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Injury-Induced Endotheliopathy: What You Need to Know

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Social Media Information

The endotheliopathy of trauma (EOT) is being increasingly recognized as an important contribution to patient outcomes. A review of the pathobiology and potential therapeutics to reverse the EOT are presented in this review article.

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ABSTRACT

The endotheliopathy of trauma (EOT) involves a complex interplay between the glycocalyx, von Willebrand factor (VWF) and platelets that leads to abnormalities in coagulation, inflammation, and endothelial cell function. The current review presents a synopsis of endothelial cell function under homeostatic conditions, the structure and function of the endothelial glycocalyx; mechanisms of endothelial cell injury and activation after trauma; pathological consequences of the EOT at the cellular level; and clinical implications of the EOT. Recent evidence is presented that links the EOT to extracellular vesicles and hyperadhesive ultralarge von Willebrand Factor (ULVWF) multimers through their roles in coagulopathy. Lastly, potential therapeutics to mitigate the EOT are discussed. Most research to date has focused on blood products, primarily plasma, and its contribution to restoring post-injury endothelial cell dysfunction. Additional therapeutic adjuvants that target the glycocalyx, ULVWF, low ADAMTS-13, and pathologic extracellular vesicles are reviewed. Much of the pathobiology of EOT is known, but a better mechanistic understanding can help guide therapeutics to further repair the EOT and improve patient outcomes.

Level of evidence: not applicable

Key words: endotheliopathy of trauma, vonWillebrands Factor, extracellular vesicles, glycocalyx, syndecan

OVERVIEW OF THE ENDOTHELIUM

Endothelial regulation of coagulation, inflammation, and vascular barrier function (Figure 1).

Under physiologic conditions, endothelial cells (EC) actively suppress coagulation by providing an antithrombotic surface to maintain blood fluidity. The most important endothelial-dependent anticoagulant mechanisms include expression of 1) heparan sulfate proteoglycans, 2) thrombomodulin (TM), 3) endothelial protein C receptor (EPCR), and 4) tissue factor pathway inhibitor (TFPI). Heparan sulfate is a glycosaminoglycan (GAG) bound to endothelial cell surface proteoglycans, including syndecan and glypican. Its anticoagulant function is mediated through interaction with circulating antithrombin (AT) via binding to a unique pentasaccharide domain, thereby localizing AT to the vessel wall and accelerating AT-mediated inhibition of both activated factor Xa (FXa) and thrombin (IIa). TM is an endothelial cell surface glycoprotein that, upon high affinity binding to thrombin, will change the substrate specificity of thrombin from procoagulant cleavage of fibrinogen and FV, to anticoagulant cleavage of protein C to generate activated protein C (aPC). This reaction is accelerated when protein C is bound to EPCR, bringing protein C into close proximity to the TM-thrombin complex. TFPI potently inhibits the initiation of extrinsic coagulation through its inactivation of the tissue factor (TF)-FVIIa complex.[1] Endothelial cells also express anti-platelet molecules, such as nitric oxide and prostacyclin (PGI_2), which suppress platelet activation.

The endothelium contributes to primary hemostatic clot formation through expression of von Willebrand Factor (VWF) on its surface. Upon exposure to sub-endothelial proteins such as collagen, VWF tethers platelets to the injured vasculature through binding of its A3 domain to

the subendothelial collagen and its A1 domain to the GP Ib-IX-V complex on platelets. ECs propagate thrombus formation through their role in secondary hemostasis, which is mediated in part through expression of TF, by interacting with cofactor FVIIa to activate FX to generate thrombin. To regulate thrombus growth and vessel patency, ECs also critically regulate fibrinolysis through their expression of tissue and urokinase plasminogen activators (tPA and uPA, respectively). Both tPA and uPA cleave plasminogen to produce plasmin, which degrades fibrin cross-linkages. These enzymes are inhibited by EC-derived plasminogen activator inhibitor type I (PAI-1), thereby inhibiting fibrinolysis.

Suppressing coagulation is an indirect mechanism, through which ECs regulate inflammation, due to cross-talk between coagulation and immune mechanisms.[2] Similar to their role in platelet inhibition, ECs also directly control activation of pro-inflammatory processes through secretion of nitric oxide and PGI_2 which reduce leukocyte recruitment, adhesion, and activation to the vessel wall. Production of nitric oxide also maintains EC quiescence though inhibition of pro-inflammatory gene transcription. ECs also prevent leukocyte adhesion through sequestration of P-selectin within its Weibel-Palade bodies (WPB). However, in response to an injury or insult, ECs undergo rapid Type I activation, characterized by exteriorization of leukocyte-interactive chemokines and degranulation of the WPB.[3] This is followed by a more sustained Type II response, which includes *de novo* transcription of cytokines (interleukins and monocyte chemoattractant protein) and chemokines including E-selectin, intracellular adhesion molecule 1 (ICAM-1), and vascular cell adhesion molecule 1 (VCAM1).[4]

