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Implications of the Regulation of Endothelial Glycocalyx Breakdown and Reconstitution in Severe Burn Injury

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ABSTRACT

Introduction: Effective initial fluid resuscitation is the cornerstone intervention in the setting of severe burn injury. Critically, few major advances in burn resuscitation have been made since the 1970s, and since that time there has been only modest improvement in overall morbidity and mortality. Recently, investigations regarding the dynamic changes of vascular endothelium, and more specifically the vascular endothelial glycocalyx, in the setting of severe burn injury and resuscitation have offered insight into the possibility of more tightly controlling fluid shifts and understanding the consequences thereof during this critical period.

Methods: We conducted a literature search of the PubMed database using the terms "burn", and "glycocalyx" limited to studies published in the English language over the past 10 y. A total of 31 articles were initially identified. Abstracts and full text were manually reviewed to identify suitable articles. Of the identified articles, 10 were deemed relevant and included within this review, along with additional articles necessary to provide background on glycocalyx structure and function as well as principles of burn injury management.

Results: Glycocalyx shedding is a process known to occur early in the setting of severe burn injury and resuscitation. The degree of shedding tends to increase with age and severity of injury. Though the role and regulation of this shedding is incompletely understood, it has direct consequences on vascular unction and permeability and likely coagulation as well.

Conclusions: Here in this research review, we examine what is known regarding the dynamic breakdown and reconstitution of the glycocalyx during burn injury and how it may be impacted by fluid resuscitation strategies. We further explore the need to more completely understand this mechanism and the consequences of its manipulation.

Introduction

Most deaths related to burn trauma occur within days after injury due to complications from the initial burn shock. Aggressive fluid replacement remains the cornerstone of resuscitation paradigms for serious burn injury, but it is evident that morbidity and mortality rates still have vast potential to improve. Unlike trauma resuscitation, where

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priorities historically have included re-expansion of volume, restoration of oxygen carrying capacity, and prevention or correction of coagulopathy, burn resuscitation largely focuses on volume re-expansion since the critical issue is loss of a functional integument rather than hemorrhage. Loss of a functional integument leads to significant insensible fluid loss from the intravascular space to the exposed interstitium.

The significant leakage of fluid out of the intravascular space can result in burn shock, which is an abnormal physiological state when tissue perfusion is insufficient for transportation of oxygen, nutrients, and waste products throughout the body. Burn shock is a culmination of hypovolemic, distributive, and cardiogenic shocks, making tissue perfusion immensely difficult to maintain since blood flow is inadequate at multiple levels. Further complicating burn shock is the fact that each type of shock can pose unique problems for individual burn patients. While treating burn shock as a whole remains complicated, discovering how to simplify and improve individual aspects would represent an important step toward optimizing burn injury treatment. At the level of the vasculature, one promising area of potential improvement involves regulation of a layer known as the endothelial glycocalyx.

A carbohydrate-rich layer found on the luminal side of the endothelium, the endothelial glycocalyx, is an essential component in regulating normal fluid homeostasis and movement from the intravascular space to the extravascular space. Under normal conditions, the glycocalyx prevents excess fluid leakage out of the intravascular space. Critically however, burn injuries and high-volume crystalloid resuscitation can cause glycocalyx shedding, which allows fluid to escape more easily into the interstitium.[1-3] This glycocalyx shedding appears to occur in a manner dependent upon injury severity and age of the patient.[1-3] While the exact function and consequences of this shedding are incompletely understood, this shedding has been demonstrated in the setting of both burn injury and nonburn trauma to correlate with increased morbidity and mortality. Using serum syndecan levels $\geq$40 ng/ml as a marker of glycocalyx shedding and endotheliopathy of trauma, Rodriguez *et al.* demonstrated increased blood product transfusion requirements and greater all-cause 30-day mortality in patients with endotheliopathy, despite similar initial base excess values and hemoglobin levels.[4] Welling *et al.* also demonstrated increased mortality in patients with endotheliopathy in the setting of either burn injury or trauma.[2] Interestingly, in both the study by Welling *et al.* and a multicenter study by the Systems Biology Coagulopathy of Trauma (SYSCOT) study group, presence of endotheliopathy was more associated with the presence of inhalational injury than total body surface area (TBSA) of the burn, and specifically in the study by the SYSCOT group, associated with abnormalities in clot fibrinolysis a potential mechanism for additional morbidity and mortality.[5] Increasingly, we also recognize that resuscitation strategies likely impact the magnitude and duration of shedding as well as the rate of reconstitution.[2,3] Therefore, understanding how to regulate the timing, magnitude, duration, and localization of glycocalyx shedding and reconstitution via fluid resuscitation may facilitate further optimization of burn injury treatments, resulting in improved clinical outcomes. The purpose of this review is to highlight literature investigating glycocalyx shedding and reconstitution in major burn injury and to consider approaches to further understand and optimize this process in the clinical setting.

