

This document is the unedited Author's version of a Submitted Work that was subsequently accepted for publication in Nano Letters, copyright © American Chemical Society after peer review. To access the final edited and published work see <https://doi.org/10.1021/acs.nanolett.1c02746>. Access to this work was provided by the University of Maryland, Baltimore County (UMBC) ScholarWorks@UMBC digital repository on the Maryland Shared Open Access (MD-SOAR) platform.

Please provide feedback

Please support the ScholarWorks@UMBC repository by emailing scholarworks-group@umbc.edu and telling us what having access to this work means to you and why it's important to you. Thank you.

Hemostatic nanocapsules to control bleeding and circumvent infusion reactions

Authors:

Nuzhat Maisha, Michael Rubenstein, Charles Bieberich, and Erin Lavik

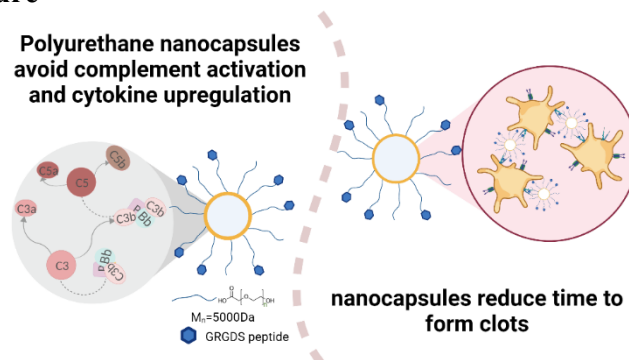
University of Maryland Baltimore County, 1000 Hilltop Circle, Baltimore, MD-21250

Corresponding author: Erin Lavik, elavik@umbc.edu

Abstract

Hemostatic nanomaterials can increase survival after traumatic injuries, which is a leading cause of death for people under 46. However, like many other intravenously administered nanomaterials, hemostatic nanomaterials can lead to complement-mediated infusion reactions. These infusion reactions are observed in about 10% of the population and can be lethal in a small fraction of people. In the context of trauma, complement proteins including C5a trigger vasodilation which exacerbates injury. Thus, nanomaterials that avoid complement and infusion responses while promoting hemostasis are critical to developing new therapeutics to manage bleeding. By screening complement responses to nanoparticles, we identified nanocapsules based on polyurethane as candidates that did not promote upregulation of C5a. We explored the PEGylation of these nanocapsules and functionalization with the GRGDS peptide to create a new class of hemostatic nanomaterials. We found that these polyurethane-based hemostatic nanocapsules do not activate complement or the major proinflammatory cytokines. We evaluated the hemostatic nanocapsules and controls using clinically relevant rotational thromboelastography (ROTEM). We determined that the hemostatic nanocapsules promote faster clotting than controls and maintain the maximum clot firmness associated with normal coagulation, which is critical for reducing bleeding and maintaining hemostasis until patients can get to definitive care. This study is a critical step in developing a new platform that is safe, effective, and translatable to preclinical models of trauma and, ultimately, the clinic.

Table of Contents Figure



Manuscript Main Text

With 30 to 40% of trauma mortality due to blood loss and 33 to 56% of this mortality occurring during the prehospital period, trauma is a leading cause of death, especially for young people (5-45 years).¹ Early post-trauma intervention is essential to maximize survival.²⁻³ Having a technology deployed in the field to manage bleeding could transform trauma care. We previously developed hemostatic nanoparticles based on block copolymers of a degradable polyester (polylactic-co-glycolic acid) (PLGA) or poly(lactic acid), poly(ethylene glycol), and the peptide RGD. These nanoparticles were highly effective in reducing bleeding in several of rodent trauma models⁴⁻⁷. However, these particles activated complement when administered in porcine trauma models⁸. We developed a variant with a nearly neutral zeta potential that effectively stopped bleeding in the porcine model without complement activation at most doses. However, at the highest doses, complement was activated, and bleeding was exacerbated by the nanoparticles, including the control nanoparticles⁸.

The complement system is the first-line defense of the immune system against pathogens, and it is active at all times controlled by several complement regulators. However, complement activation may lead to inflammatory responses as severe as anaphylaxis, an acute life-threatening respiratory failure.⁹ Due to their high surface area and rapid protein corona formation, nanoparticles have a high potential for triggering complement-mediated infusion reactions. These infusion reactions involve vasodilation, increased tissue permeability, edema, and a sharp decrease in blood pressure, leading to shock and, potentially, death¹⁰⁻¹¹. Symptoms of an infusion reaction appear within minutes of the infusion.¹⁰⁻¹¹ While complement-mediated responses have been characterized extensively in pigs¹²⁻¹³, they occur in humans as well¹⁴. A complement-mediated response incidence rate is 7% for humans, with a 0.3% chance of fatality.¹⁵

This complement response motivated us to develop a set of *in vitro* assays to investigate the components of nanomaterials that trigger complement activation¹⁶. These assays allow us to investigate a wide range of nanomaterials and molecular components rapidly. The structure of nanomaterials, as well as surface moieties, can impact surface protein adsorption. Polyurethane nanocapsules have drawn interest for a range of applications. We have previously used polyurethane nanocapsules as a drug delivery system¹⁷. In the current study, we endeavored to determine how these nanocapsules compared to our previous polyester-based nanomaterials regarding complement activation and whether they might have potential as a hemostatic system.