Finally, ECs play an essential role in maintaining the semi-permeable barrier between blood and tissue. The endothelium utilizes two distinct pathways for regulating molecular transport: 1) the transcellular pathway and 2) the paracellular pathway. The transcellular pathway regulates passage of large molecules, such as albumin, through caveolae, which are lipid rafts that transport macromolecules into and through the endothelial cytoplasm. The paracellular pathway, on the other hand, regulates passage of small molecules (i.e. water, ions). The paracellular pathway is composed of inter-endothelial junctions, including gap, adherens, and tight junctions. Connexins are gap junctional proteins that mediate the direct transfer of calcium ions, current, and signaling molecules between neighboring cells. Adherens junctions are formed by paracellular adhesion of vascular endothelial cadherin (VE-cadherin), which regulates molecular transport and barrier function through interaction with the actin cytoskeleton via binding catenins. Tight junctional proteins include occludin, claudin, and junctional adhesion molecules which regulate permeability through zona occludens-mediated linkages with the actin cytoskeleton. Inflammatory mediators result in direct dissociation/re-organization of adherens junction, reduction in EC expression of inter-endothelial junctional proteins, or induce actin stress fiber formation which can all result in disruption of a healthy endothelial barrier, with increases in paracellular permeability, and ultimately tissue edema.

Structure and Function of the Endothelial Glycocalyx (Figure 2A)

Positioned at the interface between blood and the endothelial surface, the glycocalyx is central to maintenance of a healthy endothelium. The mesh-like glycocalyx layer is composed of interwoven proteoglycans and glycoproteins to which are bound an array of GAG chains. The syndecan and glypican proteoglycans express heparan sulfate, the most abundant GAG, as well

as hyaluronan, chondroitin sulfate and dermatan sulfate. The transmembrane glycoprotein, CD44, serves as the receptor for hyaluronan. Collectively, the glycocalyx maintains vascular homeostasis and preserves normal EC function by serving as a physical impediment to circulating platelets, leukocytes, and soluble EC activating factors to the underlying endothelium.[5] Heparan sulfate and chondroitin sulfate contribute to the anticoagulant tone of the endothelium through interaction and activation of AT and cooperative interactions with TM that augment its affinity for binding thrombin, respectively. The lesser abundant GAG, dermatan sulfate, can accelerate the thrombin inhibitory activity of heparin cofactor II. Hyaluronan modulates the chemokine gradient at the EC surface and regulates permeability through its interaction with angiopoietin 1.[6] In addition to their GAG-dependent roles, syndecans also play a key role in maintaining the endothelial barrier through their core structural position and mediating outside-in signaling events.[7]

THE ENDOTHELIOPATHY OF TRAUMA

The vascular endothelium plays a central role in maintaining organ homeostasis through its regulation of vascular tone, coagulation, inflammation, and barrier function. As such, dysregulation of normal endothelial function is an important pathological feature of organ dysfunction during disease states. The term "Endotheliopathy of Trauma (EoT)" is used to describe endothelial cell injury, activation, and maladaptive responses to major injuries including tissue trauma, hemorrhagic shock, and burns.[8] Importantly, clinical evidence of EoT is strongly linked with poor outcomes, indicating that EoT is an initiating pathological event contributing to systemic microvascular thrombosis, inflammation, loss of barrier integrity, and coagulopathy that are all key hallmarks of organ failure in this population (**Figure 2B**).[9] Therefore,

understanding the underlying mechanisms contributing to EoT and therapeutic strategies to limit or reverse its development could significantly improve survival and recovery from major trauma.

Post-injury morbidity and mortality linked to EOT

Clinically, EoT has been identified using known plasma/serum biomarkers of EC damage, including soluble TM, syndecan-1, and E-selectin. Haywood-Watson et al and Johansson et provided the first clinical data demonstrating EoT, noting pronounced elevations in plasma syndecan-1 upon hospital admission in severely injured patients.[10,11] Since then, many studies have corroborated these findings and strongly linked EoT biomarkers with morbidity and mortality after major trauma. Johansson et al measured EoT in 424 level 1 trauma patients and demonstrated significant correlations between EoT and sympathoadrenal activation, as well as both early and late mortality.[9] Rodriguez et al demonstrated that admission EoT, defined by a plasma syndecan-1 level ≥40 ng/mL, was associated with a 2.23 adjusted odds of in-hospital mortality compared to those without EoT.[12] All of these studies have shown that EoT is most apparent in those patients with the highest injury severity scores and greatest metabolic indices of shock. Interestingly, recent evidence indicates that EoT occurs very rapidly after trauma with data from Naumann et al demonstrating increased soluble TM and syndecan in the pre-hospital setting, within minutes after injury.[13] Additional recent data shows that EoT is a pathophysiologic state that persists over the first 24 hours after injury and remains a significant predictor of poor outcomes throughout this time-period.[14] Utilizing innovative proteomics approaches, Krocker et al have shown that EoT is associated with a unique plasma proteome signature enriched in damage associated molecular patterns (DAMPS), inflammatory mediators,

and organ-specific intracellular markers, corroborating clinical evidence linking EoT with organ damage.[15]

Despite the robust clinical relationship between EoT and trauma-associated morbidity and mortality, the cellular and plasma triggers of EoT and precise molecular events that drive EoT are still under pre-clinical investigation. Below, we describe the most recent data that highlights mechanisms and mediators of EoT that provide a future path forward for therapeutic development.

