GLYCOMICS IN CLINICAL ACTION:

THE ENDOTHELIAL GLYCOCALYX

By: Helen Messier, PhD, MD

The recent explosion of interest in glycomics — the science of sugar molecules (glycans) — is a reminder of how much is still to be learned about the fundamentals of human biology. What we see in this field is an example of an apparently uninteresting phenomenon that turns out to be decisively important. Think glial cells in the brain, or "junk DNA."

Glycans are polymer chains of covalently linked monosaccharides and include both polysaccharides and monosaccharides. Glycans can be covalently bound to proteins or lipids forming glycoproteins or glycolipids respectively. Until quite recently, they were thought to provide mere structural support and were not actively studied. Now it is known that glycans perform essential signaling functions, playing a dynamic role in a multitude of critical physiological activities.[1]

Glycans do play structural roles in the organism, but they can also be individually recognized by other molecules, primarily glycan-binding proteins known as lectins. Intrinsic glycan-binding proteins recognize glycans from the same organism and typically mediate cell-to-cell interactions. Extrinsic glycan-binding proteins are mostly pathogens, so in this case glycan recognition plays an important role in infectious diseases.[2] This wide range of structural and signalling functions along with technical advancements in our ability to isolate and identify glycans has prompted an accelerating growth in glycomic research over the past 20 years:

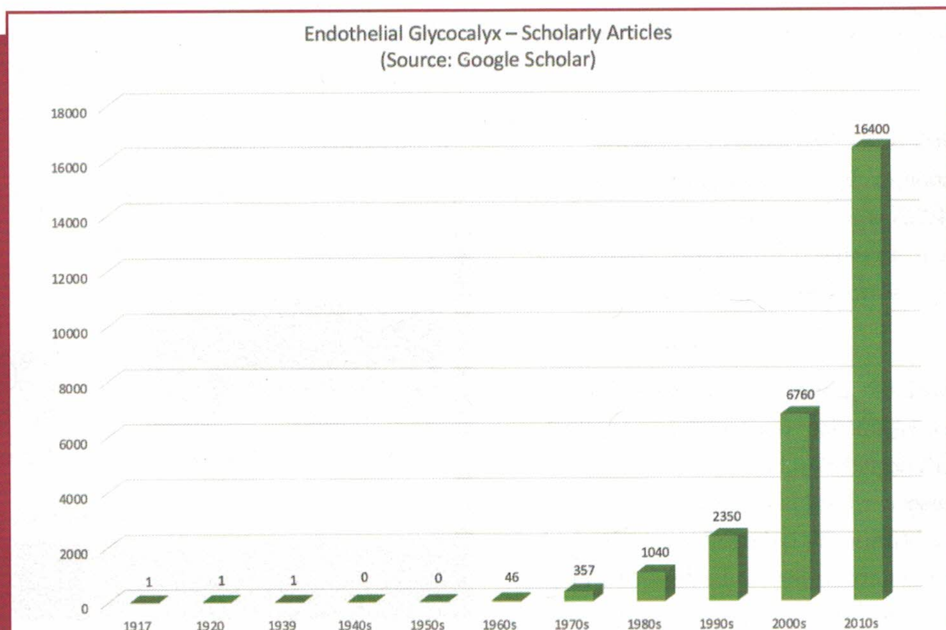
The translation of science to clinical application always takes time, but the findings of glycomic research are already bearing fruit in our understanding of immune functioning, cardiovascular disease, diabetes, kidney disease, cancer metastasis, HIV and influenza.

The Endothelial Glycocalyx

A clinically important example of the dynamic role played by glycans appears in one structure of the vascular system: the endothelial glycocalyx (EGX).[3] This is the microscopically thin gel-like layer that forms the inner lining of every blood vessel in the body, all 60,000 miles of arteries, veins and capillaries. The EGX is a selectively permeable barrier with anti-adhesive, anti-inflammatory, and antioxidative properties. It mediates biochemical and biomechanical signals in the circulating blood for optimal endothelial function.

