Structure and function of the endothelial glycocalyx in the microcirculation

Dane, M.J.C.

7

Summary and general discussion

Function and assessment of the endothelial glycocalyx

The endothelial glycocalyx (EG) covers the endothelium throughout the whole vasculature. Its strategic location, between the circulating blood and the endothelium with its underlying tissue, points towards a potentially important functional role for the glycocalyx. The layer is involved in almost all functions of the endothelium; it contributes to the permeability barrier, it protects against coagulation and inflammation and is involved in NO regulation, shear sensing and cell-cell signaling. Perturbation of this gel-like layer is associated with inflammation and various vasculature-related pathologies. Moreover, since the endothelial glycocalyx is in direct contact with pathogenic factors (e.g. elevated glucose, increased lipids and disturbed shear stresses) it has been proposed to be one of the first casualties during endothelial activation [1]. Consequently, alterations within the EG are proposed to be an early risk marker for vascular dysfunction. Monitoring changes is therefore proposed to identify early risk for cardiovascular disease. However, since it is difficult to keep the structure in its original in vivo state, visualization of this EG remains challenging.

In this thesis we studied the endothelial glycocalyx using a variety of visualization techniques. We showed an overview of the current techniques to visualize the endothelial glycocalyx in Chapter 2. By stimulating endothelial cells with prolonged shear stress, we aimed to mimic an in vivo situation and thereby stimulate EG production. With lectins and GAG-specific antibodies, we visualized compositional and dimensional changes within the endothelial glycocalyx after prolonged flow culture.

In chapter 4 we used thick freshly isolated kidney slices, to diminish the influence of processing techniques on EG structure. After staining with lectins and endothelial markers, we quantified the luminal endothelial glycocalyx thickness and density. In addition, we used electronic microscopy in combination with cupromeronic blue, a compound that stabilizes and stains the EG. This enables visualization of the exact location of the endothelial glycocalyx in high detail and revealed the presence of dense endothelial glycocalyx plugs within the endothelial fenestrae. Moreover, it revealed a structurally heterogeneous glycocalyx on top of the endothelial layer, depending most likely both on endothelial phenotype and location.

In chapter 5 and 6 we used a relatively new technique to noninvasively detect changes in the red blood cell exclusion properties of the endothelial glycocalyx, reflected by the perfused boundary region (PBR). By monitoring the sublingual vasculature with SDF imaging in human participants, this technique can be used to evaluate microvascular health and perhaps even predict cardiovascular risk. With the current technique, changes in EG in severely diseased patients have been detected noninvasively [2-4].

Structure and composition of the endothelial glycocalyx

In vitro, the endothelial glycocalyx lacks much of its barrier function when compared to the in vivo situation [5,6]. Furthermore, endothelial glycocalyx dimension is reduced in vitro [7]. Endothelial cells in culture normally lack exposure to continuous blood flow and in vivo studies revealed that a disturbed blood flow is associated with glycocalyx perturbation [8-10], while continuous laminar shear stress has been proposed to be involved in endothelial glycocalyx production [11-14]. We set out to determine the effect of prolonged shear stress on the endothelial surface glycocalyx in culture in Chapter 3. Particularly, we have focused on the role of prolonged shear stress on the compositional and functional changes in the luminal endothelial glycocalyx. We therefore reconstructed the endothelial layer to a 3-dimensional image and specifically quantified the luminal endothelial glycocalyx staining.

To determine overall changes in the endothelial glycocalyx, we used the lectin wheat germ agglutinin (WGA). We observed an increase in WGA intensity and thickness and found a more homogenous distribution of the WGA staining on top of the endothelial layer. Previously, WGA staining on endothelial cells was decreased in a model of spontaneous kidney failure in aged rats [15]. Consequently, the observed increase of WGA staining upon prolonged exposure to flow most likely reflects an improved glycocalyx structure.

