Hyperfibrinolysis Drives Mechanical Instabilities In Trauma Induced Coagulopathy

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Statement of Purpose: Traumatic injury accounts for over 5 million deaths annually and is the leading cause of death among those under 44 years old. Hemorrhage, or excessive bleeding, is the second most common cause of death in trauma patients. This is exacerbated by trauma induced coagulopathy (TIC), which occurs in at least 25% of trauma patients and results in a 4-6-fold increase in mortality. TIC has numerous phenotypes and the mechanisms governing the progression of TIC are poorly understood. Therefore, the underlying biochemical changes which dictate the phenotype of TIC must be further elucidated to ensure proper diagnosis and treatment of trauma patients in a patient specific manner. The hyperfibrinolytic phenotype of TIC corresponds to decreased clot stability and has the highest association with bleeding mortality. Using an in vitro whole blood model, Kosoutov et al. determined that whole blood diluted 15%, initiated with 45 pmol/L tissue factor (TF) and supplemented with 225ng/mL tissue plasminogen activator (tPA) recreated a hyperfibrinolytic clot formation pattern seen in TIC patients when measured using TEG. However, use of whole blood prevents the identification of the direct causes of TIC. So, we investigated the role of TIC in clot formation in a simulated model of TIC (STIC) using platelet poor pooled donor plasma. Our aim was to examine how changes in the biochemical components of coagulation result in changes in the mechanics and structure of blood clots, pointing to mechanisms of bleeding in TIC. We specifically looked at the factors which affect the fibrin mesh, hyperactivation, hemodilution and hyperfibrinolysis.

Methods: Deidentified human whole blood, obtained from blood bank donation platelet poor plasma (PPP) was pooled from 25 individual donors. 10mM CaCl2 and 0.2U/mL thrombin were used to initiate clotting in the presence or absence of additional STIC factors, TF, tPA and saline dilution. 10mM Tranexamic acid (TXA) was added to STIC samples to determine whether an antifibrinolytic agent could preventing the loss of structural integrity seen in STIC samples. Confocal microscopy was used to assess fibrin fiber network, fiber length, pore size, and fibrin density. Rheology was used to measure the viscoelastic properties of plasma, namely storage modulus (G'), loss modulus (G''), and tan delta (ratio of G''/G'). Turbidity was used to measure the optical properties of the plasma. Rheology and turbidity measurements were used to calculate the fibrin polymerization rates and fibrinolysis rates of each sample.

Results: STIC samples had faster fibrin polymerization rates within the first 300-600 seconds as compared to the control. In addition, they had a lower max storage modulus and optical density (OD) as well as a complete loss of stiffness and OD after 1200 seconds (Fig. 1). Confocal image analysis showed that STIC samples had rapid fibrin mesh formation, with shorter, more closely packed fibers than the control samples with a loss of all visible fibrin by 30 minutes. Individual STIC factor contributions (hemodilution, hyperactivation and hyperfibrinolysis) were compared to the control and STIC samples to determine individual factor contribution to the STIC model. Hemodilution lead to no significant changes in mechanical properties or polymerization rates compared to the control but had similar maximum OD as the STIC sample (Fig. 1). TF led to faster polymerization rates, similar to STIC samples, had similar max stiffness as the control, but had significantly lower OD than the control (Fig. 1). TPA lead to significant clot degradation in all testing methods between 300-600 seconds, like what was seen in the STIC sample (Fig. 1). Lastly, addition of TXA lead to an inhibition in the loss of structure seen in the STIC sample in each testing method (Fig. 1). Confocal imaging further showed that the fibrin had not degraded, but the fibers were still shorter and more densely packed than control fibers.

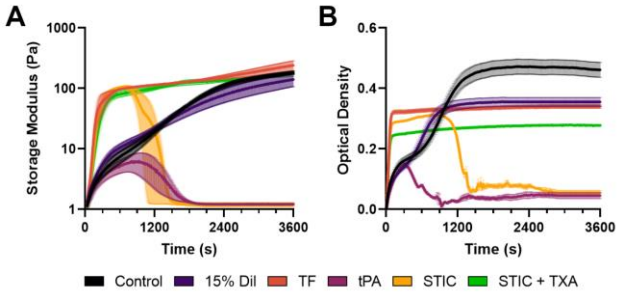

Figure 1: Clot formation profile in platelet poor plasma by **(A)** rheometer and **(B)** optical turbidity testing.

Conclusion: Distinct changes were observed when modeling the aspects of TIC. Hemodilution with saline led to decreased turbidity however this decrease was not also observed in mechanical testing. Hyperactivation with tissue factor leads to increased clotting rate and altered fibrin structure as seen with a lower OD than the control, which was also not observed in mechanical testing. Hyperfibrinolysis with tPA lead to lysis and decreased stability. TXA led to a prevention of clot lysis, but other changes due to TF and dilution were still present. A key finding was that some of these results were only apparent in one type of testing method. The development of this simulated TIC model helps to show how combinatorial analysis methods can be used to detect differences between normal clots and TIC clots, an essential process that must be improved to properly detect, diagnose, and treat patients with coagulopathy to reduce mortality. Our testing has shown limitations of each testing method, pointing to the importance of developing better diagnostic tools or combining them to improve TIC diagnosis.