INVESTIGATION OF SLIDING HYDROGELS FOR USE AS A HEMOSTATIC AGENT

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ABSTRACT

Traumatic injury is the leading cause of death for the 5-44 year old age group worldwide, and while many products have been developed to treat the external injuries involved with trauma, few options currently exist for first responders to treat internal bleeding. To illustrate the magnitude of this problem, fatal severe blood loss can occur in 5-10 minutes and is associated with over 30% of civilian deaths before the patient reaches the hospital, and 50% of mortalities in military settings. Surprisingly, these statistics have not improved in almost 40 years. Addressing this issue, the collaborative labs of Dr. Jennie Leach and Dr. Erin Lavik work to engineer hemostatic nanoparticles that would be delivered intravenously to quickly treat widespread, uncontrolled, internal hemorrhages. More specifically, the focus of this project is to investigate whether polyrotaxanes, a sliding hydrogel, could be used for hemostatic applications due to their unprecedented range of platelet like properties such as high elasticity, strength, and capacity for self-healing. The research approach aims to synthesize polyrotaxanes using literature published by Okumura & Ito at the University of Tokyo and Tong & Yang at Stanford University, then characterize the polymer using nuclear magnetic resonance (NMR) and mass spectrometry. Once a successful synthesis and molecular characterization are achieved, the next aim would be to characterize the physical properties of the hydrogel through swelling and rheology experiments Using Okumura & Ito's method, the hydrogel has not yet been successfully synthesized. However the Tong & Yang method, which remains to be conducted, appears to be a promising and biocompatible alternative.

BACKGROUND

Previously, Dr. Lavik's lab conceptualized and engineered the first hemostatic nanoparticle. The particles are designed from a nanosphere core, ~200nm in diameter [Fig. 1A], composed of PLGA (poly(lactic-co-glycolic acid))-PLL (polylysine). This nanocore is ligated with PEG (polyethylene glycol) arms, and the PEG arms are conjugated with RGD (Arg-Gly-Asp), a peptide that binds to activated platelets to assist with clotting [Fig. 1B]. Excitingly, this particle has been shown to be successful as a hemostatic agent due to its specificity to vascular injury sites, biocompatibility, and biodegradability. Additionally, this particle is ideal for first response settings because of its capacity for rapid and easy administration, and its ability to be stored dry at room temperature.

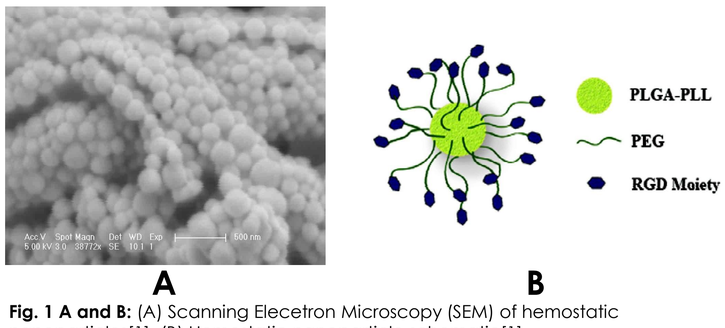


Fig. 1 A and B: (A) Scanning Elecetron Microscopy (SEM) of hemostatic nanoparticles[1]. (B) Hemostatic nanoparticle schematic[1].

To test the efficacy of the nanoparticles, trauma models using mice were designed. Essentially, the mice were exposed to a full body or head only blast trauma, at which the synthetic platelets (hemostatic nanoparticles) were administered [Figure 2]. From here, different assessments of survival [Figure 3], functional outcomes, and histological analyses were conducted.

Hemostatic Nanoparticles to Halt Bleeding Following Blast Injuries

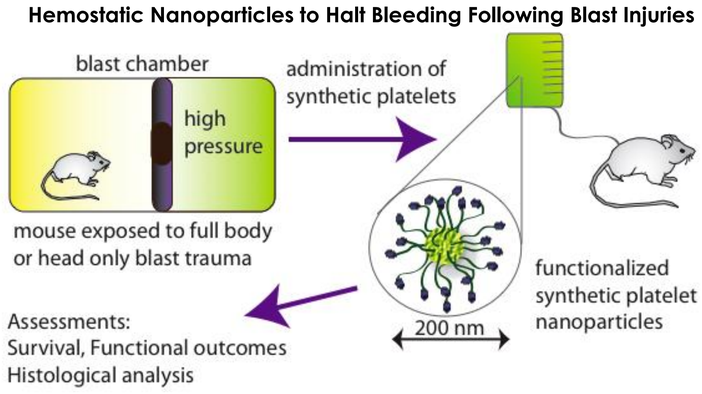


Fig. 2: Trauma model for subsequent hemostatic nanoparticle administration. Dose of synthetic platelets given at 50uL at 10, 20, 40 mg/mL