Methods

We conducted a literature search of the PubMed database using the terms “burn” and “glycocalyx” limited to studies published in the English language over the past 10 y. A total of 31 articles were initially identified. Abstracts and full text were manually reviewed to identify suitable articles. Of the identified articles, 10 were deemed relevant and included within this review, along with additional articles necessary to provide background on glycocalyx structure and function, as well as principles of burn injury management.

Structure and composition

First visualized in the late 1960s, the endothelial glycocalyx refers to a gel-like layer of various membrane-bound and soluble molecules covering the luminal surface of the vascular endothelium (see Fig. 1A).[6] In humans, the glycocalyx can be anywhere between 0.5 and 5 μm thick, depending on numerous factors but usually positively associated with vascular diameter.[7] The glycocalyx is mainly composed of proteoglycans, glycosaminoglycan (GAG) chains, glycoproteins, and soluble plasma proteins (see Fig. 1B).[6]

Glycoproteins serve as the main backbone molecules of the glycocalyx. Proteoglycans make up a subclass of glycoproteins, distinguished from other types by their relatively long linear side chains. Syndecan and glypican are membrane-bound proteoglycans, anchoring the glycocalyx to the endothelial cells. Perlecan, versican, decorin, biglycan, and mimecan are soluble proteoglycans that stack upon the foundational mesh and add to its thickness. Perlecan and versican are the largest proteoglycans measuring at around 400 kDa, while all others range between 20 and 70 kDa.[6]

Aside from their structural positions and core protein sizes, proteoglycans can also be characterized by their attachments to GAG chains. The five types of GAG chains are.

- Heparan sulfate (HS)
- Chondroitin sulfate (CS)
- Dermatan sulfate (DS)
- Keratan sulfate (KS)
- Hyaluronan, or hyaluronic acid

Syndecan, glypican, and perlecan link with HS and CS chains; versican, decorin, and biglycan link with CS and DS chains; mimecan links with KS. GAG chains are highly negative, and the different types are distinguished by their degree of disaccharide polymerization, pattern of sulfation modifications, and composition of uronic acids and hexosamines. HS and CS are considered the most abundant GAG chains in the glycocalyx.[6]

The backbone created by proteoglycans and glycoproteins makes up only a portion of the glycocalyx; additional soluble components from both the endothelium and plasma mesh