Within the endothelial glycocalyx, heparan sulfates (HS) play a crucial role in signal transduction from the vasculature to the endothelium and its underlying tissues. In particular, variation in HS disaccharide sequence and sulfation patterns determines binding properties for circulating proteins [16,17]. Consequently, HS are involved in many cellular processes such as attachment, migration, differentiation, blood coagulation, lipid metabolism, and inflammation [16]. Therefore we also investigated HS compositional changes in the glycocalyx. For this, we used the HS-antibodies 10E4 and JM403. 10E4 has been shown to need mixed HS domains, containing both N-acetylated and N-sulfated disaccharide units, for binding [18]. In contrast, JM403 binding to HS depends on the presence of N-unsubstituted glucosamine [19]. In our study we found a marked increase of JM403 staining after 1 days of culture under flow. This coincided with an increase of IL-8 gene expression. After 7 days of flow culture, the JM403 staining was hardly present. Since its presence is relatively rare it has been proposed that the N-unsubstituted glucosamine residues contribute to selective protein binding and therefore in biological and pathological cell processes [20]. This is supported by the finding that these N-unsubstituted HS domains have been demonstrated to be involved in L-selectin binding [21]. The observed decrease in JM403 binding therefore indicates a reduction in pro-inflammatory HS epitopes within the endothelial glycocalyx.

The decreased adhesion of monocytes after blocking the epitope for JM403 binding not only supports this hypothesis, but also suggests a direct involvement of this HS epitope in binding of inflammatory cells. This study shows that prolonged shear stress induces

an anti-inflammatory glycocalyx/HS phenotype. Moreover, it shows that endothelial glycocalyx composition is changed upon a shift in environment, which adds an extra layer of regulation to cell-cell communication by changing the binding capacity for specific signalling- or adhesion proteins. Consequently, modification of the endothelial glycocalyx is a highly interesting target to fine-tune receptor-ligand interactions on the endothelium. This definitely underlines the necessity for studying HS modifications in endothelial function.

The endothelial glycocalyx as a barrier in glomerular filtration

Within the glomerulus, the glomerular filtration barrier (GFB) is the main site for filtration in the kidney. The GFB consists of endothelium with its glycocalyx, a glomerular basement membrane (GBM) and podocytes. Although it is well established that every single layer contributes to the glomerular filtration barrier (GFB), the exact function of the separate layers is still unknown. As early as 1976, Ryan and Karnovsky demonstrated that plasma albumin does not pass the highly fenestrated endothelial layer of the glomerular capillary wall [22]. The observation that the endothelial fenestrae, which are big enough to allow protein passage, were filled with endothelial glycocalyx indicates that this glycocalyx functions as a first barrier within the GFB [23,24].

In chapter 4 we demonstrated a role for the endothelial glycocalyx and more specifically the subcomponent hyaluronan in the glomerular filtration barrier. We show that glomerular fenestrae are filled with dense negatively charged polysaccharide structures. Upon infusion of hyaluronidase, albumin passage across the endothelium can be observed in almost all the glomeruli. Such albumin passage was not observed for the control animals. In conclusion, in this study we have shown that the glomerular EG functions as a selective protein permeability barrier. In support of this, endothelial glycocalyx disruption by displacing non-covalently bound proteins has been demonstrated to result in an acute increase of albuminuria [25]. Moreover, glycosaminoglycan-degrading enzymes such as chondroitinase and heparinase have also been shown to alter the charge selectivity of the glomerular filter [26,27]. In addition, in aged Munich-Wistar-Fromter rats, albuminuria associates with endothelial glycocalyx loss.