We prepared polyurethane nanocapsules and PEGylated polyurethane nanocapsules through interfacial polymerization by modifying a previously published protocol¹⁷⁻¹⁸. To achieve the hemostatic attribute, we then used NHS/EDC-based bioconjugation technique to conjugate the carboxyl end group of the exposed PEG on the nano surface with the primary amine group of the peptide motif GRGDS. (Figure 1)

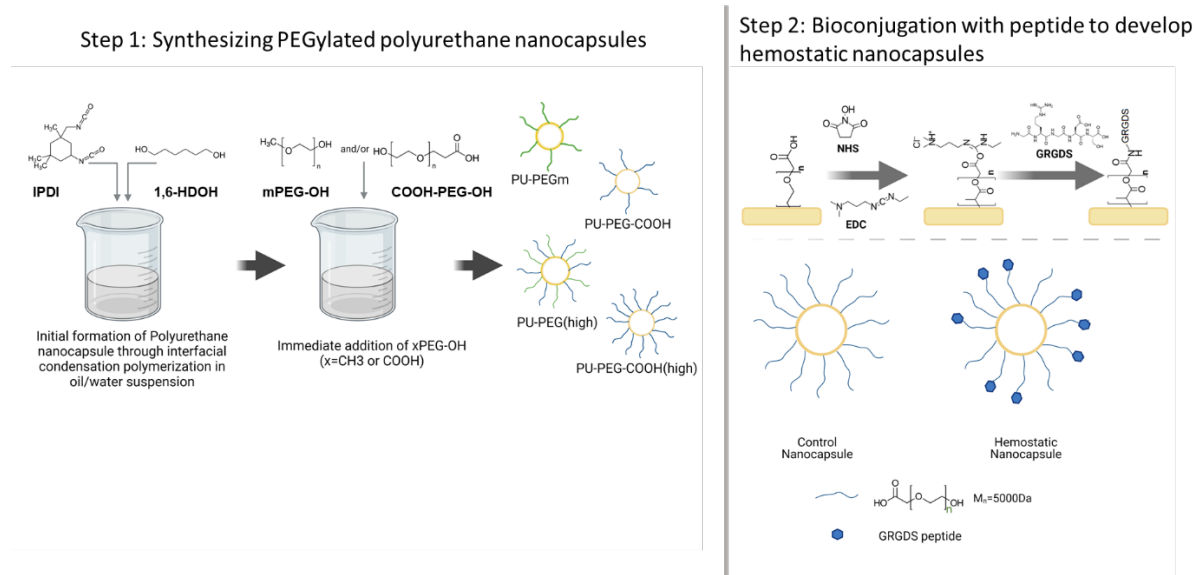


Figure 1: Synthesizing PEGylated polyurethane nanocapsules and consequent bioconjugation to prepare hemostatic nanocapsules. In the first step, polyurethane nanocapsules are synthesized through interfacial condensation polymerization between isophorone diisocyanate (IPDI) in the oil phase and 1,6 hexanediol (1,6 HDOH) in the aqueous phase. Immediately adding poly(ethylene glycol) with hydroxyl end group allows conjugation of the PEG chain to the surface. In the second step, hemostatic nanocapsules are prepared using NHS/EDC zero-length linkers. Finally, the peptide motif GRGDS is conjugated to the carboxyl end groups present in the PEG chains.

We then characterized the nanocapsules to determine their size and zeta-potential and to confirm the presence of PEG. The dynamic light scattering (DLS) data for the nanocapsules show that the nanocapsules are ~150-250 nm in size and the zeta-potential changes based on the surface composition (Figure 2 B). As previously observed,¹⁷ the nanocapsules in DLS display two peaks, with most nanocapsules in the 150-250 nm size range. A very small population of nanocapsules <10 nm was observed. Since the nanocapsules are PEGylated, the zeta-potential increases from the highly negative zeta-potential of -47.3 mV, observed in the non-PEGylated nanocapsules, as the molar amount of PEG increases. TEM of the nanocapsules was also consistent with the size of the particles observed in DLS (Figure 2C). As we modified the nanocapsules by PEGylation, we used ¹H-NMR to confirm the presence of the PEG and to quantify the amount present on the surface. To that end, we resuspended the lyophilized nanocapsules in deuterated water and used both PEGylated and non-PEGylated samples to generate the peaks. The peak corresponding to PEG appears at 3.572 ppm, which was not observed in the NMR peak for non-PEGylated polyurethane nanocapsules. (Supplementary figures 1-4). The molar ratio of PEG to IPDI in the sample was determined based on the peak observed for the cyclohexyl ring at 1.41-1.42 ppm. Increasing input PEG during synthesis leads to an increase in the amount of the chain conjugated to the surface (Figure 2B).