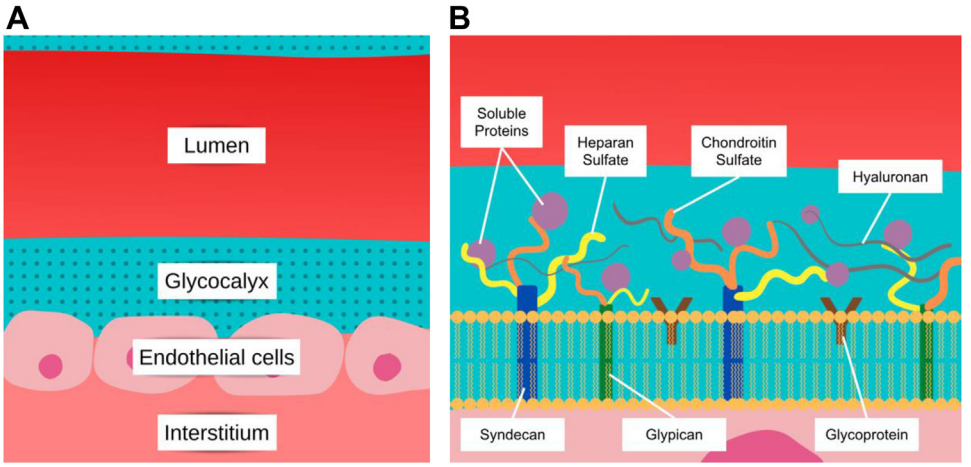

Fig. 1 – Structure and Composition of the Endothelial Glycocalyx. (A) The glycocalyx is located on the luminal side of the endothelium; (B) The glycocalyx is composed of a network of membrane-bound and soluble molecules. Note that these figures are not to scale.

into the rest of this network.[8] The solubility of the components embedded into the glycocalyx is one reason why the structural dimensions of this layer are dynamic. The glycocalyx reacts to stimuli from the rest of the body. Therefore, as some soluble components enter and exit the bloodstream, the thickness and organization of the glycocalyx consequently change.

Function

The endothelial glycocalyx maintains systemic functions as it lines the vasculature throughout the entire body, but its roles can also vary depending on localized conditions and stimuli.

Regulatory barrier

Thickening the physical barrier between the lumen and interstitium, one main function of the endothelial glycocalyx is its role as a determinant of vascular permeability. The highly negative charge created by the GAG chains makes it difficult for larger proteins and cells—mainly albumin, leukocytes, and platelets—from filtrating out of the vasculature into the interstitium.[8]

The movement of fluid and solute between the intravascular space and the interstitium has critical implications during resuscitation and as such, there have been several attempts to describe it. In 1896, Starling, using isotonic saline injected into the interstitium in a dog-hind limb model, proposed that capillaries and postcapillary venules are semipermeable and that fluid movement across them is dependent upon a gradient created by a combination of the colloid osmotic pressure generated primarily by plasma proteins, and the hydraulic and hydrostatic pressure due to the volume and heartbeat. While Starling never created an equation to describe his hypothesis, Landis *et al.* subsequently created the equation based on experiments conducted with frog mesenteric capillaries.[9-12] The classic Starling principle can be summarized by the following equation:

$$F = (P_C - P_i) - \sigma(\pi_C - \pi i)$$

Where F represents the sum of forces across the semipermeable endothelial lining, P_C is the capillary hydrostatic pressure, P_i is the interstitial fluid hydrostatic pressure, σ is the osmotic reflection coefficient, π_C is the colloid osmotic pressure of plasma, and π_i is the colloid osmotic pressure of interstitial fluid.[13,14] In a practical setting, however, calculations using the classic equation overestimates filtration, because it does not account for the effects of the endothelial glycocalyx (see Fig. 2A).[13-15]

Accounting for the endothelial glycocalyx requires revision to the classic Starling principle, as the glycocalyx creates a new type of fluid called the subglycocalyx fluid. Subglycocalyx fluid is located in the paracellular cleft between adjacent endothelial cells and in contact with the plasma and interstitial fluid (see Fig. 2B). The revised Starling equation is as follows:

$$F = (P_C - P_i) - \sigma(\pi_C - \pi_G)$$

Here π_G represents the colloid osmotic pressure of the subglycocalyx fluid. Since the glycocalyx prevents larger molecules from exiting the lumen, the subglycocalyx fluid has low protein levels and is significantly hypotonic. π_G is therefore significantly smaller than π_C, and the osmotic contribution to decreasing the rate of filtration would be expected to be greater than that predicted by the original Starling formula.[13]

Importantly, proponents of this hypothesis imply that plasma volume is normally maintained by a kind of circulation where fluid is lost to the interstitium by filtration and returns primarily by way of lymphatics, and direct fluid uptake into plasma only occurs in specific tissues such as intestinal mucosa and renal peritubular capillaries under normal conditions. Furthermore, they propose that the acute drop in capillary pressure after acute hemorrhage could