The EGX is composed of glycoproteins, proteoglycans and glycosaminoglycans.[4] Proteoglycans provide the primary binding of the EGX to the endothelial membrane. They consist of a core protein to which glycosaminoglycan chains are linked. There are five types of glycosaminoglycan in the EGX: heparan sulfate, chondroitin sulfate, dermatan sulfate, keratan sulfate, and hyaluronan. The most populous of these is heparan sulfate, making up 50 to 90% of the total amount of proteoglycans. A number of other glycoproteins are contained within the forest of glycans making up the EGX. Cell adhesion molecules along with

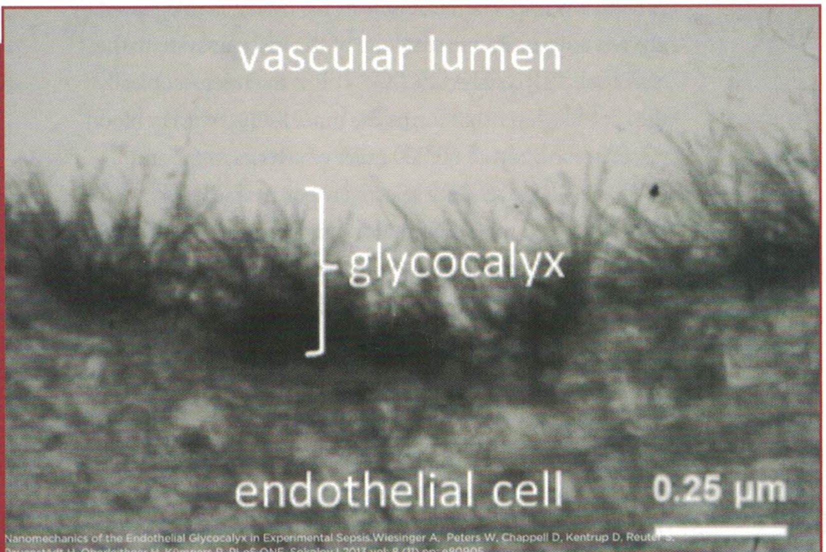
Graph showing glycomic research growth



1 Wang W, Özdemir V. Special Issue: Glycomics and Personalized Glycomedicine. OMICS. 2019;23(12):599-600. doi:10.1089/omi.2019.0177
2 Varki, Ajit, and John B. Lowe. "Biological Roles of Glycans." Essentials of Glycobiology, edited by Ajit Varki et al., 2nd ed., Cold Spring Harbor Laboratory Press, 2009, http://www.ncbi.nlm.nih.gov/books/NBK1897/.
3 VanTeeffelen JW, Brands J, Stroes ES, Vink H. Endothelial Glycocalyx: Sweet Shield of Blood Vessels. Trends in Cardiovascular Medicine. 2007;17(3):101-105. doi:10.1016/j.tcm.2007.02.002
4 Li Q, Xie Y, Wong M, Barboza M, Lebrilla CB. Comprehensive structural glycomic characterization of the glycocalyxes of cells and tissues. Nature Protocols. 2020;15(8):2668-2704. doi:10.1038/s41596-020-0350-4

coagulation and fibrinolysis factors jut out from the endothelial cell layer.[5] Closer to the flowing blood, there are several soluble proteins and proteoglycans that are embedded within the EGX mesh. The total volume of the EGX is significantT — about 2 liters, compared to about 5 liters of total blood volume.

Image of EGX structure



Nanomechanics of the Endothelial Glycocalyx in Experimental Sepsis.Wiesinger A, Peters W, Chappell D, Kentrup D, Reuter S, Pavenstädt H, Oberleithner H, Kümpers P, PLoS ONE, Sokolov I 2013 vol: 8 (11) pp: e80905

At its simplest, the EGX provides essential structural protection of the blood vessel wall.[6] Like Teflon, its slippery-smooth surface prevents the adhesion and penetration of unwanted particles in the blood. But the EGX plays a far more active role than this static picture suggests. The EGX is a "smart" barrier that actively mediates what is allowed to pass from the blood into the tissues, and what is not.[7] It also facilitates the generation of important molecules, including nitric oxide. And it plays a central role in regulating inflammation, oxidation and coagulation. With all these functions, the EGX is gaining increasing attention as the target for treatment of a range of pathologies, from atherosclerosis to renal disease to diabetic neuropathy.[8]