Blast Injury: Lethality at 1 Hour

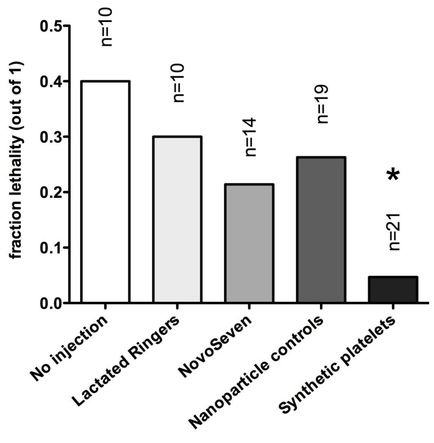
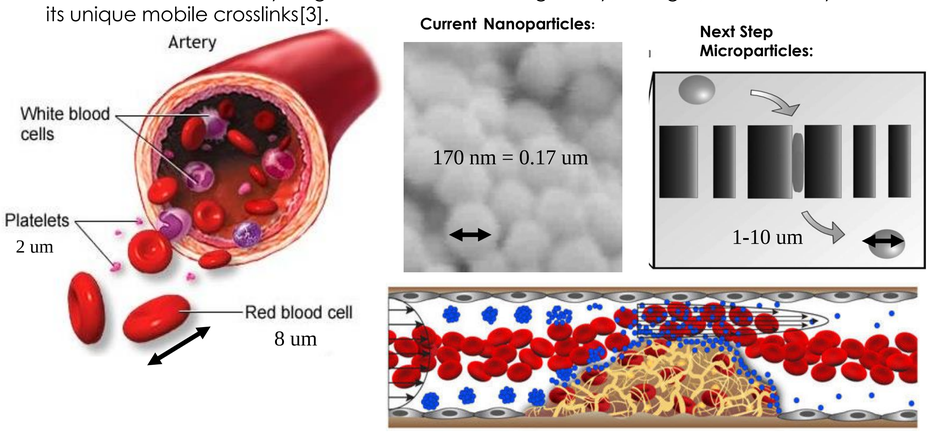


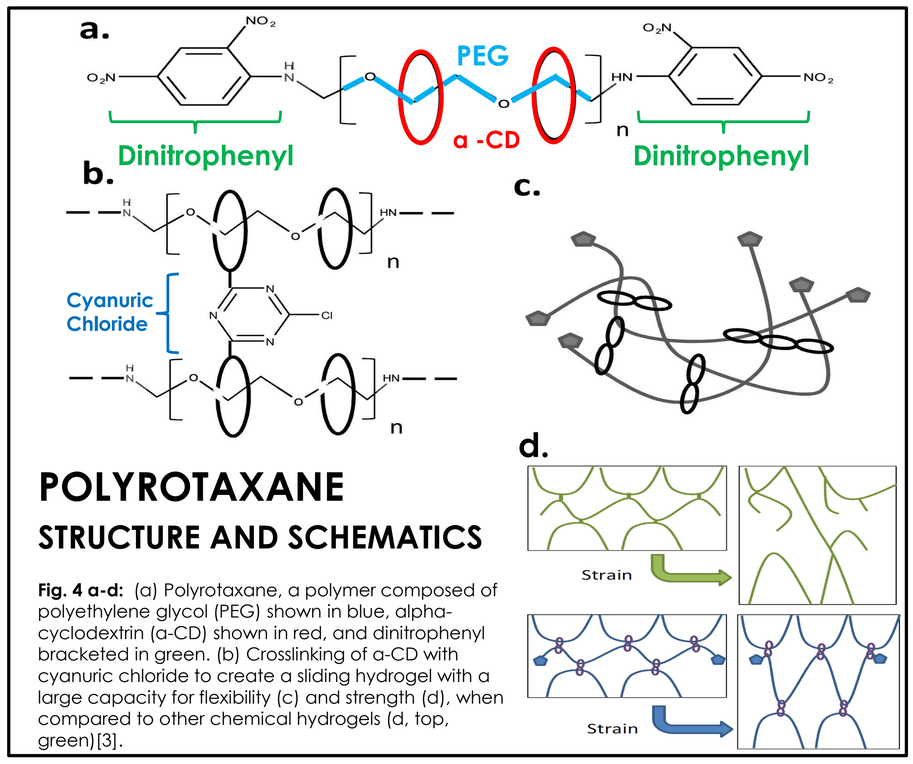
Fig. 3: 1hr after the initial blast injury and subsequent artificial platelet delivery, synthetic platelets gave an approximate 0.05 fraction of lethality (out of 1) compared to no injection which gave a 0.4 fraction of lethality. NovoSeven, a commercial coagulator, has the second lowest fraction of lethality at ~0.275. Odds ratio for synthetic platelets versus no injection = 13.3 [2]. [Not Pictured] 3 weeks post-blast: 6/7 animals treated with synthetic platelets survived their injuries [2].