Although we used a model to specifically determine the role of hyaluronan in the glomerular filtration barrier, it is tempting to speculate about the implications of glycocalyx damage in the development of renal failure. Based on the presented data we suggest that damage of the endothelial glycocalyx results in very early damage in the glomerular filtration barrier. Consequently, during diseases in which glomerular failure is observed, glycocalyx damage might be one of the first changes upon exposure to risk factors. In diabetes, for example, an increase in heparanase and hyaluronidase has been demonstrated, most likely as a consequence of increased levels of risk factors such as hyperglycemia, and hyperlipidemia [28-31]. Moreover, both hyperglycemia and diabetes

have been associated with a reduction in endothelial glycocalyx volume [32,33]. As shown in our model, the perturbation of the ESL within the glomerular filtration barrier leads to protein leakage and binding (/uptake) by podocytes. This could initiate inflammatory signalling and contribute to podocyte transformation and effacement, as was postulated by Morigi et al. [34], and matches early activation of podocytes observed in our model. When prolonging the time of damage it seems to be reasonable to expect accumulation of damage leading to changes in podocyte morphology. Eventually loss of podocyte morphology leads to a severely damaged glomerular filtration barrier. Based on this model, we propose that the endothelial glycocalyx is the first casualty during prolonged risk factor exposure. Both the subsequent activation of endothelial cells, as the passage of proteins over the filtration barrier, leads to a disturbed cell-cell signalling within the GFB which can result in activation of endothelial cells, podocytes and mesangium (**figure 1**). However, although albumin passes the filtration barrier during this stage, we hypothesize that albuminuria only develops when chronic activation results in structural damage, such as podocyte flattening, mesangial expansion and loss of endothelial fenestrae [35].

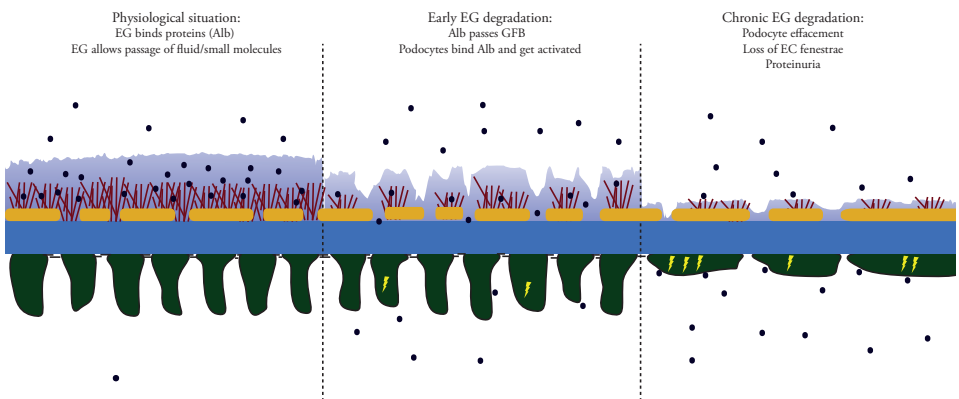

Figure 1: Schematic overview of the hypothesized mechanism of glomerular barrier failure. *During a healthy situation the glycocalyx covers the glomerular endothelium and fills the fenestrae. Proteins, such as albumin, are adsorbed by the endothelial glycocalyx. In this way they form a barrier for circulating plasma proteins, which are consequently unable to cross the filtration barrier. In contrast, the gel-like glycocalyx allows passage of smaller molecules and fluids, thus facilitating glomerular filtration (left). During early endothelial glycocalyx modification and degradation, less protein can be bound and proteins can pass the filtration barrier. Here, proteins can bind to podocytes, parietal epithelial cells and tubular epithelial cells. These cells get activated as a result of binding and uptake of proteins (middle). Upon chronic EG degradation, proteins continuously pass the filtration barrier. As a result, podocytes are chronically activated and effacement will occur. Loss of the glomerular barrier structure will eventually result in large quantities of proteins passing the glomerular filtration barrier which leads to glomerular and kidney failure (right).*