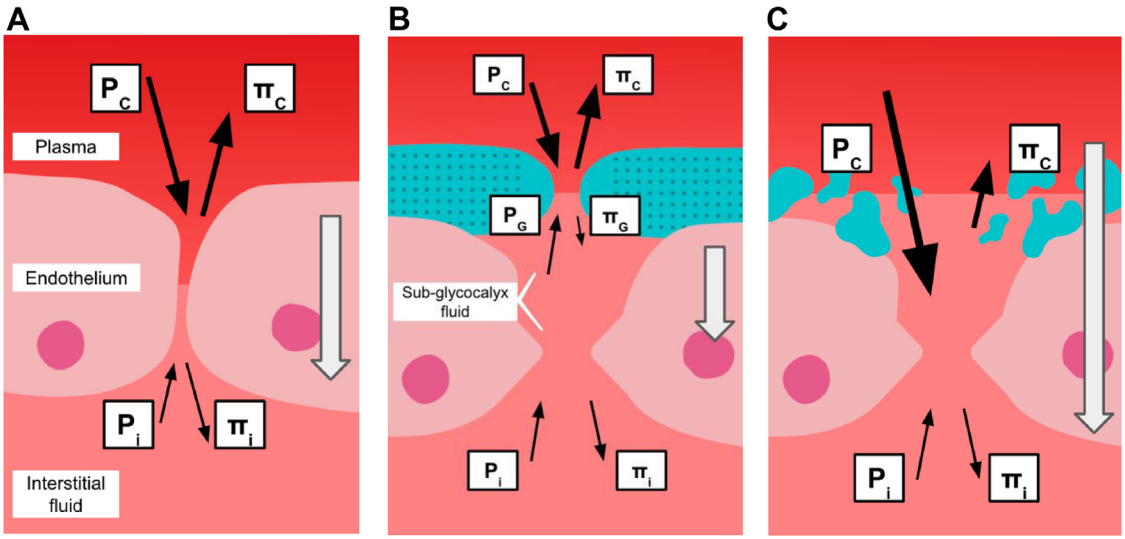

Fig. 2 – Classic and Revised Starling Principles. (A) Classic Starling principle focuses on capillary hydrostatic pressure (PC) and colloid osmotic pressure of plasma (πC) as the determining forces for filtration and absorption; (B) Revised Starling principle accounts for a normal endothelial glycocalyx, introducing new forces created by the subglycocalyx fluid that minimize filtration; (C) Revised Starling principle during initial glycocalyx shedding matches more closely to the classic Starling principle as the forces from the subglycocalyx fluid pressures do not exist. Black arrows denote direction and relative magnitudes of pressure forces. White arrows across figures compare relative expected rates of filtration.

initiate a transient, rapid fluid uptake from tissue to plasma.[9,11,13,14]

Critics of the revised Starling principle point out that there is little with respect to clinical data to support it. They point out that administration of a 20% albumin infusion could increase plasma by twice the infused amount in healthy volunteers, arguing aginst the view that fluid recruitment directly to plasma from the interstitium does not typically occur. Additionally, they argue that the frog and rat mesenteric postcapillary venule models may not accurately reflect what occurs in human muscle tissue with respect to the possibility of fluid resorption.[10,12] Nevertheless, the hypothetical model remains important and serves a basis for driving resuscitation research, particularly for severe burns where the correlation of injury severity to syndecan shedding is possibly more established than what is seen in the postsurgical setting or after trauma.

Critically, the predictions of the revised Starling principle potentially change when the glycocalyx sheds often in response to burn injury and even some forms of fluid resuscitation. In fact, expected rate of filtration during initial glycocalyx shedding may match more closely with that of the classic Starling equation when filtration is high (see Fig. 2C). This similarity is reasonable since the classic Starling principle does not account for the subglycocalyx fluid that does not exist when the glycocalyx has shed. During shedding, both fluids and proteins have easier access to escape into the interstitium; therefore, outward net flow dominates as the oncotic pressure gradient approaches equilibrium.[13,15] Additional translational and clinical research is needed to offer further insight.