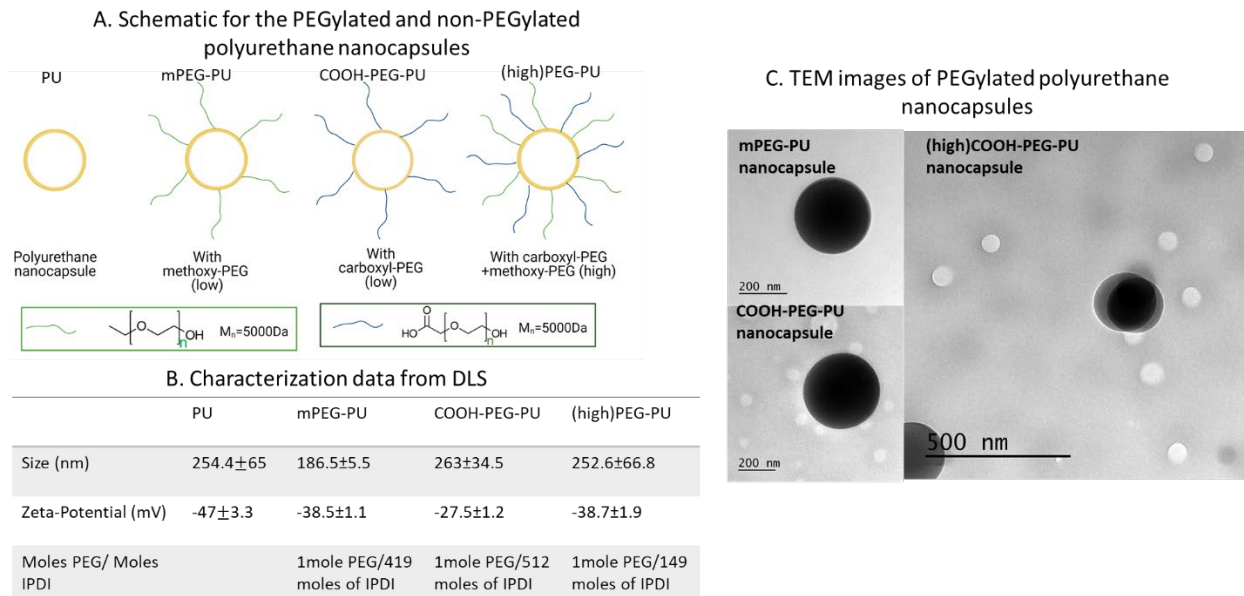


Figure 2: Characterization of PEGylated polyurethane nanocapsules. A. Schematic representation of polyurethane nanocapsules with and without PEGylation. B. A summary of the size and surface charge of the PEGylated and non-PEGylated nanocapsules. The presence of PEG was confirmed through ^1H -NMR in deuterated water. C. TEM images of the nanocapsules confirm the size observed through DLS.

To determine complement activation with the different nanomaterials, we incubated complement-protected human serum with the PEGylated and non-PEGylated nanocapsules as well as controls based on our previous work.¹⁶ The normalized change was determined compared to the C5a levels observed for complement-protected human serum incubated with PBS only. The change in complement protein was the lowest for both the bare polyurethane nanocapsules and the highly PEGylated polyurethane nanocapsules. We observed the highest change for zymosan, a known complement activator¹⁹, and positive control in this study. Remarkably, the bare nanocapsules did not activate complement. In contrast, PLA-based nanoparticles do activate complement, typically within a range of the positive control (Fig. 4), and PEGylation is essential to reduce complement activation (Fig. 4)²⁰. PEGylation of the polyurethane nanocapsules showed slight changes in complement activation, but the increase is not considered significant and is unlikely to lead to a complement response *in vivo*²¹.

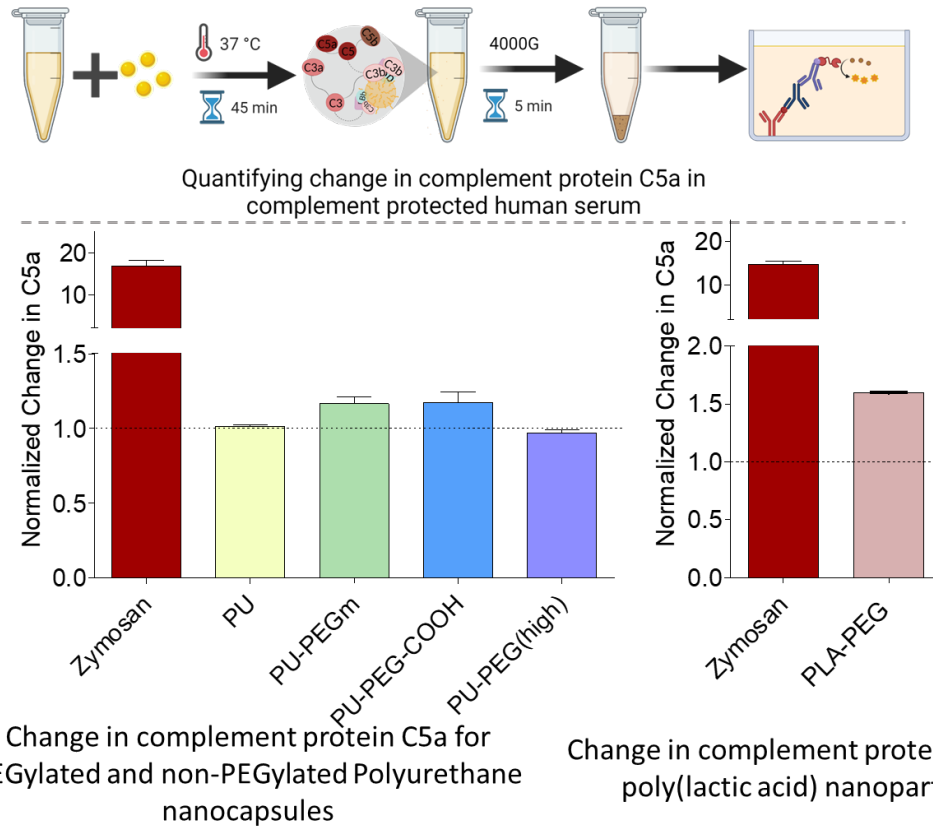


Figure 3: Quantifying complement protein for polyurethane nanocapsules. Polyurethane nanocapsules lead to low levels of complement protein C5a. Nanocapsules were incubated with complement-protected human serum and incubated for 45 minutes at 37°C. The complement protein C5a was least for the non-PEGylated nanocapsules and the highly PEGylated nanocapsules.

Complement pathway activation results in the generation of C3 convertase, which triggers the release of anaphylatoxins C3a and C5a.¹⁰ The anaphylatoxins are proinflammatory and lead to degranulation of mast cells and release of histamines,^{10,22} vasodilation, increased permeability of blood vessels²³ among some of many impacts of complement activation. We compared the polyurethane-based systems with and without PEGylation to poly(lactic acid) based nanomaterials which are more prone to complement activation. We found that compared to highly reactive poly(lactic acid) cores, both PEGylated and non-PEGylated polyurethane nanocapsules did not trigger changes in complement protein C5a (Fig 3.) The levels remained close to that in heparinized human whole blood incubated with PBS. In our previous studies with the polyester-based hemostatic nanoparticles, the particles that exhibited signs of complement activation in vivo²⁴ led to 3-4 fold increases in complement protein C5a in vitro¹⁶. The lack of complement activation with the polyurethane nanoparticles in vitro is promising for translation to large animal models and clinical applications.