The observation that albuminuria in aged Munich Wistar Fromter rats associates with glycocalyx loss, suggests that widespread loss of the endothelial glycocalyx might link albuminuric kidney disease with systemic vascular dysfunction. Based on our data and these earlier studies, we propose that endothelial glycocalyx dysfunction is a central mediator in the development of both renal and cardiovascular failure [15,36]. This is supported by the Steno hypothesis, proposed by Deckert et al in 1989 [37]. In the Steno hypothesis, perturbation of the extracellular matrix, more specifically of the HS within this matrix, was proposed to be a key factor in the development of both renal failure and cardiovascular disease. We propose a revised hypothesis: Perturbation of the luminal extracellular matrix, thus endothelial glycocalyx, is the central mediator for the development of both renal and cardiovascular failure. More detailed studies, for example in a model in which one could follow both the development of glycocalyx damage as the effects on macrovascular- and renal pathologies in time, are of utmost importance to study this hypothesis. When this hypothesis can be supported by future studies, the endothelial glycocalyx might become a crucial target for therapeutics to prevent vascular damage and the subsequent risk for, among others, CVD and renal disease.

Estimating endothelial glycocalyx changes in patients using SDF imaging

As discussed in chapter 2, it is difficult to measure glycocalyx dimensions in patients. Until recently, shed syndecan-1 has been used, since glycocalyx shedding (e.g. in inflammatory conditions) is thought to contribute to plasma levels of soluble syndecan-1 [38]. However, the exact underlying mechanisms and dynamics of proteoglycan shedding and its link with various vascular pathologies are largely unknown, making it difficult to interpret this parameter. Moreover, the absence of shedding of one specific proteoglycan might not per definition mean that endothelial glycocalyx properties are unaltered. In chapter 5 we used and validated a newly developed technique to estimate endothelial glycocalyx changes in a fast and non-invasive manner. We used this automated, and easy to apply approach to measure the ability of RBC to penetrate the ESL, quantitatively defined as the perfused boundary region (PBR) [3,39]. Overall, measuring PBR shows to be an easier and less invasive method than measuring shed markers like syndecan and thrombomodulin. However, more studies are necessary to study the selectivity and sensitivity of the PBR as a marker for early vascular damage.

In chapter 5 we have shown that patients with an impaired kidney function have a larger PBR, which reflects a loss of endothelial surface layer dimensions. Also, ESL dimensions in patients with a stable kidney transplant were indistinguishable from healthy controls, while patients developing IFTA (Interstitial Fibrosis and Tubular Atrophy) revealed a larger PBR, i.e. ESL loss. These changes in PBR are reflected by the presence of the shed ESL components sTM and syndecan-1.

Previously, renal failure was shown to correlate with increased concentrations of shed hyaluronan [3], and release of the endothelium specific proteoglycan thrombomodulin coincides with diabetes and diabetic nephropathy [40,41]. Here, we showed that patients with renal failure have increased circulating levels of syndecan-1, which could be reversed by kidney transplantation. In addition a correlation between PBR, the glycocalyx shedding markers sTM and syndecan-1 and the endothelial activation marker Ang2 was observed. This also validates both glycocalyx measurements within this study.[42]

Plasma and serum biomarkers and PBR measurements corroborate the observation that loss of renal function is associated with endothelial activation and loss of ESL thickness. Furthermore, we show that we are able to discriminate between a group of participants with and without renal failure, thereby validating the PBR measurements. Although the causality still needs further study, the association of glycocalyx damage, renal failure and endothelial activation shown in this study further supports the earlier proposed role for endothelial glycocalyx perturbation as a central mediator in the development of both renal failure and CVD.

In Chapter 6, we set up a study to try to answer the question whether endothelial glycocalyx damage is predictive for the development of vasculature related pathologies. The NEO

study, described in this chapter, is a prospective cohort study of 6,673 individuals aged between 45 and 65 years, to study the mechanisms underlying the development of disease in obesity. Among others, we have estimated the ESL thickness in these participants as baseline measurement for future studies. Although in general this population is yet only at risk for cardiovascular disease, due to oversampling of BMI > 25, a high variation in PBR was observed. Nonetheless, no correlations with other known risk markers were observed, which might be explained by the observed high inter-individual variation. Future follow-up of this study cohort, allowing events to occur, should address whether PBR associates with the development of cardiovascular disease.

