Glycocalyx, cardiometabolic disease and inflammation
Broekhuizen, L.N.

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Endothelial Glycocalyx as potential diagnostic and therapeutic target in cardiovascular disease


LN Broekhuizen [1]
HL Mooij [1]
JJP Kastelein [1]
ESG Stroes [1]
H Vink [1,3]
M Nieuwdorp [1,2]

[1] Department of Vascular Medicine, Academic Medical Center, Amsterdam, the Netherlands
[2] Department of Internal Medicine, Academic Medical Center, Amsterdam, the Netherlands
[3] Department of Physiology, Cardiovascular Research Institute Maastricht, Maastricht University, the Netherlands
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Purpose of review

The endothelial glycocalyx has emerged as a potential orchestrator of vascular homeostasis. Under physiological conditions, the glycocalyx is an important contributor to the regulation of vascular permeability for macromolecules as well for the adhesion of circulating cells. In line, the potential role of the glycocalyx in maintaining the anti-atherogenic properties of the vessel wall may have important clinical implications. In the present review, we provide an overview of recent developments and a glance at the future of establishing endothelial glycocalyx as a crucial player in cardiovascular protection.

Recent findings

Novel methods to estimate glycocalyx dimensions in vivo (using Orthogonal Polarization Spectral imaging (OPS) or Sideview Darkfield imaging (SDF)) as well as progressive insight into the enzymes involved in glycocalyx synthesis will be crucial in the assessment of this structure as a potential surrogate marker or therapeutic target for cardiovascular risk. The validation of these 'imaging' techniques and the integration with glycocalyx degradation products in plasma will allow us to test the value of the endothelial glycocalyx in estimating cardiovascular risk.

Summary

The endothelial glycocalyx, protecting the vascular wall against atherogenic influences, could be used for cardiovascular risk stratification. For this purpose, new methods to estimate glycocalyx dimension are promising.

The glycocalyx forms a gel-like layer covering the endothelial lining, shielding endothelial cells from direct contact with circulating blood cells. This strategic position already implies its potential role in several crucial balances at the level of the vessel wall, including the vasoconstrictor-vasodilator, the pro- and -anticoagulant, the pro- and anti-inflammatory as well as the pro- and anti-oxidative balance. This commentary will discuss the latest insights into glycocalyx-related functions in patients at risk for cardiovascular disease and future options to use the glycocalyx as a diagnostic tool or even a therapeutic target for preventive strategies in clinical practice.

Synthesis and role of glycocalyx in vascular homeostasis

The composition of the glycocalyx consists of a number of glycoproteins and proteoglycans, including the core proteoglycans (syndecans, glypicans, CD-44 and versecan) with a firm connection to the endothelial cell membrane and soluble proteoglycans (like perlecan, mimecan and byglican). These proteoglycans in turn provide the backbone for binding of various glycosaminoglycans (GAGs), comprising the negatively charged, sulphated GAGs heparan sulphate, chondroitin sulphate and to a lesser extent keratan sulphate and dermatan sulphate. An important uncharged GAG in the
glycocalyx is hyaluronan, which differs from other GAGs in that it is linked to only one proteoglycan, CD-44. Hyaluronan is crucial for the structure and the maintenance of the entangled network providing stability to the glycocalyx due to its water-retaining properties. Finally, a dynamic layer of soluble components like plasma proteins and proteoglycans, which form the selectively charged barrier, covers these GAGs. This high concentration of carbohydrate chains results in an overall hydrodynamically relevant glycocalyx dimension of approximately 0.5-3 µm, as determined by fluorescent dye exclusion technique and microparticle image velocimetry (µ-PIV) (Figure 1) 1, 2. Endothelial glycocalyx is present in macro- as well as microvasculature and its thickness progresses with increasing vascular diameter 3, 4. Since more than 95% of the endothelium is located within the microvascular capillaries, the vast majority of the endothelial mass resides within the microvasculature 5. Glycocalyx dimension is dependent upon the balance between biosynthesis versus degradation (‘shedding’) of glycocalyx components 6. Markedly, more than 5% of the genome (named the Glycome) is involved in generating these tissue-specific glycans on each cell type in humans 7. The biosynthesis of these glycan chains occurs mostly in compartments of the Endoplasmic Reticulum-Golgi pathway in stepwise reactions involving specific nucleotide sugar transporters, glycosyltransferases, glycosidases and other glycan-modifying enzymes 8. Subsequently, depending on nature of the endothelial GAG, specific enzymes elongate and modify sugar chains (Figure 2); relative amounts and localisation of GAG synthesizing proteins in the Golgi apparatus determine the structure of the endothelial glycocalyx 9, 10.