Beyond endovascular changes, burn injury also causes integument damage that expands the parameters of net filtration. Damaged or lost integument such as broken skin allows even more fluid to escape the body (see Fig. 3A). In this case, this particular set of Starling forces would calculate an even greater rate of filtration and fluid loss. Not only is P_C potentiating filtration at the level between lumen and interstitium, but interstitial pressures create a vacuum that drives a continuous loss of fluid and proteins out of the intravascular space, through the interstitium, and to the environment. Without a healthy skin barrier, the Starling forces cannot reach equilibrium within the body and rather allow blood to continuously exit out of the vasculature. After glycocalyx reconstitution however, fluids and proteins may still easily escape the body, but the subglycocalyx forces are able to counteract the filtration to slow down overall blood loss (see Fig. 3B).[15]

Exposed directly to blood flow, the glycocalyx also participates in mechanotransduction to sense hemodynamic shear stresses. For example, mechanical distortions of proteoglycans due to shear stress from blood flow can signal the endothelial cells to release nitric oxide to dilate the vessels and decrease blood pressure.[16]

Anti-inflammatory and anti-oxidative response

When healthy, the glycocalyx has anti-inflammatory functions because some GAG chains bind to anticoagulant mediators, such as antithrombin. On the other hand, glycocalyx shedding is proinflammatory as the damaged barrier exposes selectins that facilitate leukocyte migration.[17,18]

Additionally, the glycocalyx has protective functions against free radicals because some enzymes, such as extracellular superoxide dismutase, can easily access and bind to the glycocalyx to protect the endothelial cells against oxidative stress.[19]

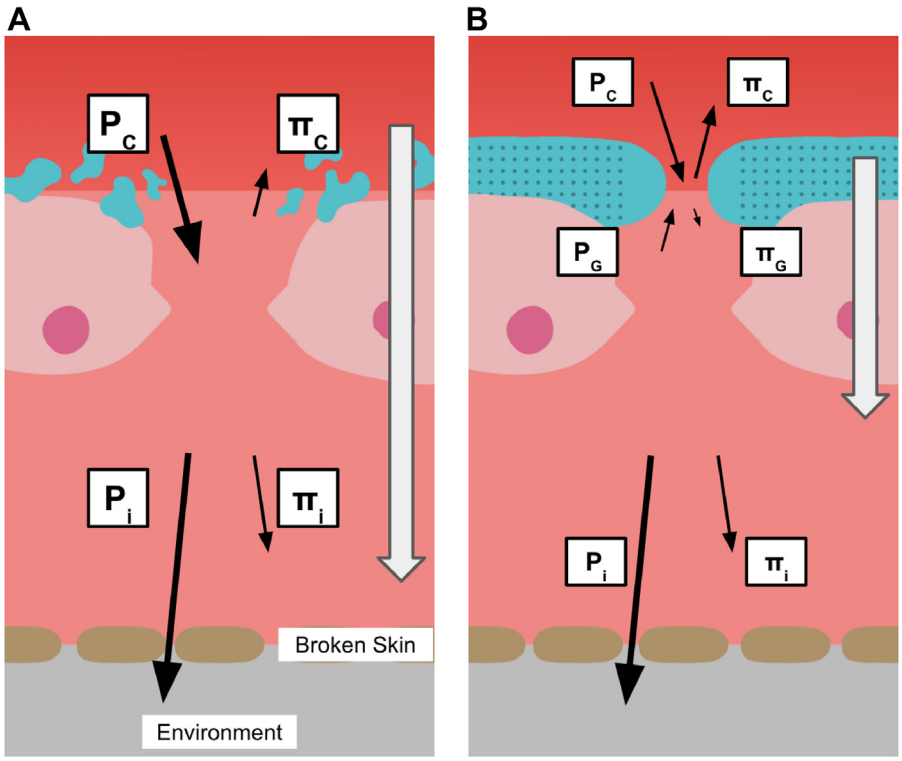

Fig. 3 – Starling Forces upon Burn Injury. (A) Damaged integument allows blood to escape the body, and the initial absence of the glycocalyx is unable to prevent blood from leaving the vasculature; (B) While the damaged integument still enhances blood loss, a reconstituted glycocalyx recreates the oncotic pressure gradient that maintains blood volume within the vasculature. Similar to Figure 2, black arrows denote direction and relative magnitudes of pressure forces, and white arrows across figures compare relative expected rates of filtration.