Glycocalyx damage in the development of EoT

Clinical data demonstrating pronounced shedding of EC surface components into the circulation holds key insights into mechanisms driving EoT. Loss of the endothelial glycocalyx is emerging as a unifying mechanism linking EoT with microvascular inflammation, barrier leakage, coagulopathy, and organ injury. Rahbar et al demonstrated prominent shedding of both syndecan-1 and GAG (heparan sulfate, chondroitin sulfate, and hyaluronan) components of the glycocalyx after major trauma.[16] These findings are validated by pre-clinical animal data demonstrating pronounced thinning of the entire glycocalyx layer after induction of hemorrhagic shock.[17,18] It is important to note that autologous shedding of some membrane-associated glycoproteins, such as TM or EPCR, are physiologic responses to EC activation that ensure appropriate hemostasis; however, loss of EC glycocalyx components are thought to result from soluble and cell-bound sheddases. During major trauma and hemorrhagic shock, large quantities of pro-inflammatory mediators are released from the damaged tissue into the plasma. Cytokines such as TNFα instigate the release of proteases that degrade glycocalyx structures from activated

leukocytes, platelets, ECs, and mast cells. This includes matrix metalloproteinases (MMPs) that non-specifically cleave syndecans, with identified roles for MMPs 2, 9, 13, and 15 during chronic or inflammatory disease states.[19] In addition, activation of the coagulation system may contribute to shedding of the glycocalyx as thrombin, plasmin, and elastase all act as sheddases in vitro.[20] Given the important role of syndecans as core proteoglycans, shedding of syndecans destabilize the glycocalyx as a whole and contributes to loss of EC surface GAG expression. In addition, the GAG-specific enzyme, heparanase I, cleaves heparan sulfates from the proteoglycan core.[21] Soluble heparan sulfate fragments can further propagate EoT by acting as DAMPS that signal through toll-like receptors.[22] The GAG hyaluronidase is secreted by activated leukocytes and degrades hyaluronan from the CD44 receptor, further destabilizing the glycocalyx layer. Neutrophil elastase has also been shown to cleave hyaluronan.[20] Non-enzymatic depolarization of GAGs by reactive oxygen species further contributes to non-specific GAG shedding.[23]

Systemic shedding of the EC glycocalyx layer has a number of important pathologic consequences that contribute to EoT and subsequent organ injury. First, loss of anticoagulant GAG molecules from the cell surface creates a procoagulant environment. Syndecan proteolysis, as well as enzyme-specific cleavage of GAGs, results in loss of EC expression of heparan, chondroitin, and dermatan sulfates that collectively suppress the generation or activity of thrombin at the cell surface, thereby contributing to microvascular thrombosis. Second, shedding of the glycocalyx results in loss of a physical barrier between soluble or cellular inflammatory mediators and the endothelium. This then allows binding of soluble cytokines and DAMPs to EC receptors that induce intracellular signaling events (i.e. activation of NFkB) that increase EC pro-

thromboinflammatory gene transcription. In addition, circulating activated leukocytes are now able to adhere to the EC surface where they release proteases and reactive oxygen species that further degrade membrane-bound proteins, as well as neutrophil extracellular traps (NETs) that facilitate formation of fibrin-rich microvascular clots. Third, glycocalyx shedding results in loss of vascular barrier integrity and hyperpermeability. This results, in part, due to loss of albumin-mediated control of intracellular calcium.[24] In addition, loss of syndecan expression hinders mechanotransduction signaling that is crucial to maintenance of cytoskeletal structure and junctional protein expression. Activated leukocytes secrete soluble factors that can directly disrupt paracellular VE-cadherin linkages. Further, recent data from Richter et al demonstrates that heparan sulfate cleavage upregulates EC production of angiopoietin-2, resulting in suppression of Tie2 receptor-mediated maintenance of intercellular junction protein expression.[25]

Contribution of Extracellular Vesicles to EoT

ECs subjected to traumatic, ischemic, and inflammatory insults undergo rapid changes that not only deplete the surface protective glycocalyx layer but also result in release of bioreactive molecules and EC apoptosis. These changes not only define trauma-induced endotheliopathy but also cause and disseminate secondary injuries following trauma. A key causal and disseminating factor is extracellular vesicle (EV) release from activated or apoptotic ECs that can be detected in peripheral blood samples of patients with trauma and in samples from experimental animals subjected to traumatic injury. These endothelial EVs have been widely used as markers for endothelial injury, and together with EVs from other cells, are increasingly recognized as a new class of biologic mediators.