Figure 1. Electron microscopy image of coronary endothelial glycocalyx (courtesy of B. van den Berg, Maastricht University).
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Figure 2. Proteoglycans and GAGs involved in endothelial glycocalyx.

Abbreviations: GAG, glycosaminoglycan; HAS, hyaluronan synthase; HYAL, hyaluronidase; NO, nitric oxide; TLR, toll like receptor; EXT, exostosin; NDST, N-deacetylase/N-sulphotransferase; OST, O-sulfotransferase.

Under physiological conditions, the endothelial glycocalyx has several well-defined functions aimed at preserving the integrity of the vessel wall. First, the glycocalyx serves as an inert barrier that precludes direct ‘endothelial’ contact, and at the same time creates a selectively permeable structure contributing to the generation of the osmotic pressure gradient across the vessel wall. Second, the glycocalyx serves as an active reservoir containing major enzymatic systems as well as their cofactors, for instance lipoprotein lipase (LPL), extracellular superoxide dismutase (ec-SOD), antithrombin III (AT III), anticoagulant heparansulphates and thrombomodulin 4. These enzymes, involved in regulating lipid homeostasis, oxidation status and (anti)coagulant responses, determine in part the capacity of the vessel wall to respond towards noxious stimuli 11-16. Third, the glycocalyx has an important role as mechano-transductor transferring shear-stress into shear-dependent endothelial responses. One of the major shear-dependent reaction products includes the release of nitric oxide (NO), which is a key determinant of the anti-atherogenic, vasorelaxant capacity of the endothelial lining 1, 17. Interestingly, shear stress also stimulates the synthesis and incorporation of hyaluronan, heparan- and chondroitinsulphate in the glycocalyx, thereby increasing glycocalyx thickness 7.
On the other hand, damage to the endothelial glycocalyx is associated with influx of lipoproteins, leakage of macromolecules and adhesion of circulating cells to the endothelium. Concomitantly, loss of glycocalyx may contribute to an imbalance in enzymatic systems such as coagulation and anti-oxidant defence as well as an impaired NO release. In fact, there appears to be an intimate relation between risk factors for atherosclerosis and the damage to endothelial glycocalyx. As GAG synthesis is under direct negative feedback of inflammatory and oxidative stimuli, this indicates that a pro-atherogenic environment has a direct impact on both GAG composition as well as glycocalyx dimension. In turn, both a decreased rate and aberrant composition of GAG synthesis may contribute to vascular inflammation and vascular dysfunction.

Glycocalyx and Inflammation

In recent years, low-grade inflammation has emerged as a hallmark of atherosclerosis, resulting in increased cellular influx into the vessel wall contributing to early lesion formation as well as plaque destabilisation at later stages of atherogenesis. Recently, Henry et al. demonstrated that a TNF-α stimulus in hamsters increased permeation of macromolecules into the glycocalyx, which in turn may promote inflammation-associated edema formation by decreasing the trans-glycocalyx oncotic pressure gradient. In line, Marechal observed that endotoxin administration in rats induced microvascular dysfunction in conjunction with immediate glycocalyx degradation pointing out a role for Toll Like Receptors (TLRs) in glyocalyx perturbation. Markedly, infusion of activated protein C, an anticoagulant enzyme residing within the endothelial lining, could prevent the endotoxin-induced glycocalyx perturbation. These findings imply that glycocalyx constituents are actively involved in fine-tuning of inflammatory responses. In fact, experimental data have corroborated that endothelial heparansulfates are also involved in ‘scavenging’ of cytokines. Thus, the ‘state’ of endothelial glycocalyx (e.g. presence of hyaluronan and sialic acid, which cover these cytokine binding places on selectins) actively determines whether and to what extent cytokines will be able to induce localized vessel wall inflammation.