In this short overview, we'll take a brief look at how the EGX was discovered, and the essential functions that have been identified. Then we'll consider EGX degradation, both its causes and consequences, and the associated pathologies. We'll give special focus to atherosclerotic plaque, because this is the pathway to the single largest cause of human mortality, cardiovascular disease (CVD). Then we'll turn our attention to the challenge of restoring the EGX, well-researched ways to achieve this, and the clinical benefits.

The Research Trajectory: An Exemplary Field of Glycomic Science

Research into the EGX has grown exponentially, in tandem with the larger field of glycomic science. Observing its history shines a useful light on what this critical structure means for human health. When we grasp how the EGX at first eluded notice, was then misunderstood, and was finally revealed, we see more clearly what an important physiological role it plays. We also understand why even today, so few medical professionals are acquainted with it.

The endothelium, a single-cell layer lining the interior of every blood vessel, has long been known to science. Before the EGX was discovered, it was assumed that the flowing blood directly contacted the endothelium. However, with the beginning of advanced microscopy, a puzzling gap was detected between the lumen and the vessel wall. It appeared to be an empty space, so the first explanation was some kind of repulsive electrical field. Improved visualization revealed the presence of a physical barrier, which was named the endothelial glycocalyx. It had never been detected in autopsies because it was so thin and fragile. However, as technologies improved, its full structure and constitution was uncovered.

In today's imaging, the EGX appears as a forest of small hair-like elements protruding, rather like villi, from the vessel wall. Biochemical analysis of the EGX revealed a composition of negatively charged sulfates organized in a carbohydrate-rich mesh of membrane molecules.

Healthy Glycocalyx | Compromised Glycocalyx

Van den Berg 2006

Image Credit: Gouverneur, et al., Journal of Internal Medicine (2006), **259: 393-400**

5 Reitsma S, Slaaf DW, Vink H, van Zandvoort MA, oude Egbrink MG. The endothelial glycocalyx: composition, functions, and visualization. Pflugers Arch. 2007;454(3):345-359. doi:10.1007/s00424-007-0212-8
6 Harding IC, Mitra R, Mensah SA, Nersesyan A, Bal NN, Ebong EE. Endothelial barrier reinforcement relies on flow-regulated glycocalyx, a potential therapeutic target. Biorheology. 2019;56(2-3):131-149. doi:10.3233/BIR-180205
7 Alphonsus CS, Rodseth RN. The endothelial glycocalyx: a review of the vascular barrier. Anaesthesia. 2014;69(7):777-784. doi:10.1111/anae.12661
8 Facchini L, Bellin A, Toro EF. Modeling Loss of Microvascular Wall Homeostasis during Glycocalyx Deterioration and Hypertension that Impacts Plasma Filtration and Solute Exchange. Curr Neurovasc Res. 2016;13(2):147-155. doi:10.2174/1567202613666160223121415

From a glycomics perspective, what is striking about the EGX is its remarkably dynamic character.[9,10] The exact composition, thickness and architecture of the EGX is in constant change, responding to the flow and contents of the blood. It is the dynamic character of the EGX that enables it to perform such a wide range of physiologically important functions.

The EGX is universal in the vascular system, including the smallest capillaries where it constitutes a significant percentage of their total volume.

The Functions That Matter

The primary function of the EGX is to provide a semi-permeable barrier between the lumen and the endothelium. It normally allows only the smallest molecules (<70 kDa) through to the endothelium. This inhibits the passage or adhesion of LDL, Lp(a), platelets and other blood contents.[11]

We've mentioned that glycans can function as signaling molecules, and in the case of the EGX this capability has special importance. The shear stress of flowing blood triggers the EGX to send a signal to the endothelium, prompting the generation of nitric oxide. As a vasodilator, NO plays a vital role in maintaining blood pressure and the overall performance of the microvasculature.