EVs are ≤ 1 μm cellular vesicles and include membrane fragments, intracellular organelles, exosomes, and associated cargo molecules. They are released from cells undergoing apoptosis or active microvesiculation and can cause endothelial dysfunction and coagulopathy during acute injury through distinct but closely related pathways. First, membrane EVs often express anionic phospholipids, which are key cofactors in coagulation initiation and propagation. Second, EVs from peripheral blood samples of mice subjected to traumatic brain injury (TBI) induce vasoconstriction in-vivo and in-vitro, resulting in tissue ischemia.[26] This EV activity does not appear to derive from specific factors carried by the EVs but due to the specific structure of EVs because neither the protein nor the lipid fraction extracted from these EVs induces vasoconstriction individually.[26] Third, EVs carry bioactive factors derived from parental cells or captured from plasma that can activate or injure ECs.

VWF in the development of EOT

VWF is synthesized primarily in endothelial cells and megakaryocytes as a single-chain propolypeptide of 2,813 amino acids that contains multiple domains.[27] Pro-VWF first dimerizes through the CK domain. A variable number of dimers then multimerize through N-terminal disulfide bonds. Newly synthesized VWF multimers are either constitutively released into the circulation and subendothelial matrix or stored in the Weibel-Palade bodies of ECs and the ά-granules of megakaryocytes/platelets, where multimerization continues. As a result, VWF multimers stored in these granules are enriched in ultra-large (UL) forms and are released after ECs and platelets are activated.[28] VWF multimers differ not only in molecular mass but also in adhesive activity.[27] VWF circulating in the resting state binds platelets poorly unless they are immobilized on the exposed subendothelium at the site of vascular injury. In contrast, ULVWF

freshly released from the storage granules is intrinsically hyperadhesive and spontaneously binds platelets and endothelial cells.[29] A key structural difference between the two forms of VWF is that the platelet-binding A1 domain is hidden in the globular structure of plasma VWF but exposed on the surface of hyperadhesive ULVWF.[30] Upon release, hyperadhesive ULVWF multimers are anchored to ECs, stretched by the shear stress of blood flow, and rapidly cleaved at the Y^{1605}-M^{1606} peptide bond in the A2 domain by the metalloprotease ADAMTS-13 (A Disintegrin And Metalloproteinase with a ThromboSpondin type 1 motif, member 13).[31] The cleavage releases smaller plasma forms of VWF multimers found in the circulation. The proteolysis therefore serves as a safeguard to maintain the hemostatic activity of plasma VWF without causing spontaneous thrombosis by ULVWF. This safeguard is lost or altered during acute trauma and hemorrhagic shock through multiple pathways. First, as an acute phase reactant, VWF is kept in the storage granules of ECs to ensure its quick release at the site of vascular injury. In contrast, ADAMTS-13 is constitutively released upon synthesis from hepatic stellate cells, ECs, and platelets.[32-34] As a result, the homeostatic balance between ADAMTS-13 and VWF is disrupted during acute trauma by the substantial release of VWF without a parallel release of ADAMTS-13. Second, ADAMTS-13 synthesis and activity are inhibited by inflammatory cytokines and post-translational citrullination.[35] Both VWF and ADAMTS-13 can also be oxidized in selective cysteine and methionine residues in the oxidative stress environment of trauma, rendering the former resistant to cleavage and the latter less able to cleave VWF.[36] Third, VWF cleavage by ADAMTS-13 is facilitated by the fluid shear stress of blood flow.[37] The cleavage is therefore likely suppressed in the hypoperfusion state caused by hemorrhagic shock. Finally, because of their multidomain multimeric structure, EV- and cell-bound VWF can serve as a coupling factor that tethers EVs to the surface of ECs through P-

selectin, integrin $\alpha_v\beta_3$, or other molecules to enhance their vascular activities. Surface-anchored VWF multimers also bring leukocytes to ECs to facilitate or enhance vascular inflammation.

It is interesting that modifying VWF synthesis and release may affect endothelial integrity as VWF deficiency upregulated the tight junction protein claudin-5 to protect the integrity of the blood-brain barrier in mice.[38] Conversely, the microRNA miR-143-3p upregulated VWF expression to improve acute trauma-induced coagulopathy.[39] Desmopressin (DDAVP), which increases VWF release from the storage pool of ECs, along with a 1:1:1 transfusion strategy improved shock parameters and maintained coagulation in rats undergoing polytrauma and shock compared to transfusion alone controls.[40] While the study attributed the protective effect of DDAVP to the release of VWF from ECs, it is equally possible that the drug depleted the intracellular pool of VWF, as found in VWF deficient ECs.[38] These observations thus raise the question whether intracellular (stored) and extracellular (released) VWF multimers differentially regulate endothelial integrity and function.