EXPANDING UPON PRIOR RESEARCH

With the success of the Lavik Lab experiments, the Leach Lab is currently investigating whether a different approach in materials may yield more optimal results. We hypothesize that microparticles , as opposed to nanoparticles, could work more effectively as a hemostatic agent because they more closely resemble the size of naturally occurring platelets. However, due to this increase in size, it is critical that these particles are both durable and flexible. The durability is needed so the particle can work effectively at the injury site without premature degradation. Similarly, the flexibility is essential so the particles can "squish" to pass through the smallest capillaries of the body (8 um), as to not clot in inappropriate locations; unwanted clotting has the potential to cause strokes, heart attacks, and other serious events.

The material chosen for this investigation is polyrotaxane, a polymer that when crosslinked, creates a hydrogel known for its swelling ability, strength, and flexibility thanks to its unique mobile crosslinks[3].

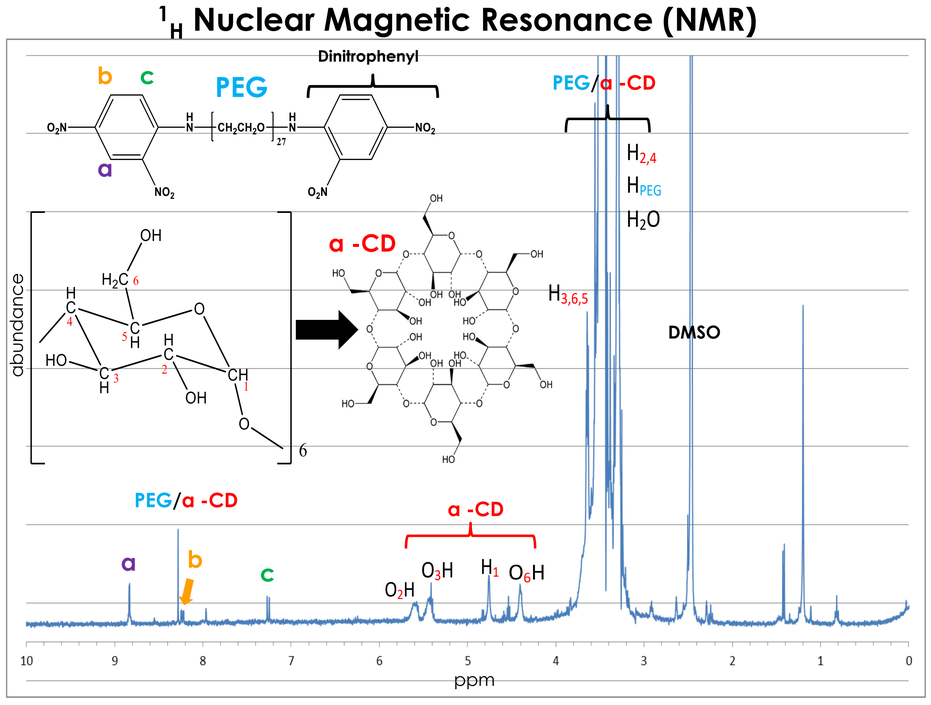




POLYROTAXANE
STRUCTURE AND SCHEMATICS

Fig. 4 a-d: (a) Polyrotaxane, a polymer composed of polyethylene glycol (PEG) shown in blue, alpha-cyclodextrin (a-CD) shown in red, and dinitrophenyl bracketed in green. (b) Crosslinking of a-CD with cyanuric chloride to create a sliding hydrogel with a large capacity for flexibility (c) and strength (d), when compared to other chemical hydrogels (d, top, green)[3].

Molecular Characterization of Polyrotaxane via ^{1}H Nuclear Magnetic Resonance (NMR)