Relevant to blood pressure, the EGX also buffers blood sodium. The glycosaminoglycans (GAG) in the EGX are negatively charged due to their high sulfate content. They bind to and inactivate sodium as non-osmotic sodium storage, preventing accumulation into the endothelium and vascular smooth muscle.

The EGX mediates inflammation in the vascular system. The relevance of this becomes clearer as CVD is increasingly recognized as an inflammatory disorder. Specifically, the EGX affects the interaction of leukocytes with the endothelial wall. A healthy EGX, with its slippery anti-adhesive surface, supports the "rolling" of leukocytes as they pass through the lumen. Without this protection, leukocytes are liable to adhere to, and penetrate, the endothelium.[12]

The EGX has an important antioxidant function because it houses sodium oxide dismutase (SOD) which is critical in the ceaseless combat with corrosive free radicals and cellular oxidation. Again, absent an intact EGX, this function collapses with important implications for vascular aging and overall health.

The EGX houses both coagulation and anticoagulation factors. As a result, its presence reduces the likelihood of blood clots forming in the lumen in the event of inflammatory disturbance of the endothelium.[13]

Degradation of the EGX

The EGX is extremely thin and fragile. It degrades easily, and the degradation can be seen both directly through microscopic visualization, and indirectly by identifying EGX components and sheddases floating in the blood.[14] The breakdown tends to first occur at bifurcations in the vascular system where there is greatest turbulence in the blood flow. As a structure the EGX is unusually dynamic, capable of both collapsing and rebuilding quickly. In fact, a healthy EGX exhibits a constant turnover, balancing shedding and new synthesis. However, chronic loss of the EGX can pose serious threats to the vascular system, and hence to the health of the entire body.

Causes of chronic EGX degradation include the familiar enemies of cardiovascular health:

- **High sugar diet**
- **Lack of exercise**
- **Stress and inflammation**
- **Infections**
- **High blood pressure**
- **Aging**

Problems arise when these causative factors are sustained over time, preventing the natural rebound of the EGX. Other causes

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10 Curry FE, Adamson RH. ENDOTHELIAL GLYCOCALYX: PERMEABILITY BARRIER AND MECHANOSENSOR. Ann Biomed Eng. 2012;40(4):828-839. doi:10.1007/s10439-011-0429-8

11 Liu X, Fan Y, Deng X. Effect of the endothelial glycocalyx layer on arterial LDL transport under normal and high pressure. Journal of Theoretical Biology. 2011;283(1):71-81. doi:10.1016/j.jtbi.2011.05.03

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13 Buchheim J-I, Enzinger MC, Choukèr A, Bruegel M, Holdt L, Rehm M. The stressed vascular barrier and coagulation - The impact of key glycocalyx components on in vitro clot formation. Thromb Res. 2020;186:93-102. doi:10.1016/j.thromres.2019.12.015

14 Becker BF, Jacob M, Leipert S, Salmon AHJ, Chappell D. Degradation of the endothelial glycocalyx in clinical settings: searching for the sheddases. Br J Clin Pharmacol. 2015;80(3):389-402. doi:10.1111/bcp.12629

can include trauma, electrolyte imbalances, surgery and the administration of IV fluids.

Consequences of EGX Degradation

Once the multiple functions of the EGX are fully understood, the seriousness of its degradation becomes apparent.[15] The body suffers loss of an essential barrier function throughout the vascular system. This results in increased leukocyte and platelet adhesion to the endothelium. In addition, an important trigger of NO production is lost, as well as a key antioxidant function. There's a reduction in coagulation control, leading to increased thrombin generation, and greater exposure to inflammation both systemic and local.

These consequences have very real clinical implications. Most obviously, EGX dysfunction is a factor in CVD and stroke. The importance of this factor is increasingly apparent, as the inflammatory character of atherosclerosis is better understood. We address this in more detail below.

Because of the vasodilation role of NO, loss of the EGX is almost certainly implicated in hypertension. It's also likely to be involved in a range of other conditions where the microvasculature plays an essential role. Examples include peripheral artery disease, Raynaud's, renal disease, diabetic neuropathy, macular degeneration, and erectile dysfunction.