Effect of Fluid Resuscitation on Glycocalyx Integrity

At the level of the vasculature, the body's natural response to burn injury involves shedding of the endothelial glycocalyx.[20] As previously explained in Figure 3A, initial glycocalyx shedding after burn injury potentiates fluid loss. Since fluid loss is a macroscopically evident physiological issue, burn treatment paradigms have historically prioritized treating intravascular volume reexpansion. Recently however, some fluid resuscitation paradigms for general trauma have begun shifting focus to treating endotheliopathy. With numerous fluid resuscitation models available to treat a wide range of trauma, it is important to outline how the types of resuscitation fluids and their methods of administration affect the integrity of the glycocalyx.

Resuscitation fluids

While glycocalyx shedding is the natural immediate response of the body to burn injury, evidence suggests that the glycocalyx can naturally be quickly reconstituted over time. Kozar *et al.* demonstrated initiation of reconstitution within an hour in a rodent model of hemorrhagic shock through both cessation of syndecan shedding and upregulated expression of glycocalyx protein components.[21,22] Potter *et al.* demonstrated glycocalyx restoration within 5-7 d in a murine cremasteric venule model of enzymatic glycocalyx degradation, which perhaps was more damaging than what might be expected from a hemorrhagic model.[23] The type of resuscitation fluids employed after injury potentially can either prolong the shedding or expedite the natural reconstitution process.

Crystalloids

One of the most common crystalloid products used to treat burn injuries is known as Lactated Ringer's (LR) solution, which consists of sodium, chloride, potassium, calcium, and lactate mixed into a solution with an osmolarity of 273 mOsm/L and pH of 6.5. Burn patient outcomes have been shown to favor high-volume fluid resuscitation with LR, especially within the first 24 h of trauma, as it successfully accomplishes the primary goal of re-expanding intravascular fluid volume.[24] LR has been demonstrated in animal models to escalate syndecan shedding and glycocalyx breakdown.[13] Critically, a consequence of over-resuscitation with crystalloids, such as LR, is a phenomenon known as "fluid creep," characterized by complications caused by high-volume resuscitation, including edema. Since LR and other crystalloid products are known to facilitate glycocalyx shedding, fluid creep might be further potentiated by fluid passing more freely into the interstitium when the glycocalyx is shed, causing edema and hypovolemia.[25] In keeping with this inference, Osuka *et al.* found in a prospective cohort study that

after adjustment for age, sex, percent of TBSA burned, and inhalation injury, plasma syndecan-1 (SDC-1) was an independent parameter for increased fluid requirement and development compartment syndrome.[3] At this time, the specific role of glycocalyx breakdown and reconstitution in fluid creep is incompletely defined.

While the evolution of high-volume crystalloid resuscitation formulas, such as the Parkland formula, the modified Brooke formula, or the rule of nines guidelines used in the Tactical Combat Casualty Care guidelines of the United States military, has yielded more successful patient outcomes and lower mortality rates over past century, one statistic particularly stands out: mortality rates associated with burn injuries have not improved in 30 y.[26,27] Recent developments about the role of the glycocalyx call for deeper research into the effectiveness of fluid resuscitation from a new perspective.

Blood Products

Most blood product forms of resuscitation fluids are restorative and protective of the glycocalyx but by different—and unclear—mechanisms. Albumin is one of the easier blood product treatments to implement, and it transports sphingosine-1-phosphate (S1P) which has protective endothelial effects.[8,28,29] Meta-analysis to this point has suggested that albumin administration may improve outcomes in burn injury though evidence is limited at this point.[29]