These research and clinical findings also raise the question of why and under what conditions the hemostatic factor VWF becomes detrimental in the acute trauma setting. Since plasma levels of VWF and ADAMTS-13 vary significantly among healthy individuals, one can speculate that subjects with intrinsically high VWF and/or low ADAMTS-13 are more likely to develop VWF-driven endotheliopathy and coagulopathy during acute trauma and hemorrhagic shock. For example, subjects with O blood type have low baseline levels of VWF, whereas blood type B subjects have the highest.[41] ABO blood group could therefore be a confounding factor for VWF-associated pathologies in acutely injured patients.[42] In a study of 268 patients, type O patients

were more likely to develop hyperfibrinolysis than non-O patients and also had increased odds of massive transfusion after adjustment for injury severity score.[43] Preexisting conditions associated with hyperadhesive VWF could also exaggerate the detrimental activities of VWF. The role of coagulation factor VIII in trauma-induced endotheliopathy and coagulopathy is largely unknown, even though it circulates together as a complex with VWF and affects VWF cleavage. These knowledge gaps call for more extensive mechanistic studies to define the role of VWF in the pathogenesis of and recovery from trauma and hemorrhagic shock.

EOT Contributes to Traumatic Coagulopathy

As a key component of hemostasis, trauma-induced endothelial injury triggers local hemostasis at the site of injury. This process can become exaggerated and disseminated systemically, resulting in consumptive coagulopathy through several interconnected pathways. First, the endothelium, which provides an anticoagulant and platelet-repellent surface in the resting state becomes highly procoagulant and attracts platelets and leukocytes when its protective glycocalyx is depleted. Hyperadhesive VWF also tethers EVs and leukocytes to ECs remote from the injury site. Second, ECs express anionic phospholipids, such as phosphatidylserine (PS) on their surface and tissue factor (TF) when they are activated or injured, to trigger coagulation on the surface of ECs, as demonstrated by fibrin deposition to the vessel wall.[44,45] As a transmembrane protein, endothelial EVs carry TF and PS to promote the formation of the tenase complex to generate thrombin, which is a potent EC activator, and to disseminate intravascular coagulation.[46] Though this is best described as occurring following traumatic brain injury (TBI), increasing similarities between TBI and hemorrhagic shock-induced coagulation abnormalities are being identified. Third, VWF multimers, especially those with the exposed A1 domain, bind

and activate platelets to promote microvascular thrombosis, intravascular coagulation, and the release of procoagulant and proinflammatory EVs. Fourth, the transmembrane thrombomodulin (CD141), which acts with protein C and endothelial protein C receptor to form the anticoagulant system on the surface of ECs. However, the shedding of thrombomodulin from activated ECs by multiple enzymes weakens this anticoagulant system.[47] Soluble and EV-bound thrombomodulin remain active, further increasing the risk of coagulopathy.[48] Similarly, shedding of heparan sulfates from the glycocalyx layer further diminishes EC anticoagulant functions, thereby upregulating thrombin production. Fifth, when perfused over cultured human ECs, plasma from trauma patients increased VWF release from ECs and this activity was prevented by the anti-fibrinolytic agent tranexamic acid, providing a direct link between endotheliopathy and the state of fibrinolysis during acute trauma.[49] In addition, ADAMTS-13 activity measured in the early stage of trauma is significantly correlated with changes in prothrombin time and fibrin/fibrinogen degradation product, and ADAMTS-13 activity at < 50% of normal levels correlates with the development of disseminated intravascular coagulation and the need for transfusion of fresh frozen plasma.[50] Finally, activated ECs release large intracellular pools of tPA, which has been shown to drive plasmin generation and hyperfibrinolysis in coagulopathic trauma patients. Collectively, these EC functional changes caused by EOT are likely to drive an early consumptive coagulopathic phenotype, driven by rapid upregulation of thrombin production and tPA-mediated fibrinolysis, that later shifts to a hypercoagulable state resulting from sustained EC activation in the post-acute setting.[51]

POTENTIAL THERAPEUTICS TO MITIGATE THE EOT

Blood products

Clinical studies demonstrating improved survival by the early use of plasma in patients in hemorrhagic shock led to the hypothesis that the benefit of plasma entailed more than just achieving hemostasis to also include protection of the endothelium.[52-54] In pre-clinical studies, plasma has been shown to restore the endothelial glycocalyx via restoration of syndecan-1.[55] Subsequent mechanistic studies sought to determine the molecule(s) in plasma responsible for its endothelial protective effects. Both antithrombin III and adiponectin have been shown to contribute to plasma's protection of the endothelium.[56,57] Two separate groups of investigators determined that fibrinogen was also a key molecule.[58,59] Fibrinogen binds to cell surface endothelial syndecan-1 to stabilize it on the cell membrane, likely through binding to the heparan sulfate GAGs of the glycocalyx.[59] Wu et demonstrated that this binding activates an intra-cellular PAK-1 signaling pathway which disassembles stress fibers to reduce endothelial permeability and maintain barrier integrity.[60] DeBot et al demonstrated that RhoA GTPase is activated after trauma and reduces endothelial permeability.[61] RhoA GTPAse is upstream of PAK-1and likely is involved in the same pathway of endothelial protection. As low fibrinogen levels are associated with worse outcomes after trauma, fibrinogen supplementation beyond that found in plasma has been investigated.[62-64] Correction of trauma-induced hypofibrinogenemia in the US is primarily via cryoprecipitate administration, thus studies in this country have focused on cryoprecipitate. An in-vitro study by Wu et al found that cryoprecipitate restored endothelial cell barrier function comparable to fresh frozen plasma.[65] This was followed by an in-vivo study by Barry et al verifying cryoprecipitate's endothelial protection.[66] The short post-thaw storage time of cryoprecipitate, however, prevents its early use clinically. To address this