From a clinical perspective, patients with chronic inflammatory disorders, such as rheumatoid arthritis (RA) or systemic lupus erythematoses are indeed characterized by an increased prevalence of coronary heart disease, which cannot be fully explained by the traditional risk factors. In this respect, the concept has emerged that the vessel wall in patients with chronic inflammatory disease (CID) is ‘somehow’ more sensitive towards pro-atherogenic stimuli. Conversely, recent studies confirm reversal of vessel wall dysfunction upon adequate suppression of the pro-inflammatory activity. In view of the experimental evidence reporting glycocalyx loss upon inflammatory challenges, we recently extrapolated this concept to the human setting. It was shown that a controlled inflammatory challenge leads to instantaneous shedding of glycocalyx constituents and leukocyte activation in conjunction with a profound decrease in glycocalyx dimension. Pre-treatment with Etanercept, a soluble TNF-α receptor, prevented leukocyte activation as well as shedding of glycocalyx.
compounds 16. These data point towards the glycocalyx as a protective shield against inflammatory stimuli and suggests that prevention of glycocalyx damage may attenuate the impact of inflammatory stimuli in the atherosclerosis and sepsis.

Glycocalyx and hyperglycaemia

Another disease associated with increased risk of cardiovascular disease is diabetes mellitus, characterized by profound vascular pathology comprising both macrovascular as well as microvascular complications. A hallmark of diabetic vascular pathology is an increased permeability of the vessel wall for macromolecules, such as albumin and lipoproteins. The exact mechanisms contributing to the generalized ‘vascular’ dysfunction in diabetes have not been fully elucidated. Recently, we showed that acute hyperglycaemia resulted in a profound perturbation of the endothelial glycocalyx with a concomitant increase in vascular permeability 13,15. Mechanistically, several pathways may contribute to the loss of glycocalyx during hyperglycaemia. For instance, oxygen radicals produced during hyperglycaemia could directly damage the glycocalyx structure but hyperglycaemia might also elevate the activity of enzymes that degrade the glycocalyx 13,14. Indeed, glycocalyx decrease was found to be most pronounced in type 1 diabetes mellitus (DM1) patients with pre-existent microalbuminuria 14. In this respect, it is interesting to note that GAGs within the glycocalyx layer may contribute to the size and charge selectivity in the glomerulus 35-37.

In fact, degradation of the glycocalyx lining within the glomeruli by specific enzymes leads to a profound increase of albuminuria 36. Moreover both hyaluronan and hyaluronidase plasma levels were found to be increased in DM1 patients with a reduced glycocalyx 14. Presumably, activation of glycocalyx degrading enzymes as a result of the hyperglycaemia in DM may cause chronic systemic glycocalyx injury leading to albuminuria and increased influx of macromolecules like the LDL particle. The concomitant activation of the inflammation and coagulation cascades, as a result of glycocalyx loss may thus contribute to the propensity towards cardiovascular disease in patients with diabetes 15.

Glycocalyx and hypercholesterolemia

As discussed previously, hyperlipidemia may be an important contributor to glycocalyx perturbation. Recently published data show an intricate relationship between increased plasma LDL cholesterol levels, reduced expression of endothelial GAGs and increased wall thickness at carotid lesion-prone sites 38,39. These data implicate that intact glycocalyx may serve as a molecular sieve for LDL, thus preventing accumulation and subsequent (syndecan) receptor mediated uptake of atherogenic LDL 40. These data concur with a recent publication showing an inverse relationship between plasma LDL cholesterol levels and glycocalyx dimensions in patients with familial hypercholesterolemia (FH), whereas cholesterol-lowering treatment resulted in partial recovery of glycocalyx 41. In line, to study the role of glycocalyx degrading enzymes on atherosclerosis progression, Meuwese et al investigated the effect of chronic glycocalyx injury through enzymatic degradation of hyaluronan by chronic
hyaluronidase infusion in ApoE -/- knockout mice. Indeed, endothelial glycocalyx was significantly reduced in the active hyaluronidase treatment group, accompanied by increased vascular permeability for albumin. Moreover disruption of the carotid intima/lesion border was observed in these mice, which may imply the formation of a more unstable plaque phenotype compared to controls. In conclusion, these findings underscore the potential of GAG metabolism in the pathophysiology of atherosclerosis and warrant further exploration.

Role for glycocalyx in cardiovascular risk stratification

In view of the protective effects of the glycocalyx, it is tempting to speculate that, in analogy to e.g. intima media thickness as a surrogate for structural atherosclerotic changes, visualization of the glycocalyx may provide an attractive surrogate marker for subclinical atherosclerotic changes. However, measurements of endothelial glycocalyx are hampered by methodological difficulties. Most importantly, to gain acceptance as potential surrogate cardiovascular disease marker, the measurement should be reproducible and should predict cardiovascular events. At present, the three available techniques to estimate glycocalyx are all compromised by specific disadvantages (Table 1). In the first technique, systemic glycocalyx volume is measured using the glycocalyx permeable tracer Dextran 40, versus a glycocalyx impermeable tracer; fluorescein-labeled erythrocytes. The calculated difference between the two intravascular volumes that these tracers occupy renders a reasonable estimation of whole body glycocalyx volume. However, the invasive nature and the time consuming preparations precludes its use in larger cohorts. Moreover, criticism has arisen as to whether this method considers the endothelial glycocalyx as uniformly distributed throughout the vasculature. In spite of potential shortcomings, this technique may be of use in order to obtain a first indication of systemic glycocalyx dimensions in humans.

