The glycocalyx: Pathobiology and repair

Although in early days the glycocalyx was assumed to simply provide a protective coating for cells, work over the last several decades has revealed the glycocalyx to be an intricate and highly-nuanced structure of multifunctional nature. Today, the glycocalyx is recognized as a critical mediator of human health and disease that, when intact, contributes to the maintenance of homeostatic cellular processes and, when damaged or modified, promotes pathologic cellular responses. Burgeoning of the field of glycocalyx biology has occurred in parallel to the development of novel tools that enable visualization and characterization of this delicate yet highly important structure, leading to a surge in research interest as reflected by the increasing number of publications within the field (Fig. 1).

The goal of this *Matrix Biology Plus* special issue entitled "Glycocalyx: Pathobiology and Repair" is to present both original research articles and reviews that together provide cutting edge updates from leaders in the field of glycocalyx biology with particular emphasis on glycocalyx damage during disease and potential for its repair. This special issue is comprised of 13 articles from a total of 65 contributing authors. Many of the articles focus specifically on the endothelial glycocalyx, highlighting mechanisms that promote vascular damage in response to traumatic injury or in critical illnesses and revealing novel strategies to protect or repair the glycocalyx as a means for preserving endothelial functions. Additionally, several articles present novel research describing the glycocalyx's role in cancer mechanisms that promote metastasis or disease progression. As an introduction, we provide a brief overview of the glycocalyx and describe its role in pathobiology with reference to the original research and review manuscripts that are included in this special issue.

Glycocalyx structure and function

The term "glycocalyx" or "sweet husk" (from the Greek glycus = sweet, calyx = husk) was first noted in 1963 by H. Stanley Bennett following his recognition that cells contained an extracellular coating of polysaccharides [1]. Further studies revealed that the glycocalyx comprises an extensive coating present over the plasma membrane of all cells and is visualized by electron microscopy as a bulky "fuzzy" layer (elegantly depicted in the contribution of Drost, et al. in this special issue [2]). It is composed predominantly of sugars, most of which are attached to proteins anchored within or on the plasma membrane thus forming an important interface between the cell and the extracellular matrix. These protein-glycan conjugates include glycoproteins and proteoglycans that together make up the bulk of the glycocalyx. Glycans within the glycocalyx can also be in the form of glycolipids or they can reside within the glycocalyx free of any protein or lipid constituent. The abundance of glycans include a broad array of sugar types many with complex structures. Common sugars that comprise these structures include glucose, galactose, mannose, *N*-acetylneuraminic acid, *N*-acetyl glucosamine, *N*-acetylgalactosamine, glucuronic acid, xylose and fucose [3]. This complexity of glycan structures enables the glycocalyx to function in multiple ways that impact cell behavior and cell response to external stimuli.

Notably, heparan sulfate proteoglycans (HSPGs; e.g., syndecan and glypican) are highly sulfated protein-glycan conjugates whose ubiquitous expression contributes immensely to the structure and function of the glycocalyx. The processes that regulate heparan sulfate synthesis are complex and highly nuanced resulting in wide permutations in both the sequencing and sulfation patterning of disaccharides that give rise to extraordinary diversity in the structural features of HSPGs expressed within the glycocalyx layer. Despite this heterogeneity, HSPG expression is highly regulated, and alterations in HSPG synthesis and sulfation can have important impact on biological functions of the glycocalyx that regulate health and disease (a topic that is reviewed in relation to the endothelial glycocalyx by Pretorius et al. [4]).

The review by Jiang and Goligorsky points out that the synthesis of a unified model of the glycocalyx and how it functions has been hampered by the fragmentary nature of our existing knowledge that has been accumulated by diverse physical, biophysical and biological approaches, techniques, assumptions and interpretations [5]. The goal of their review is to attempt fusion of the discoveries in cell biology

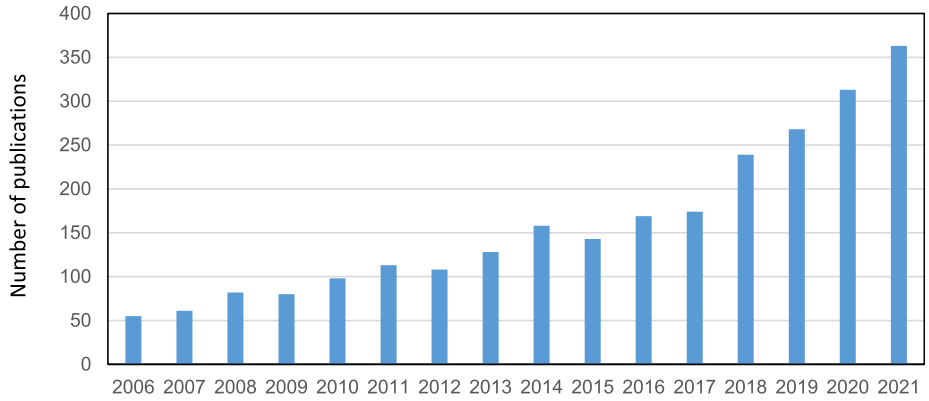

Fig. 1. Increase in publications related to the glycocalyx (2006–2021). The PubMed database was searched for the term "glycocalyx" present in either the title or abstract. Note the dramatic acceleration in publication numbers since 2017.

and mechanical engineering to produce a comprehensive biophysical model of the endothelial glycocalyx with the hope that it will further stimulate crosspollination of these fields and lead to deeper understanding of the glycocalyx.