limitation, there is now a pathogen-reduced five-day post thaw shelf-life cryoprecipitate (INTERCEPT Fibrinogen Complex) and a lyophilized cryoprecipitate product that is under development. In a mouse model of polytrauma and hemorrhagic shock, both conventional and lyophilized cryoprecipitate protected the endothelium comparable to plasma, while lyophilized cryoprecipitate further increased the ADAMTS13:VWF ratio beyond both conventional cryoprecipitate and plasma, suggesting potential additional benefit.[67] Both the 5-day shelf life and the lyophilized product also resulted in sustained organ protection in a long-term rodent model of hemorrhagic shock and prolonged hypotensive resuscitation.[68]

Clinical studies are on-going to investigate the potential benefit of prothrombin complex concentrates (PCCs) on hemostasis after severe trauma. In a pre-clinical mouse model of hemorrhagic shock, Pati et al demonstrated that PCCs comparably reduced vascular permeability to that of plasma.[69]

Therapeutic Adjuncts to Blood Products

To date, efforts have focused on reversing endotheliopathy using blood component therapy as part of damage control resuscitation, but additional adjunctive strategies are needed. Potential therapeutic adjuncts with implications for trauma due to their endothelial protective properties are presented.

Targeting the glycocalyx

Given the important role of glycocalyx shedding in the pathogenesis of EOT, therapeutics that target responsible sheddases, or even upstream activation of leukocytes that produce such

sheddases, have garnered increasing attention in a variety of preclinical models.[70] Examples of these include etanercept, angiopoetin-1, hydrocortisone and heparin, though none of the reported benefits are specific to trauma.[70-73] In addition, in a clinical study, patients randomized to receiving doxycycline, an MMP inhibitor, exhibited reduced glycocalyx shedding in response to cardiopulmonary bypass.[74] While syndecan-1 provides structural support to the glycocalyx, the attached GAGs can also play an important role in protection. Heparan sulfate is the most abundant proteoglycan of the endothelial glycocalyx and is robustly shed in response to trauma and hemorrhagic shock. In particular, heparan sulfates endowed with a rare 3-O-sulfate modification possess both anticoagulant and anti-inflammatory properties through high affinity interaction with AT and the ability to bind and sequester circulation DAMPs. A recent preclinical study demonstrated that a synthetic 3-O sulfated heparan sulfate, dekaparin, had similar anti-inflammatory and organ protective properties to plasma in a mouse model of trauma and hemorrhagic shock.[75] These data indicate the important biological role of this unique heparan sulfate molecule and demonstrate that restoration of shed glycocalyx components could be a novel therapeutic avenue for future development.

Targeting ADAMTS-13 and VWF

VWF-mediated EV-induced vascular activity after trauma can be blocked by exogenous ADAMTS-13, VWF antibody, or a recombinant A2 protein, which binds the A1 domain exposed on hyperadhesive VWF in rodent models of trauma and hemorrhagic shock or traumatic brain injury.[36] These research findings are consistent with clinical observations that high plasma levels of VWF, low ADAMTS-13 activity, and/or an altered VWF-to-ADAMTS-13 ratios are associated with endotheliopathy, coagulopathy, and other measures linked to poor outcomes of

patients with severe injury.[76-80] These findings suggest that repletion of ADAMTS-13 may be beneficial. A study by Zhou et al in a rodent model of renal ischemia/reperfusion found that recombinant(rh) ADAMTS-13 reduced inflammation and improved endothelial cell function.[81.] Similarly, Wu et al found in a mouse model of traumatic brain injury that rhADAMTS-13 was effective in mitigating cerebral vascular leakage and coagulopathy and improved neurologic outcomes and survival.[82] Kleinveld et al examined rhADAMTS in a rat model of shock and found that rhADAMTS13 decreased lung permeability and lung and kidney injury and minimized syndecan-1 shedding comparable to that of plasma.[76] However, only rhADAMTS13 was able to restore the ADAMTS13:VWF ratio. There are several clinical trials ongoing or recently completed using rhADAMTS-13 in patients with thrombotic thrombocytopenic purpura (TTP) and sickel cell, raising the possibilty of repletion after trauma and hemorrahage may be feasible.