In some cases, research has already been conducted on the role of the EGX in the associated pathology. Mostly, we can draw conclusions from what is known about the vascular aspect of these conditions, and the total dependence of the microvasculature on a healthy EGX. For any condition that is exacerbated by compromised blood flow, or by inflammation of the arteries, or by dysfunction of the capillaries, clinical attention should be paid to the condition of the EGX.

The Case of Atherosclerosis

EGX dysfunction plays an important role in atherogenesis and the specific threat posed by vulnerable plaque. Any insult to the artery wall may prompt an influx of macrophages, and if there's an opening, these are liable to penetrate the endothelium. Especially in areas of high turbulence, LDL may enter into the subendothelial space, where it can be oxidized and be ingested by macrophages to form foam cells. These can accumulate to become the most dangerous form of plaque — the unstable, vulnerable type with a lipid-rich necrotic core. If the fragile cap on vulnerable plaque breaks, clots can form and erupt into the lumen.

It's important to note that this mechanism does not require high volumes of circulating LDL,[16] which may explain why 50% of myocardial infarctions afflict individuals whose blood lipid levels are "normal". The importance of the EGX lies at the beginning of the story. Simply put, LDL can only pose a risk if excessive amounts penetrate the endothelium. A healthy EGX will inhibit this from happening, or it will support reverse transport (efflux) if excessive LDL penetration does occur.

Restoration of the EGX

It should be clear that maintaining a robust EGX is essential for sustained health, and restoring a compromised EGX should be a priority in the treatment of any disorder where vascular dysfunction is an issue. As mentioned the EGX, while fragile, is also resilient. It can be repaired rapidly, though as yet no pharmaceutical solution has been specifically designed to meet this need. Lifestyle factors are always important, of course, and any indication of EGX dysfunction should prompt attention to the core issues of diet, exercise, sleep and stress management. A number of glycocalyx regenerating compounds (GRCs) have shown promise in recent years. These include albumin, NAC, and heparin mimetics (Sulodexide).

Beyond these options, an understanding of glycomics can help the practitioner. Knowing that the EGX is comprised primarily of glycans, the question becomes: which natural glycans might be provided to help regenerate a compromised EGX?

Over the past twenty years, there have been studies of one restorative agent in particular in relation to cardiovascular health that holds promise for this role, rhamnan sulfate (RS). Like many polysaccharides, RS has a complex structure and not all versions found in nature are equally useful.

One particular form, derived from a rare seaweed, Monostroma nitidum, has been researched specifically for its potential as

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16 Fernández-Friera Leticia, Fuster Valentín, López-Melgar Beatriz, et al. Normal LDL-Cholesterol Levels Are Associated With Subclinical Atherosclerosis in the Absence of Risk Factors. Journal of the American College of Cardiology. 2017;70(24):2979-2991. doi:10.1016/j.jacc.2017.10.024

a GRC.[17,18] In one study, a microfluidic chip was developed to examine the impact of various GRCs on a living EGX in laboratory conditions. The study showed that marine-sourced RS was the most effective GRC and quickly rebuilt an EGX compromised by sugar exposure.

Conclusion

For clinicians, the most important conclusion to be drawn from this overview is the decisive role in human health that is played by a physiological structure that is still widely unknown: the endothelial glycocalyx. Becoming acquainted with the EGX, learning to recognize its dysfunction, and researching ways to restore and protect it, are worthwhile priorities for every practitioner. More broadly, the role of the EGX exemplifies the growing significance of glycomics, and we can expect a wave of new discoveries to open up new therapeutic opportunities in the near future.

Dr. Messier earned a PhD in molecular immunology and an MD in family medicine. She practices and teaches functional medicine. Her passion is bringing scientific understanding into clinical practice so that patients can benefit from research findings sooner.

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18 Okamoto T, Akita N, Terasawa M, Hayashi T, Suzuki K. Rhamnan sulfate extracted from Monostroma nitidum attenuates blood coagulation and inflammation of vascular endothelial cells. J Nat Med. 2019;73(3):614-619. doi:10.1007/s11418-019-01289-5