Glycocalyx pathobiology

Many pathologic conditions result in aberrations in glycocalyx expression that, in turn, influence cellular mechanisms of disease. Changes in glycocalyx biosynthesis or post-translational structural modifications, for example, have been linked to mechanisms that disrupt anti-coagulant and anti-inflammatory properties of the vascular endothelium [4]. Furthermore, glycocalyx erosion is a hallmark of many critical illnesses, and byproducts of glycocalyx degradation can be detected in serum and measured as a marker of disease/illness severity and prognosis. Cleavage of the glycocalyx from endothelial cells, in particular, has widespread consequences for vascular functions that regulate permeability, immune cell trafficking, vasoreactivity, mechanosignaling and coagulation. As an example, Fig. 2 shows an activated platelet binding to the denuded endothelium within the pulmonary microvasculature of an injured mouse, illustrating how glycocalyx damage permits endothelial cell interactions with circulating blood cells that are capable of mediating coagulation and immune responses. Finally, glycocalyx ectodomains and shed fragments that are liberated from the cellular surface constitute an additional level of control over cell signaling since cleavage products are capable of initiating autocrine or paracrine signaling to direct cellular functions.

Several review articles included in this special issue provide overviews of the mechanistic factors that contribute to glycocalyx cleavage and/or modifications during states of acute and chronic pathologic conditions. Within the context of sepsis, Sullivan et al. reviews factors contributing to the activation of "sheddases" that cleave proteoglycans and glycosaminoglycans from the endothelial cellular surface, highlighting the systemic and local consequences of glycocalyx shedding on sepsis pathobiology [6]. Likewise, Masola et al. also provides an overview of the endothelial glycocalyx and brings to light the important functions of the heparan sulfate-degrading endoglucuronidase, heparanase-1, in mediating glycocalyx remodeling that contributes to disease progression [7]. The enzymatic activity of heparanase-1 is also reviewed by Pape et al., and the counterbalancing role of heparanase-2 to regulate or inhibit heparanase-1 activity is described [8]. Interestingly, an original research report by Henriksen et al., reveals that patients admitted with endotheliopathy of trauma (defined by shed syndecan-1 within the plasma of > 40 ng/ml) have detectable differences in metabolites within their plasma that suggests the possibility of impaired thromboxaneA2 and LTC4 synthesis [9]. Reduced thromboxaneA2 and leukotrienes impairs vasoconstriction leading the authors to speculate that these metabolic changes may contribute to endotheliopathy, shock and high mortality within this patient population.

The narrative review by Richter et al. provides a unique perspective into the endothelial glycocalyx in pediatric critical illnesses, including severe infection and sepsis, trauma/hemorrhage, burns, and extracorporeal life support [10]. Up-to-date

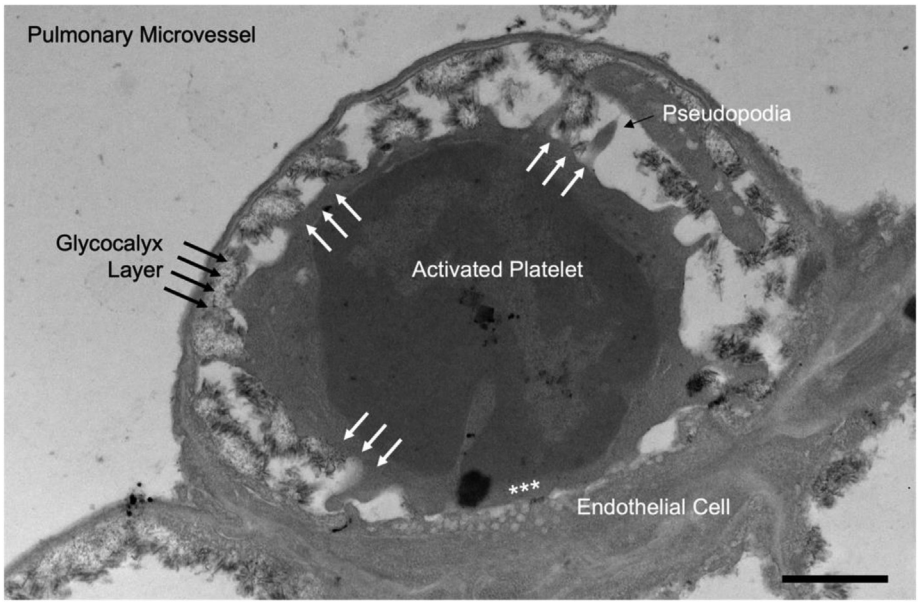

Fig. 2. Transmission electron micrograph depicting an activated platelet interacting with the pulmonary microvascular glycocalyx and denuded endothelium of a mouse treated with heparinase III from *Flavobacterium heparinum* (Sigma-Aldrich; 1 unit). The glycocalyx was fixed and prepared for electron microscopy as previously described [16]. White arrows denote platelet pseudopodia extensions and interactions with the damaged glycocalyx layer. Asterisks denote platelet attachment to the denuded endothelial cell surface. Scale bar is 1 μm.

knowledge of the endothelial glycocalyx in children is described, highlighting the important influence that ageing, immature immune system development and childhood comorbidities can have on endothelial glycocalyx integrity and overall vascular health.