Within the baseline measurements of the NEO study we did observe an association between changes in endothelial glycocalyx properties (PBR) and estimates of microvascular perfusion in the sublingual microcirculation. The strongest association was observed between PBR and RBC filling percentage, which represents the microvascular perfusion changes over time. Although this population is only at risk for cardiovascular disease, the high variation in PBR and negative correlation of PBR with perfusion estimates might indicate the onset of the development of vascular dysfunction in a subset of these participants.

In conclusion, changes in the ESL have been postulated to associate with, and maybe even precede, vascular and renal damage [1]. In this thesis we confirm a correlation between glycocalyx dimensions, perfusion, renal failure and vascular activation. Because measuring PBR in the microcirculation is a non-invasive and fast method to assess changes in the ESL in patients, this is a promising new method for clinical monitoring of the systemic microvasculature. Furthermore, follow-up of the NEO study cohort should address whether PBR associates with, or might even predict the development of cardiovascular disease.

Conclusion and future perspectives

The endothelial glycocalyx is a highly interactive matrix that facilitates protein-protein and protein-receptor interactions and thereby serves as a key modifier of endothelial function. Both structural dimension as well as biochemical composition determines its properties as a bioactive scaffold.

The structure and modifications of carbohydrates are highly complex and exceed the complexity of proteins and genes. Studying glycocalyx composition has proven very challenging due to the structural fragility and the complex sugar biochemistry involved. However, modern imaging technology, as discussed in this thesis, opens up opportunities to take the glycocalyx into account when studying vascular and renal physiology. While it is still technically impossible to unequivocally chemically characterize HS and HA variability, most of our knowledge on disaccharide modification - function relationships needs to be derived from experiments where enzymes that edit the chemical composition of the glycocalyx are conditionally knocked out. The development of new methods to study the exact structure and composition of this luminal endothelial glycocalyx is therefore of key importance to obtain more insight in the endothelial glycocalyx function.

Together with earlier published data [43], our data in chapter 4 and 5 points towards a central role for endothelial glycocalyx perturbations in the development of both cardiovascular disease and renal failure. Consequently, the role of endothelial glycocalyx damage in development of vascular related pathologies needs to be further studied. The development of the SDF camera might enable us to study glycocalyx perturbations in large cohorts. For now, structural dimensions of the glycocalyx have been measured using SDF imaging in severely diseased patients [3,44,45]. However, to be able to detect early glycocalyx damage and thus predict the development of cardiovascular disease, further development and optimization of this technique is required.

Altogether, development of further insight in structure-function relationships of the glycocalyx will create new perspectives in our understanding of physiology and provide opportunities to innovate medicine. Development of technological innovations that enable the further interrogation and the modulation of glycocalyx function will prove to be a critical success factor.