The platelet-binding A1 domain of VWF is exposed on hyperadhesive ULVWF multimers, but is hidden in the globular structure of physiologic plasma VWF multimers. The A1 domain is hidden because it forms a complex with the adjacent A2 domain. This A1-A2 complex is formed when the two vicinal cysteine residues (C^{1669}-C^{1670}) in the A2 domain are reduced (free thiols), but it disassociates when these cysteines are oxidized.[83] A human recombinant A2 protein which lacks the vicinal cysteines to allow binding of A1 in an oxidative environment such as is found in trauma has been developed and is being tested. In models of sepsis, Nguyen et al found that rhA2 inhibited platelet interaction with fibrin to lessen intravascular thrombosis.[84] To further explore its potential benefits, Xu et al administered rhA2 protein in a mouse model of traumatic brain injury and found it markedly reduced mortality and improved neurologic

function.[85] Additionally, A2 protected the endothelium as evidenced by reduced vascular leakage in the brain, reduced lung edema and reduced endothelial activation, which the authors attributed to a reduction of brain-derived EVs.[85]

Targeting EVs

VWF multimers secreted after trauma and other pathologic conditions activate platelets and endothelial cells to release procoagulant and proinflammatory EVs. These VWF-bound EVs provoke vascular hyperpermeability, a critical component of endotheliopathy. When EVs were isolated from severely injured patients and injected into naïve mice, endothelial cell injury, as demonstrated by syndecan-1 shedding, coagulopathy and organ injury, were all significantly increased as compared to mice receiving EVs from minimally injured patients.[86] Similarly, Dyer et al found that platelet EVs released after trauma were pro-hemostatic but also pro-thrombotic and contributed to enhanced venous thrombosis.[87]

Lactadherin, (milk fat globule-epidermal growth factor 8) can remove pathologic EVs by coupling them to macrophages to facilitate phagocytosis. Lactadherin has an integrin-binding RGD sequence and two C-terminal domains that bind phosphatidyl serine with a high affinity. Zhou et al have shown that lactadherin promotes the clearance of EVs, lessens coagulopathy, prevents permeability, and improves neurologic outcomes and survival in mice subjected to severe TBI.[88] As trauma causes widespread tissue injury and global ischemia it is likely that severely injured patients have more circulating EVs than those with isolated TBI. Lactadherin should therefore also prevent endotheliopathy and coagulopathy after trauma and hemorrhage, but this has not yet been studied.

Although EVs secreted after trauma are injurious, EVs can also be therapeutic when obtained under physiologic conditions.[89] For example, EVs from activated astrocytes carry miR-873a-5p, which inhibits NF-κB signaling to reduce microglia-mediated neuroinflammation in TBI mice.[90] EVs from neutrophils increase the release of transforming growth factor β1 (TGFβ1) and downregulate macrophage activity to suppress injury-induced inflammation.[91] Platelet derived-EVs tempered development of hemorrhagic shock, stabilized the endothelium and maintained endothelial cell barrier integrity.[92,93] An advantage of platelet EVs is that they can be stored frozen and potentially dried or lyophilized to extend their clinical applications.

Cellular Therapeutics

Mesenchymal stem cells (MSCS), which are stromal progenitor cells isolated from bone marrow, have been studied in-vitro, in-vivo, and clinically for use after trauma. They can facilitate angiogenesis to promote endothelial repair and in models of hemorrhagic shock can reduce endothelial permeability.[94] Additionally, comparable protection was afforded by EV-derived MSCs as MSCs themselves, suggesting MSC EVs may represent a viable cell-free therapeutic adjunct after trauma.[95,96] However, distinguishing the protective effects of EVs from their detrimental effects can be challenging because these diverse effects can be specific for parental cells, target cells, and cargo contents, i.e., the same EVs can both injure and protect ECs. Continued efforts are therefore needed to minimize detrimental effects while enhancing the protective effects of EVs in regulating trauma pathogenesis.

In conclusion, the endotheliopathy of trauma involves a complex interplay between the glycocalyx, VWF and platelets that lead to abnormalities in coagulation, inflammation and

endothelial cell function. The current review has focused on mechanisms of endothelial injury and the pathologic consequences of EOT as well as potential therapeutic targets. Most research to date has focused on blood products, primarily plasma, to restore endothelial cell dysfunction though a number of additional adjuncts are now being investigated. Much of the pathobiology of EOT is known, but a better mechanistic understanding can help guide therapeutics to further repair the EOT and improve patient outcomes.