Three original research publications within this special issue address the role of the glycocalyx in various aspects of cancer. A novel study by Kanyo et al. reveals the potential importance of studying single cell adhesitivity of cancer cells [11]. In this work, the glycocalyx of HeLa cells was degraded by exposure to different levels of the chondroitin sulfate degrading enzyme chondroitinase ABC. Using a high spatial resolution optical biosensor to monitor adhesion at the single-cell and cell population level, the authors discovered that digestion of the glycocalyx enhances cell heterogeneity in respect to cell adhesitivity. Moreover, when cells were exposed to a low level of chondroitinase ABC, a small population of cells with high adhesitivity was identified, while exposure to a high level of enzyme a subpopulation of cells with very low adhesitivity was revealed. Differences in cell adhesitivity have important implications for regulating tumor cell motility and metastasis. Motility and metastasis can also be activated by interstitial flow that is detected by the glycocalyx. Moran et al. addressed this using high and low metastatic counterparts of a renal cell carcinoma cell line [12]. Inhibition of heparan sulfate synthesis inhibited metastasis of the highly metastatic cells in response to interstitial flow. Similarly, they found that interfering with hyaluronic acid synthesis inhibited cell migration and metastasis. Another family of glycocalyx components are the sialoglycoproteins. These are highly expressed in the tumor cell glycocalyx and bind to Siglec receptors often present on immune cells leading to immunosuppression. Metcalf et al. hypothesized that an association between tumor fibrosis and increased production of immunosuppressive sialoglycoproteins could provide a functional link for fibrosis-induced immune suppression [13]. A comprehensive histological analysis and cellular census of human breast cancer tumors revealed that sialoglycan levels are increased in regions of high fibrosis and aggressive breast cancer subtypes. Importantly, the more aggressive subtypes had enhanced infiltration of immunosuppressive Siglec receptor positive myeloid cells. These findings led the authors to speculate that the sialic acid-Siglec axis may represent an attractive therapeutic target for breast cancer.

Glycocalyx repair

Therapeutic strategies to protect and restore the glycocalyx and its downstream functions are also highlighted in several of the manuscripts included in this special issue. In the review by Almahayni et al., a comprehensive overview of small

molecule inhibitors of glycosylation is presented [14]. Although many of these inhibitors currently have limitations and exhibit cytotoxic effects, it may in the future be possible to use these inhibitors strategically to diminish the impact of aberrant glycosylation within the glycocalyx that can occur during disease progression. Moreover, regulation of glycosylation has the potential to facilitate orderly repair of the glycocalyx following injury. Within the context of sepsis, Drost et al. and Sullivan et al. provide overviews of targeted approaches to limit glycocalyx damage and the resulting breakdown of the vascular barrier or, alternately, therapies to accelerate the repair of the endothelial barrier [2,6]. Strategies are highlighted that protect or repair the glycocalyx by inhibiting heparanase-1, substituting glycocalyx components or precursors to endogenously seal the damaged glycocalyx or promote its expression, and other pharmacologic approaches to limit glycocalyx sheddases. Likewise, Pape et al. provide an overview of therapies that could help promote a balance between heparanase-1 and heparanase-2 to attenuate sepsis-associated glycocalyx injury [8]. The article by Barry et al. discusses the use of plasma and platelet transfusions to maintain glycocalyx integrity and protect the vascular endothelium from damage caused by traumatic injury and hemorrhagic shock [15]. Together these reviews paint a hopeful picture indicating future progress in glycocalyx research that may lead to effective therapies.

Conclusion and outlook

As research continues to reveal new insights into the structural and functional properties of the glycocalyx and as the field now turns towards developing strategies to promote glycocalyx repair as a means of targeting disease mechanisms, we anticipate a continued upward trajectory of publications related to glycocalyx biology in the years to come. The intense interest in both the normal structure of the glycocalyx and changes in structure that occur during various disease states as well as the mechanisms that drive those changes will continue under dedicated scrutiny. Further defining glycocalyx-degrading enzymes and their potential inhibitors are ongoing focus areas that provide hope for the development of rapid interventions that can be applied to patients with glycocalyx damage. Finally, therapeutic approaches that promote synthesis or fine-tune structural features of glycocalyx constituents show potential for being able to mitigate pathologic mechanisms and restore homeostatic health. We eagerly await these scientific advances and anticipate the development of novel therapies that, based on the field of glycocalyx biology, may mitigate disease and critical illness.

DATA AVAILABILITY

No data was used for the research described in the article.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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