Complement, though, is only part of the infusion response. When blood encounters nanoparticles, distinct complement proteins can further exacerbate inflammation, which could be

detected from the generated proinflammatory cytokines. To investigate these nanocapsules further regarding their potential safety for intravenous administration, we incubated the hemostatic nanocapsules or controls in heparinized human whole blood. We performed a cytokine array panel to determine the impact of the different nanoparticles on inflammatory responses. We screened polyurethane nanocapsules and poly(lactic acid) nanoparticles using whole blood along with highly PEGylated polyurethane nanocapsules and polyurethane-PEG-GRGDS nanocapsules. This approach is not quantitative in an absolute manner since the molarity of chemokines generated is not determined, but it provides robust relative responses. The integrated pixel density for heparinized human whole blood incubated with minimum essential media (MEM) was used as a control. The cytokine panel includes complement, which facilitates validation across assays. The change in the complement protein biomarker C5/C5a was most elevated for PLA, while there was no change for PU and PU-PEG (Figure 4). The proinflammatory cytokine IL-8 did not change with the incubation of any of the nanoparticles or nanocapsules. IL-16 was decreased in the presence of all of the nanoparticles screened. The proinflammatory cytokines IL-6, IL-1 β , IFN- γ , and TNF- α , were not detected by the cytokine assay in whole blood with or without nanoparticles.

We did see that CXCL-1 was higher for PU-PEG nanocapsules than controls. However, it was not elevated for the hemostatic nanocapsules (PU-PEG-GRGDS) or the polyurethane nanocapsules that were not functionalized with PEG. CXCL-1 is a proinflammatory cytokine most often associated with neutrophils and macrophages, but we were less concerned since the overall increase was slight and was not seen in any of the other groups. Nonetheless, it warrants further investigation in the future. Macrophage migration inhibitory factor (MIF) was lower for the polyurethane-based nanocapsules compared to controls, but again, the changes, while significant, were small. MIF can trigger hypersensitivity responses but typically with increases not seen here²⁵. Low levels of MIF suggest that the nanocapsules do not trigger proinflammatory cascades²⁵.

Complement protein C5a can stimulate Plasminogen activator inhibitor-1 (PAI-1) production and lead to vascular injury.²⁶ However, we saw no significant differences in PAI-1. Some of the iron-based nanomedicines, depending on the physicochemical properties of the final formulation, can lead to an elevation in IL-1 β and IL-6.²⁷ Some of the other mediators of inflammation include the cytokines IFN- γ and TNF- α along with IL-1 β generated at higher amounts in vitro from lymphocytes due to carbon nanotubes.²⁸ Moreover, IL-1 β , TNF α , IL-6, and IL-8 are found to be upregulated from mesothelial cells and THP-1 macrophages upon incubation with carbon nanotubes as well.²⁹ Thus, it was exciting to see that these molecules were not upregulated upon incubation with the polyurethane-based nanocapsules.