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Figure Legends

Figure 1. Endothelial mechanisms that regulate coagulation, inflammation, and vascular barrier integrity. Endothelial cells control activation of the coagulation system by balancing both pro- and anticoagulant and fibrinolytic processes. Key anticoagulant mechanisms include 1) expression of heparan sulfates which are bound to a syndecan core and bind antithrombin (AT) to inhibit thrombin (IIa); 2) expression of thrombomodulin (TM) and endothelial protein C receptor (EPCR), which cooperatively generate activated protein C (aPC); and 3) expression of tissue factor pathway inhibitor (TFPI), which suppresses thrombin generation through inhibition of the tissue factor (TF):factor VIIa (FVIIa) complex. Endothelial cells can potently activate the coagulation system through secretion of von Willebrand Factor (vWF), which tethers platelets to the subendothelium and expresses TF. Clot dissolution is regulated through expression of pro-fibrinolytic enzymes tissue and urokinase plasminogen activators (tPA and uPA, respectively) and antifibrinolytic plasminogen activator inhibitor type 1 (PAI-1). Endothelial cells prevent adhesion and activation of circulating platelets and immune cells through secretion of nitric oxide (NO) and prostacyclin (PGI_2). They can also selectively mediate cellular adhesion through expression of selectins and intracellular or vascular adhesion molecules (ICAM-1 and VCAM-1, respectively). Endothelial barrier function is regulated by the transcellular pathway through calveolae and the paracellular pathway through expression of gap, adherens, and tight junctional proteins. (Image created with BioRender.com.)

Figure 2. Endothelial glycocalyx structure and function during healthy and post-injury states. Under healthy conditions, the endothelial glycocalyx is composed of proteoglycans (syndecans) and glycoproteins (CD44 and glypican) that are interconnected by an array of

glycosaminoglycans (GAG), including heparan sulfate, hyaluronan, and chondroitin sulfate. When intact, the glycocalyx functionally suppresses production of thrombin (IIa) from prothrombin (II), prevents platelet and leukocyte adhesion to the endothelial surface, and regulates molecular transport and transduction of shear stress signals. However, following major injury and hemorrhagic shock, syndecan and GAGs are enzymatically cleaved from the cell surface by sheddases resulting in release of these fragments into circulation. Loss of the endothelial glycocalyx layer contributes to coagulation at the cell surface, loss of vascular barrier integrity, and leukocyte adhesion and transmigration. (Image created with BioRender.com.)

Figure 1

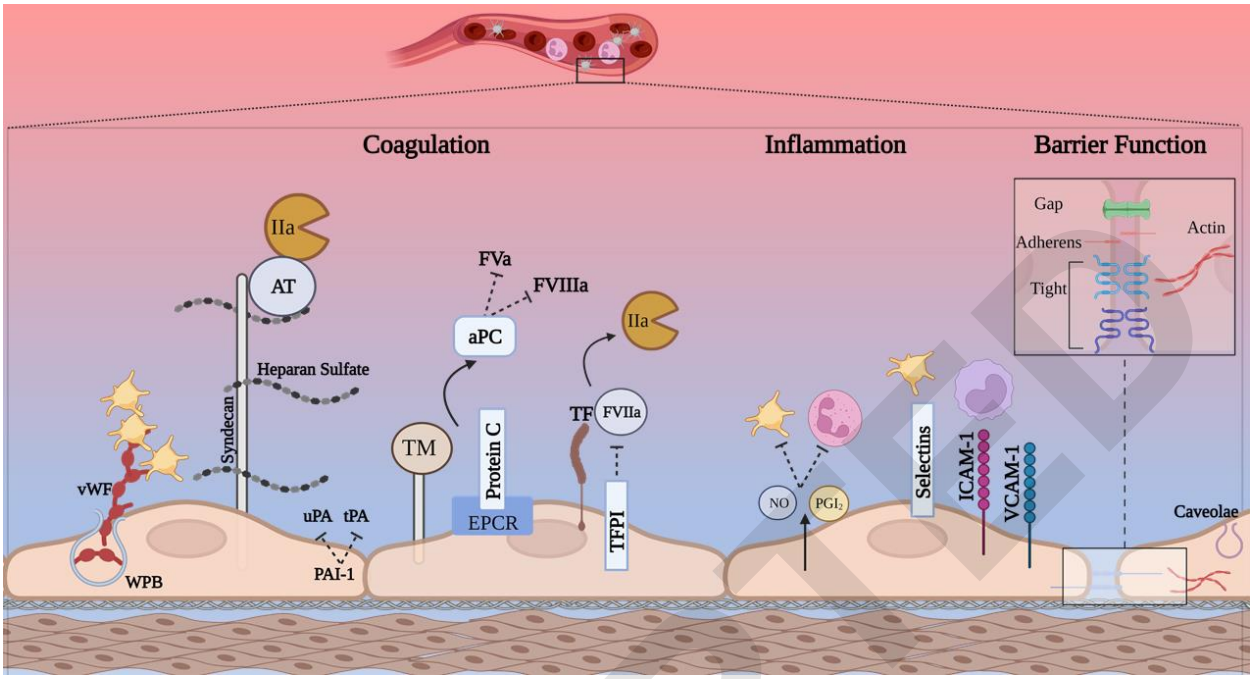

Figure 2