NMR ANALYSIS

Fig. 5: (a)Singlet of the dinitrophenyl at 8.84ppm with an integration of 0.078. (b)Doublet of the dinitrophenyl at 8.23ppm with an integration of 0.057. (c) Doublet of the dinitrophenyl at 7.75ppm with an integration of 0.062. Peaks a, b, and c have similar and miniscule integrals due to the relatively low abundance of the dinitrophenyl when compared to the PEG chain (notice the 27 subscript of the brackets) and the a-CD (notice the 6 subscript, which signifies the number of monomers to create 1 a-CD [5], of which there are approximately 2 a-CD for every PEG repeat (27) in one molecule polyrotaxane [4]. At 8.27 ppm there is a peak shared by protons in both the PEG and a-CD with an integral of 0.060. Further, between 4 and 6 ppm lies the protons labeled in the a-CD monomer, with higher integrals: 0.25, 0.30, 0.26, and 0.51 (moving downfield to upfield respectively). Finally, between 4 and 3ppm lies overlapping multiplets associated with PEG, the remaining protons labeled in the a-CD monomer, and water. These peaks have extremely high integrals at 207.07 and 39.57 due to the large number of monomer repeats in the molecule. The chemical shifts were referenced to the solvent DMSO at ~2.5ppm [4] and the remaining upfield peaks are currently unknown but are speculated to be excess reagents (such as poly(ethylenegycol)bis(amine)) or impurities.

CONCLUSION

Although the NMR spectrum for our sample looked promising, ultimately we were not able to successfully complete the final step of the hydrogel synthesis. In other words, our sample did not gel but remained in a liquid state. Future investigation utilizing mass spectrometry will be conducted to determine whether percent modification is needed to perfect the chemistry. For example, perhaps there was not a sufficient percentage of crosslinks (cyanuric chloride) or a-CDs to create the hydrogel mesh. Otherwise, the incomplete synthesis is likely due to an excess of reagents or impurities that interfered with the gel synthesis. To address this in the future, we would purify the polyrotaxane product (via dialysis) for a longer period of time.

ONGOING RESEARCH

Using the information we gathered over the past few months, we now have a better direction for our future synthetic process. As we continue to investigate the cause of the aforementioned unsuccessful synthesis (conducted using literature published by Okumura et al.), we are beginning a new synthesis of polyrotaxane using the method described in Tong and Yang's publication. We are optimistic that the synthetic protocol in this paper may work better for our purposes because, fittingly, Tong and Yang's variety of polyrotaxane has been shown to be biocompatible [6].

Biocompatible Sliding Hydrogel

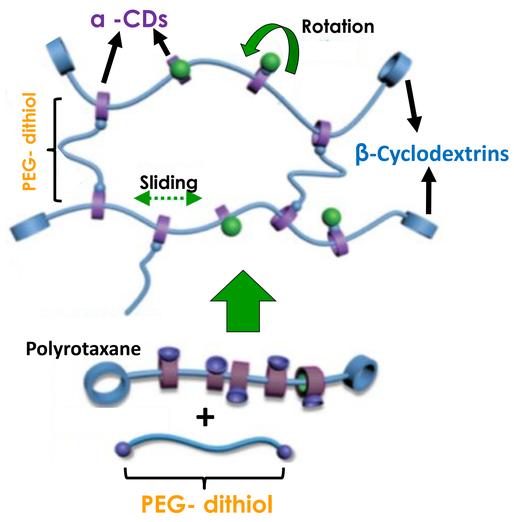


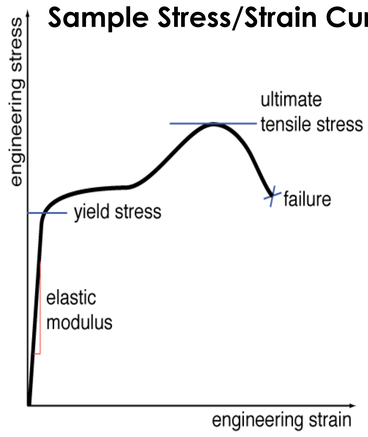
Fig. 6:

The biocompatibility of Tong and Yang's hydrogel can be attributed to the use of β-cyclodextrins (significantly larger than a-CDs)[6] instead of the dinitrophenyl end groups in Okumura's synthesis [figure 4a] which is synthesized from dinitrofluorobenzene, a highly toxic substance. Additionally, Tong and Yang utilize benign PEG-dithiol as a crosslinker [6] instead of cyanuric chloride [figure 4b], another extremely health hazardous material.

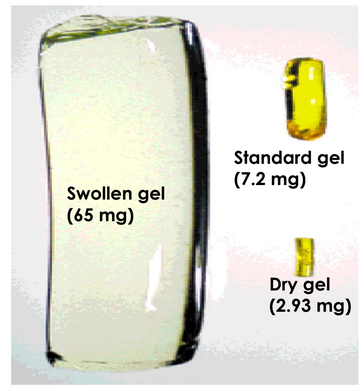
FUTURE DIRECTIONS

After the sliding hydrogel is synthesized, its mechanical properties will be tested using swelling [4] and stress/strain experiments [7]. These experiments will help determine how well the material could function within the vascular system.

Sample Stress/Strain Curve



Polyrotaxane Swelling in Water



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