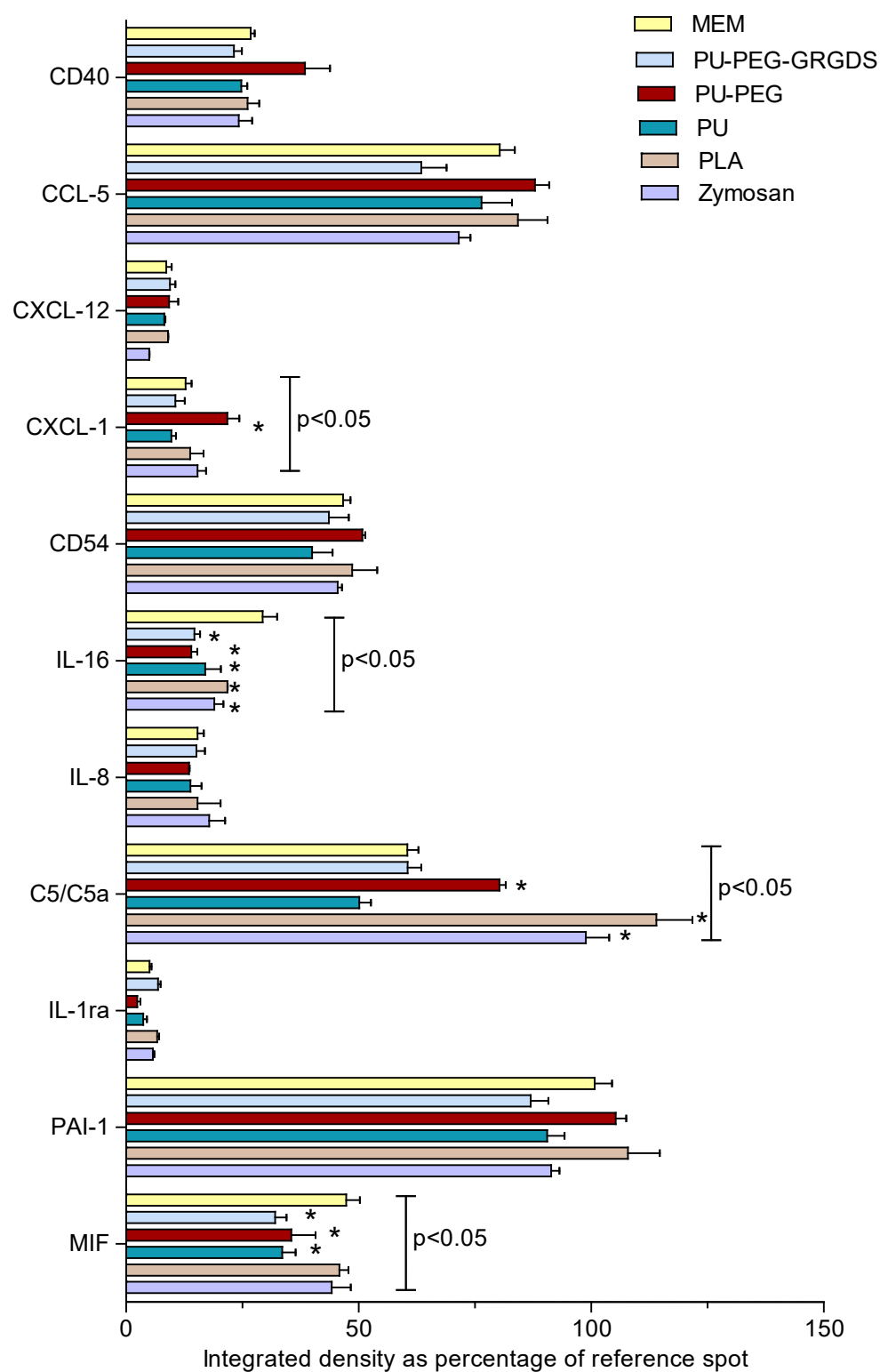


Figure 4: Normalized changes in the integrated pixel densities for the detected cytokines in vitro in heparinized human whole blood.

Armed with these data, we sought to determine the hemostatic properties of the nanocapsule system using rotational thromboelastography (ROTEM), a standard technique used both in the clinic and research labs determine blood hemostatic behavior³⁰⁻³³. The *in vitro* coagulation assay was performed using the NATEM test of ROTEM for citrated blood collected from Sprague-Dawley rats through cardiac puncture. A pin is inserted in a cup, and rotates back and forth while a load cell measures the force. As the blood clots, the ability to rotate the pin decreases, and this is marked on a ROTEM plot by the growth of the clot. The clotting time, CT is the time to initial clotting, and the clot formation time is the time at which an amplitude of 20 mm is reached. CT+CFT is a standard marker for clot formation.

We performed ROTEM on rodent blood samples with hemostatic nanocapsules, control nanocapsules, and MEM alone. There are four chambers in the ROTEM system, and MEM alone was run in each run. Since blood coagulation is impacted by time after the blood draw, all samples were normalized to the MEM values for their particular run. At a concentration of 2.5 mg of nanocapsules per ml of MEM, there were no differences in the CT+CFT for either the control nanocapsules or the hemostatic nanocapsules. While it appears the value for the hemostatic nanocapsules is lower than controls, the difference is not significant. However, at a concentration of 5 mg of nanocapsules/ml of MEM, the hemostatic nanocapsules led to a significant reduction in clotting time (CT+CFT). The 5 mg/ml dose is consistent with ROTEM results from our previous hemostatic nanoparticles based on polyester cores^{5-6, 34}.

The maximum clot firmness (MCF) is an essential value from ROTEM analysis to assess whether nanoparticles maintain or disrupt clots. Mechanically weak clots would be highly problematic. Both the hemostatic nanocapsules and control nanocapsules at the two doses show no significant differences from MEM alone.

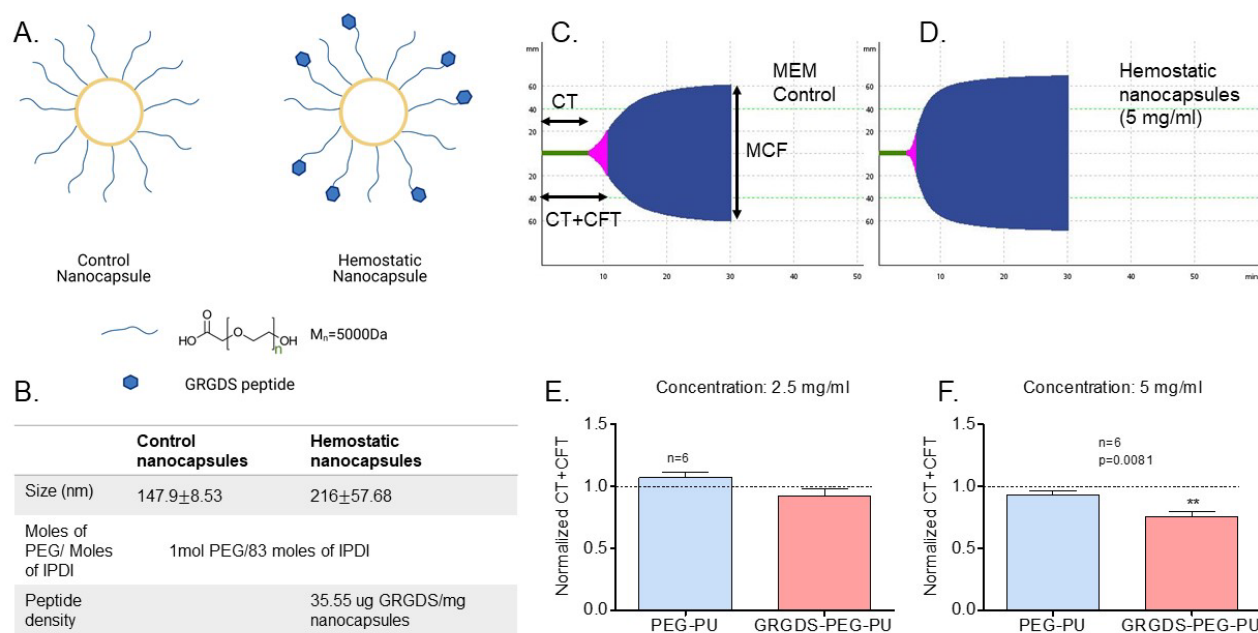


Figure 5: Evaluating the impact on coagulation *in vitro*. A. A schematic representation of the control and hemostatic nanocapsules. B. Table with materials characteristics of the control and hemostatic nanocapsules. C. ROTEM curve for representative MEM control with labels for CT,

CT+CFT and MCF. D. ROTEM curve for representative hemostatic nanocapsules at 5 mg/ml dose. E. The clotting time was lower for the hemostatic nanocapsules at 2.5mg/ml concentration but not significantly different. F. The clotting time was the least for the hemostatic nanocapsules at 5mg/ml concentration and significantly different as evaluated using t-test.

