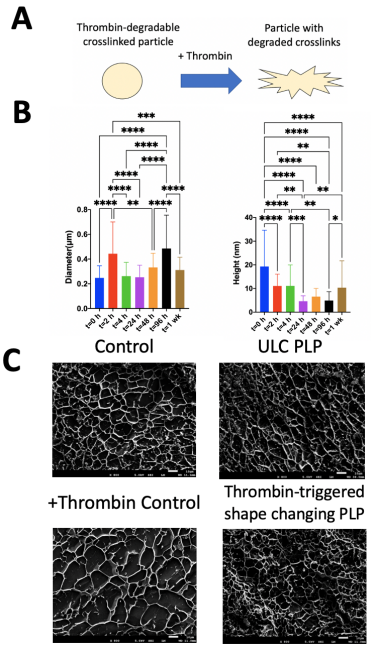

Fig 1: Thrombin-sensitive crosslinks results in nanogel shape change and clot retraction. A: Schematic of thrombin-degradable particle function. B: Summary of AFM Diameter and Height following exposure to thrombin. C: Representative CryoSEM images of clots including ULC PLPs and thrombin-triggered shape changing PLPs. Denser fibrin network is observed in clots including ULC PLPs and thrombin-triggered shape changing PLPs.

Cryo-SEM analysis demonstrated that clots incorporated with thrombin-sensitive PLPs and then overlaid with thrombin displayed denser fibrin networks (Figure 1C) with significantly smaller mean pore areas compared to controls ($p<0.05$), indicative of clot retraction.

Conclusions: In conclusion, a nanogel with thrombin-cleavable peptide was successfully synthesized. Thrombin degradation experiments suggest that exposure to thrombin results in partial degradation of crosslinks and increased particle deformability, but does not result in complete particle degradation. On-going studies are investigating these degradation mechanisms in more detail. Though complete nanogel degradation was not observed, it appears that the degradation is sufficient to induce a nanogel shape change that promotes clot retraction. On-going studies are investigating the clot retraction dynamics of the thrombin-sensitive particles.

References: [1]Nandi S. Exp Biol Med. 2016; 241(10):1138-1148. [2]Brown AC. Nature mats. 2014; 13(12):1108. [3]Du H. Mat Horizns. 2016;3(6):556-562.