<table>
<thead>
<tr>
<th>Types of endothelial glycocalyx measurements</th>
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<th>Contra</th>
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<tr>
<td>Systemic glycocalyx volume</td>
<td>- Total body glycocalyx estimation</td>
<td>- Invasive technique</td>
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<tr>
<td></td>
<td>- Reproducible measurement</td>
<td>- Unsuitable for large cohorts</td>
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<tr>
<td></td>
<td>- Non invasive</td>
<td>- Indirect estimation of endothelial glycocalyx</td>
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<tr>
<td></td>
<td>- High throughput due to semi-automated analyses</td>
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<tr>
<td></td>
<td>- Reproducible</td>
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<tr>
<td>Microvascular glycocalyx (SDF)</td>
<td>Pathophysiological insight</td>
<td>High number of involved genomic genes (5%)</td>
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<td></td>
<td>High throughput</td>
<td>Lack of reliable ELISA antibodies for shedded glycocalyx components in plasma</td>
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<tr>
<td>Glycocalyx genes/biochemistry markers</td>
<td>Applicable in existing cohorts</td>
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Abbreviations: SDF, Sideview Darkfield imaging; CVD, CardioVascular Disease.
Second, the non-invasive, semi-automated imaging method to estimate glycocalyx thickness by OPS (Orthogonal Polarization Spectral imaging) or SDF (Sideview Darkfield imaging) imaging has been developed to image the superficial microvasculature. Based on the observation that the glycocalyx limits the proximity of erythrocytes to the capillary endothelial cells, the imaging method uses the erythrocyte-endothelium gap of the capillaries in the image to quantify glycocalyx thickness. In a pilot study, these estimations of glycocalyx thickness were shown to be reproducible and correlated with cardiovascular risk factors. Moreover, in this study systemic glycocalyx volume estimations were significantly correlated with microvascular glycocalyx, which seems to be reasonable as the majority of endothelial surface area is located in the microcirculation. Notwithstanding, it remains to be established whether measurements performed within the microcirculation accurately reflect macrovascular glycocalyx damage. Measurements in large patient cohorts are thus warranted to validate the value of this non-invasive method in cardiovascular risk stratification.

Finally, we observed that in a large cohort of DM1 patients' changes in glycocalyx biochemistry (e.g. plasma hyaluronan and hyaluronidase) were associated with carotid intima media thickness, an established marker of cardiovascular disease. These findings support the concept that glycocalyx perturbation leads to changes in plasma GAG products, which in turn are related to other markers of cardiovascular disease. However, it should be realized that glycocalyx derived carbohydrate structures are complex in structure and thus difficult to analyze. Recent developments in glycomics (including sensitive ELISAs, mass spectrometry and lectin based microarrays) will hopefully help elucidate differences in glycocalyx composition. Concomitantly, Rehm et al recently showed that glycocalyx perturbation was paralleled by increased plasma levels of 'shedded' syndecan-1 and GAG hepan sulphates in ischemia-reperfusion injury. These findings are in line with recently published experimental data showing that increased oxygen-radical stress induces glycocalyx damage following ischemia-reperfusion injury. In summary, a critical appraisal of glycocalyx degradation products in peripheral blood or urine is needed to identify the important partakers in atherosclerosis and to validate these parameters as potential biomarkers for (early) cardiovascular risk.

**Conclusion**

Studies investigating the role of endothelial glycocalyx in conditions associated with accelerated progression of atherosclerosis have consistently shown that injury to the glycocalyx may be an important step in the development of vascular dysfunction. More recently, novel approaches have been developed allowing for reproducible estimation of glycocalyx dimension in vivo. Validation and integration of these ‘imaging’ techniques with glycocalyx degradation products in plasma will allow us to test the concept of endothelial glycocalyx as a surrogate marker for atherosclerosis in patients with unexplained increased cardiovascular risk.
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