Upon intravenous infusion, nanoparticles are immediately engulfed in a protein corona, and the subsequent interaction can lead to complement-mediated hypersensitivity responses. This complement-mediated response, termed an infusion reaction³⁵⁻³⁶, is of concern for nanoparticle systems broadly, including the liposomal nanoformulation, Doxil³⁷⁻³⁸, inorganic nanoparticles such as iron oxide and metallic nanoparticles used as contrast agents for imaging³⁹⁻⁴⁰, organic nanoparticles such as poly(lactic-co-glycolic acid)-b-poly(ethylene glycol) and polystyrene^{24, 35, 41-42} based nanoparticles. The vast differences in materials and surface architecture indicate that materials properties impact this innate immune response.^{39-40, 43-45} The hypersensitivity reaction is usually due to the first infusion, and as the system gets multiple exposures through subsequent bolus administrations, such reactions can be controlled.⁴⁶

In treating uncontrolled bleeding, time is essential, and management of infusion reactions may not be possible. Hemostatic nanoparticles are effective: they can stop bleeding⁴⁷, improve survival⁷, and lead to better neurological outcomes following blast trauma⁴⁸. However, if potential infusion reaction issues cannot be addressed, clinical translation of hemostatic nanoparticles will be difficult.

The polyurethane-based nanocapsule system described here opens the possibility for a safe and effective intravenous hemostatic treatment with clinical translation potential. The lack of complement activation coupled with the absence of cytokine responses and hemostatic efficacy at low doses represents a significant advancement in the field. Having a polyurethane nanocapsule at the core of this system holds many possibilities for further potential treatment avenues for trauma. For example, polyurethane-based biomaterial has been used as a coating on implants to release antibodies in a controlled manner, preventing pathogen colonization and virulence in implant sites.⁴⁹ Such a system could be beneficial for prolonged field care.

Polyurethane nanocapsule-based hemostasis opens new possibilities for treating trauma safely. Having a system that does not activate complement or trigger cytokine responses but promotes rapid hemostasis is critical to move the field of infusible nanotechnologies forward. This work lays the foundation for future testing preclinical models of trauma and for eventual clinical translation of polyurethane nanocapsule-based hemostatic treatments.

Acknowledgments

Some of the figures are created using Biorender. We would like to thank Tagide deCarvalho, PhD and Sara Larson for assisting with the TEM imaging. We would also like to thank Binapani Mahaling for providing the PLA nanoparticles. This work was supported by the AIMM Research award (DOD) (Award Number# W81XWH1820061)

References

1. Kauvar, D. S.; Lefering, R.; Wade, C. E., Impact of hemorrhage on trauma outcome: an overview of epidemiology, clinical presentations, and therapeutic considerations. *Journal of Trauma and Acute Care Surgery* **2006**, *60* (6), S3-S11.
2. Cloonan, C. C., Treating traumatic bleeding in a combat setting. *Mil Med* **2004**, *169* (12 Suppl), s8-s10.
3. Okada, K.; Matsumoto, H.; Saito, N.; Yagi, T.; Lee, M., Revision of 'golden hour' for hemodynamically unstable trauma patients: an analysis of nationwide hospital-based registry in Japan. *Trauma Surg Acute Care Open* **2020**, *5* (1), e000405.
4. Hubbard, W. B.; Lashof-Sullivan, M. M.; Lavik, E. B.; VandeVord, P. J., Steroid-Loaded Hemostatic Nanoparticles Combat Lung Injury after Blast Trauma. *ACS Macro Lett* **2015**, *4* (4), 387-391.
5. Lashof-Sullivan, M. M.; Shoffstall, E.; Atkins, K. T.; Keane, N.; Bir, C.; VandeVord, P.; Lavik, E. B., Intravenously administered nanoparticles increase survival following blast trauma. *Proc Natl Acad Sci U S A* **2014**, *111* (28), 10293-8.
6. Shoffstall, A. J.; Atkins, K. T.; Groynom, R. E.; Varley, M. E.; Everhart, L. M.; Lashof-Sullivan, M. M.; Martyn-Dow, B.; Butler, R. S.; Ustin, J. S.; Lavik, E. B., Intravenous hemostatic nanoparticles increase survival following blunt trauma injury. *Biomacromolecules* **2012**, *13* (11), 3850-7.
7. Lashof-Sullivan, M.; Holland, M.; Groynom, R.; Campbell, D.; Shoffstall, A.; Lavik, E., Hemostatic Nanoparticles Improve Survival Following Blunt Trauma Even after 1 Week Incubation at 50 degrees C. *ACS Biomater Sci Eng* **2016**, *2* (3), 385-392.
8. Onwukwe, C.; Maisha, N.; Holland, M.; Varley, M.; Groynom, R.; Hickman, D.; Uppal, N.; Shoffstall, A.; Ustin, J.; Lavik, E., Engineering Intravenously Administered Nanoparticles to Reduce Infusion Reaction and Stop Bleeding in a Large Animal Model of Trauma. *Bioconjug Chem* **2018**, *29* (7), 2436-2447.
9. Ricklin, D.; Lambris, J. D., Complement in immune and inflammatory disorders: pathophysiological mechanisms. *Journal of immunology (Baltimore, Md. : 1950)* **2013**, *190* (8), 3831-8.
10. Benjamini, E., *Immunology: a short course*. Vol. 77.
11. Moghimi, S. M.; Simberg, D., Complement activation turnover on surfaces of nanoparticles. *Nano Today* **2017**, *15*, 8-10.
12. Szebeni, J.; Bedocs, P.; Rozsnyay, Z.; Weiszhar, Z.; Urbanics, R.; Rosivall, L.; Cohen, R.; Garbuzenko, O.; Bathori, G.; Toth, M.; Bunger, R.; Barenholz, Y., Liposome-induced complement activation and related cardiopulmonary distress in pigs: factors promoting reactogenicity of Doxil and AmBisome. *Nanomedicine* **2012**, *8* (2), 176-84.
13. Szebeni, J.; Bedocs, P.; Dézsi, L.; Urbanics, R., A porcine model of complement activation-related pseudoallergy to nano-pharmaceuticals: Pros and cons of translation to a preclinical safety test. *Precision Nanomedicine* **2018**, *1* (1), 63-73.
14. Szebeni, J.; Bawa, R., Human Clinical Relevance of the Porcine Model of Pseudoallergic Infusion Reactions. *Biomedicines* **2020**, *8* (4).
15. Szebeni, J., Hemocompatibility testing for nanomedicines and biologicals: predictive assays for complement mediated infusion reactions. *European Journal of Nanomedicine* **2012**, *4* (1).
16. Maisha, N.; Coombs, T.; Lavik, E., Development of a Sensitive Assay to Screen Nanoparticles in Vitro for Complement Activation. *ACS Biomaterials Science & Engineering* **2020**, *6* (9), 4903-4915.
17. Menikheim, S.; Leckron, J.; Bernstein, S.; Lavik, E. B., On-Demand and Long-Term Drug Delivery from Degradable Nanocapsules. *ACS Applied Bio Materials* **2020**, *3* (11), 7369-7375.
18. Guo, J.; Pan, Q.; Huang, C.; Zhao, Y.; Ouyang, X.; Huo, Y.; Duan, S., The role of surfactant and costabilizer in controlling size of nanocapsules containing TEGDMA in miniemulsion. *Journal of Wuhan University of Technology-Mater. Sci. Ed.* **2009**, *24* (6), 1004.
19. Fearon, D. T.; Austen, K. F., Activation of the alternative complement pathway due to resistance of zymosan-bound. *Proceedings of the National Academy of Sciences* **1977**, *74* (4), 1683-1687.