Fresh frozen plasma (FFP) is another effective blood product treatment, and its protective effects may be due to its fibrinogen component and ability to restore SDC-1 after hemorrhagic shock.[25,29] Kozar *et al.* demonstrated augmented upregulation of syndecan expression following FFP administration in rodent hemorrhage models.[21,22] Cruz *et al.* compared resuscitation with LR alone, LR with FFP, and LR with albumin in the setting of 10%, 30%, or 40% TBSA burns in a rat model.[28] Critically, while addition of FFP did not significantly decrease SDC-1 shedding as determined by plasma levels, FFP did significantly decrease Evans blue dye extravasation from lung vasculature following 30% and 40% burns. Notably however, addition of albumin to LR for resuscitation did not decrease Evans blue dye extravasation in comparison to LR alone. These findings suggest that FFP may be more effective at acute functional glycocalyx restoration than albumin, though albumin resuscitation in burns continues to be a topic of active investigation.[30] The overall impact of these findings on eventual outcome with respect to morbidity and mortality however remains unclear. The impact of earlier functional glycocalyx restoration on all survival, timing of wound healing, or risk of tissue infection for example are not measured in this study and the significance of the timing of functional glycocalyx breakdown and restoration are incompletely understood. Additionally, it remains possible that the contribution of albumin in the model may not be appreciated by the measurement of SDC-1 shedding or Evans blue dye extravasation.

Resuscitation with red blood cells is another option for treatment as they, like albumin, are a major source of S1P in the body. Platelets are also rich in S1P, but the protective endothelial effect may rather be due to their release of cytokines and growth factors that maintain low vascular permeability.[28]

Discussion

Despite the initial steady improvements in fluid resuscitation models since the introduction of the Burn Budget Formula in 1947, advancements in treatment and mortality rates have recently reached a plateau, with little improvement in mortality for severe burns in the past 50 y.[25] Modern resuscitation formulas centered on intravascular volume re-expansion with high-volume crystalloid infusion have focused largely on addressing fluid shifts due to increased vascular permeability and loss of functional integument. These formulas were a significant improvement in acute care for burn patients at the time of their introduction.[15,25] Future evolution of resuscitation interventions may specifically target the endothelial dysfunction that can be seen in burn injury. The potential consequences of this endothelial dysfunction may include development of significant interstitial edema, increased tissue inflammation and leukocyte recruitment, and exacerbation of systemic coagulopathy.[5,14,15,18,25,31] Critically, these consequences may potentially be increased in severity by current high-fluid volume resuscitation paradigms for burn injury, as has been seen in trauma resuscitation, where high-volume crystalloid resuscitation has correlated with increased organ failure and respiratory distress.[31–36] Further research on how to optimally regulate endothelial glycocalyx shedding, and reconstitution glycocalyx during fluid resuscitation may facilitate development of a more precise and deliberate resuscitation algorithm.

Importantly, this recent shift in focus has led some current resuscitation paradigms to aim to hasten the restoration of an intact glycocalyx as a means of limiting endothelial dysfunction. It is anticipated that such approaches both in the setting of burn injury and nonburn trauma, may limit vascular permeability, trauma-induced coagulopathy, and secondary organ injury.[37] While rapid restoration may potentially prevent the initial potentiation of fluid loss caused by shedding, the role of glycocalyx degradation, and potential consequences of rapid restoration are incompletely understood. This lack of clarity calls for more research to be done to discover exactly how the glycocalyx should be most effectively modulated to optimize fluid shifts, coagulation, inflammation, infection control, and wound healing. A more complete teleological understanding of the role of glycocalyx shedding is critical in development of resuscitation interventions that maximize benefit and minimize risk of complication or morbidity. Understanding how to regulate the individual factors of timing, magnitude, duration, and location of glycocalyx shedding and reconstitution offers a promising avenue to optimize resuscitation for burn patients using a precision medicine approach. Until such factors of the endothelial glycocalyx are more clearly understood, progress in treatment and mortality rates are yet to be seen.

Author Contributions

All authors listed authors contributed to the writing and review of this manuscript. Vincent Basas wrote the bulk of the text of the manuscript and generated the figures and figure

legends used in the manuscript. Linda Schutzman reviewed the manuscript added content regarding burn shock to the introduction. Ian Brown as senior author reviewed the manuscript, wrote the abstract and revisions, and contributed to the discussion as well.

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