20. Pannuzzo, M.; Esposito, S.; Wu, L. P.; Key, J.; Aryal, S.; Celia, C.; di Marzio, L.; Moghimi, S. M.; Decuzzi, P., Overcoming Nanoparticle-Mediated Complement Activation by Surface PEG Pairing. *Nano Lett* **2020**, *20* (6), 4312-4321.
21. Molino, N. M.; Bilotkach, K.; Fraser, D. A.; Ren, D.; Wang, S. W., Complement activation and cell uptake responses toward polymer-functionalized protein nanocapsules. *Biomacromolecules* **2012**, *13* (4), 974-81.
22. Peng, Q.; Li, K.; Sacks, S. H.; Zhou, W., The role of anaphylatoxins C3a and C5a in regulating innate and adaptive immune responses. *Inflammation & Allergy-Drug Targets (Formerly Current Drug Targets-Inflammation & Allergy)* **2009**, *8* (3), 236-246.
23. Ember, J.; Jagels, M.; Hugli, T.; Volanakis, J.; Frank, M., The human complement system in health and disease. *Marcel Dekker* **1998**, 241-84.
24. Onwukwe, C.; Maisha, N.; Holland, M.; Varley, M.; Groynom, R.; Hickman, D.; Uppal, N.; Shoffstall, A.; Ustin, J.; Lavik, E., Engineering Intravenously Administered Nanoparticles to Reduce Infusion Reaction and Stop Bleeding in a Large Animal Model of Trauma. *Bioconjugate chemistry* **2018**, *29* (7), 2436-2447.
25. Calandra, T.; Roger, T., Macrophage migration inhibitory factor: a regulator of innate immunity. *Nature reviews. Immunology* **2003**, *3* (10), 791-800.
26. Wojta, J.; Kaun, C.; Zorn, G.; Ghannadan, M.; Hauswirth, A. W.; Sperr, W. R.; Fritsch, G.; Printz, D.; Binder, B. R.; Schatzl, G., C5a stimulates production of plasminogen activator inhibitor-1 in human mast cells and basophils. *Blood, The Journal of the American Society of Hematology* **2002**, *100* (2), 517-523.
27. Verhoef, J. J.; de Groot, A. M.; van Moorsel, M.; Ritsema, J.; Beztsinna, N.; Maas, C.; Schellekens, H., Iron nanomedicines induce Toll-like receptor activation, cytokine production and complement activation. *Biomaterials* **2017**, *119*, 68-77.
28. Andersen, A. J.; Wibroe, P. P.; Moghimi, S. M., Perspectives on carbon nanotube-mediated adverse immune effects. *Adv Drug Deliv Rev* **2012**, *64* (15), 1700-5.
29. Murphy, F. A.; Schinwald, A.; Poland, C. A.; Donaldson, K., The mechanism of pleural inflammation by long carbon nanotubes: interaction of long fibres with macrophages stimulates them to amplify proinflammatory responses in mesothelial cells. *Part Fibre Toxicol* **2012**, *9*, 8.
30. Letson, H. L.; Dobson, G. P., Differential contributions of platelets and fibrinogen to early coagulopathy in a rat model of hemorrhagic shock. *Thromb Res* **2016**, *141*, 58-65.
31. Pluta, J.; Nicinska, B.; Grzeszczyk, M.; Kolacz, M.; Jureczko, L.; Kwiatkowski, A.; Durlik, M.; Trzebicki, J., Assessment of the Hemostatic Parameters and Platelet Function on Thromboelastometry and Impedance Aggregometry in Hemodialysis Patients Qualified for Kidney Transplantation: Preliminary Report. *Transplant Proc* **2016**, *48* (5), 1431-4.
32. Boudreau, R. M.; Johnson, M.; Veile, R.; Friend, L. A.; Goetzman, H.; Pritts, T. A.; Caldwell, C. C.; Makley, A. T.; Goodman, M. D., Impact of tranexamic acid on coagulation and inflammation in murine models of traumatic brain injury and hemorrhage. *J Surg Res* **2017**, *215*, 47-54.
33. Müller, M. C. A.; Meijers, J. C.; van Meenen, D. M.; Thachil, J.; Juffermans, N. P., Thromboelastometry in critically ill patients with disseminated intravascular coagulation. *Blood Coagul Fibrinolysis* **2019**, *30* (5), 181-187.
34. Shoffstall, A. J.; Everhart, L. M.; Varley, M. E.; Soehnlen, E. S.; Shick, A. M.; Ustin, J. S.; Lavik, E. B., Tuning ligand density on intravenous hemostatic nanoparticles dramatically increases survival following blunt trauma. *Biomacromolecules* **2013**, *14* (8), 2790-7.
35. Wibroe, P. P.; Anselmo, A. C.; Nilsson, P. H.; Sarode, A.; Gupta, V.; Urbanics, R.; Szebeni, J.; Hunter, A. C.; Mitragotri, S.; Mollnes, T. E.; Moghimi, S. M., Bypassing adverse injection reactions to nanoparticles through shape modification and attachment to erythrocytes. *Nature nanotechnology* **2017**, *12* (6), 589-594.
36. Szebeni, J.; Bedöcs, P.; Csukás, D.; Rosivall, L.; Bünger, R.; Urbanics, R., A porcine model of complement-mediated infusion reactions to drug carrier nanosystems and other medicines. *Advanced Drug Delivery Reviews* **2012**, *64* (15), 1706-1716.

37. Szebeni, J.; Bedőcs, P.; Rozsnyay, Z.; Weiszhar, Z.; Urbanics, R.; Rosivall, L.; Cohen, R.; Garbuzenko, O.; Báthori, G.; Tóth, M., Liposome-induced complement activation and related cardiopulmonary distress in pigs: factors promoting reactogenicity of Doxil and AmBisome. *Nanomedicine: Nanotechnology, Biology and Medicine* **2012**, *8* (2), 176-184.
38. Chanan-Khan, A.; Szebeni, J.; Savay, S.; Liebes, L.; Rafique, N. M.; Alving, C. R.; Muggia, F. M., Complement activation following first exposure to pegylated liposomal doxorubicin (Doxil): possible role in hypersensitivity reactions. *Ann Oncol* **2003**, *14* (9), 1430-7.
39. Wang, G.; Chen, F.; Banda, N. K.; Holers, V. M.; Wu, L.; Moghimi, S. M.; Simberg, D., Activation of Human Complement System by Dextran-Coated Iron Oxide Nanoparticles Is Not Affected by Dextran/Fe Ratio, Hydroxyl Modifications, and Crosslinking. *Frontiers in immunology* **2016**, *7*, 418.
40. Banda, N. K.; Mehta, G.; Chao, Y.; Wang, G.; Inturi, S.; Fossati-Jimack, L.; Botto, M.; Wu, L.; Moghimi, S. M.; Simberg, D., Mechanisms of complement activation by dextran-coated superparamagnetic iron oxide (SPIO) nanoworms in mouse versus human serum. *Particle and fibre toxicology* **2014**, *11* (1), 64.
41. De Sousa Delgado, A.; Léonard, M.; Dellacherie, E., Surface Properties of Polystyrene Nanoparticles Coated with Dextrans and Dextran-PEO Copolymers. Effect of Polymer Architecture on Protein Adsorption. *Langmuir* **2001**, *17* (14), 4386-4391.
42. Fornaguera, C.; Caldero, G.; Mitjans, M.; Vinardell, M. P.; Solans, C.; Vauthier, C., Interactions of PLGA nanoparticles with blood components: protein adsorption, coagulation, activation of the complement system and hemolysis studies. *Nanoscale* **2015**, *7* (14), 6045-58.
43. Ruiz, A.; Alpizar, A.; Beola, L.; Rubio, C.; Gavilán, H.; Marciello, M.; Rodríguez-Ramiro, I.; Ciordia, S.; Morris, C. J.; Morales, M. d. P., Understanding the Influence of a Bifunctional Polyethylene Glycol Derivative in Protein Corona Formation around Iron Oxide Nanoparticles. *Materials* **2019**, *12* (14), 2218.
44. Hamad, I.; Christy Hunter, A.; Rutt, K. J.; Liu, Z.; Dai, H.; Moein Moghimi, S., Complement activation by PEGylated single-walled carbon nanotubes is independent of C1q and alternative pathway turnover. *Molecular immunology* **2008**, *45* (14), 3797-803.
45. Hamad, I.; Al-Hanbali, O.; Hunter, A. C.; Rutt, K. J.; Andresen, T. L.; Moghimi, S. M., Distinct polymer architecture mediates switching of complement activation pathways at the nanosphere– serum interface: implications for stealth nanoparticle engineering. *ACS nano* **2010**, *4* (11), 6629-6638.
46. Szebeni, J.; Bedocs, P.; Urbanics, R.; Bunger, R.; Rosivall, L.; Toth, M.; Barenholz, Y., Prevention of infusion reactions to PEGylated liposomal doxorubicin via tachyphylaxis induction by placebo vesicles: a porcine model. *J Control Release* **2012**, *160* (2), 382-7.
47. Bertram, J. P.; Williams, C. A.; Robinson, R.; Segal, S. S.; Flynn, N. T.; Lavik, E. B., Intravenous hemostat: nanotechnology to halt bleeding. *Sci Transl Med* **2009**, *1* (11), 11ra22.
48. Hubbard, W. B.; Lashof-Sullivan, M.; Greenberg, S.; Norris, C.; Eck, J.; Lavik, E.; VandeVord, P., Hemostatic nanoparticles increase survival, mitigate neuropathology and alleviate anxiety in a rodent blast trauma model. *Scientific reports* **2018**, *8* (1), 10622.
49. Rojas, I. A.; Slunt, J. B.; Grainger, D. W., Polyurethane coatings release bioactive antibodies to reduce bacterial adhesion. *Journal of Controlled Release* **2000**, *63* (1-2), 175-189.