References

1. Rabelink TJ, de Boer HC, van Zonneveld AJ (2010) Endothelial activation and circulating markers of endothelial activation in kidney disease. Nat Rev Nephrol 6: 404-414.
2. Donati A, Damiani E, Domizi R, Romano R, Adrario E, et al. (2013) Alteration of the sublingual microvascular glycocalyx in critically ill patients. Microvasc Res.
3. Vlahu CA, Lemkes BA, Struijk DG, Koopman MG, Krediet RT, et al. (2012) Damage of the endothelial glycocalyx in dialysis patients. J Am Soc Nephrol 23: 1900-1908.
4. Mulders TA, Nieuwdorp M, Stroes ES, Vink H, Pinto-Sietsma SJ (2013) Non-invasive assessment of microvascular dysfunction in families with premature coronary artery disease. Int J Cardiol 168: 5026-5028.
5. Potter DR, Damiano ER (2008) The hydrodynamically relevant endothelial cell glycocalyx observed in vivo is absent in vitro. Circ Res 102: 770-776.
6. Smith ML, Long DS, Damiano ER, Ley K (2003) Near-wall micro-PIV reveals a hydrodynamically relevant endothelial surface layer in venules in vivo. Biophys J 85: 637-645.
7. Chappell D, Jacob M, Paul O, Rehm M, Welsch U, et al. (2009) The glycocalyx of the human umbilical vein endothelial cell: an impressive structure ex vivo but not in culture. Circ Res 104: 1313-1317.
8. van Thienen JV, Fledderus JO, Dekker RJ, Rohlena J, van Ijzendoorn GA, et al. (2006) Shear stress sustains atheroprotective endothelial KLF2 expression more potently than statins through mRNA stabilization. Cardiovasc Res 72: 231-240.
9. Dekker RJ, van Thienen JV, Rohlena J, de Jager SC, Elderkamp YW, et al. (2005) Endothelial KLF2 links local arterial shear stress levels to the expression of vascular tone-regulating genes. Am J Pathol 167: 609-618.
10. Dekker RJ, van Soest S, Fontijn RD, Salamanca S, de Groot PG, et al. (2002) Prolonged fluid shear stress induces a distinct set of endothelial cell genes, most specifically lung Kruppel-like factor (KLF2). Blood 100: 1689-1698.
11. Ebong EE, Lopez-Quintero SV, Rizzo V, Spray DC, Tarbell JM (2014) Shear-induced endothelial NOS activation and remodeling via heparan sulfate, glypican-1, and syndecan-1. Integr Biol (Camb) 6: 338-347.
12. Koo A, Dewey CF, Jr., Garcia-Cardena G (2013) Hemodynamic shear stress characteristic of atherosclerosis-resistant regions promotes glycocalyx formation in cultured endothelial cells. Am J Physiol Cell Physiol 304: C137-146.
13. Gouverneur M, Spaan JA, Pannekoek H, Fontijn RD, Vink H (2006) Fluid shear stress stimulates incorporation of hyaluronan into endothelial cell glycocalyx. Am J Physiol Heart Circ Physiol 290: H458-452.
14. Bai K, Wang W (2013) Shear stress-induced redistribution of the glycocalyx on endothelial cells in vitro. Biomech Model Mechanobiol.
15. Salmon AH, Ferguson JK, Burford JL, Gevorgyan H, Nakano D, et al. (2012) Loss of the endothelial glycocalyx links albuminuria and vascular dysfunction. J Am Soc Nephrol 23:1339-1350.
16. Esko JD, Selleck SB (2002) Order out of chaos: assembly of ligand binding sites in heparan sulfate. Annu Rev Biochem 71: 435-471.
17. Rops AL, Jacobs CW, Linssen PC, Boezeman JB, Lensen JF, et al. (2007) Heparan sulfate

on activated glomerular endothelial cells and exogenous heparinoids influence the rolling and adhesion of leucocytes. Nephrol Dial Transplant 22: 1070-1077.
18. van den Born J, Salmivirta K, Henttinen T, Ostman N, Ishimaru T, et al. (2005) Novel heparan sulfate structures revealed by monoclonal antibodies. J Biol Chem 280: 20516-20523.
19. van den Born J, Gunnarsson K, Bakker MA, Kjellen L, Kusche-Gullberg M, et al. (1995) Presence of N-unsubstituted glucosamine units in native heparan sulfate revealed by a monoclonal antibody. J Biol Chem 270: 31303-31309.
20. Wei Z, Deakin JA, Blaum BS, Uhrin D, Gallagher JT, et al. (2011) Preparation of heparin/ heparan sulfate oligosaccharides with internal N-unsubstituted glucosamine residues for functional studies. Glycoconj J 28: 525-535.
21. Norgard-Sumnicht K, Varki A (1995) Endothelial heparan sulfate proteoglycans that bind to L-selectin have glucosamine residues with unsubstituted amino groups. J Biol Chem 270:12012-12024.
22. Ryan GB, Karnovsky MJ (1976) Distribution of endogenous albumin in the rat glomerulus: role of hemodynamic factors in glomerular barrier function. Kidney Int 9: 36-45.
23. Rostgaard J, Qvortrup K (2002) Sieve plugs in fenestrae of glomerular capillaries--site of the filtration barrier? Cells Tissues Organs 170: 132-138.
24. Rostgaard J, Qvortrup K (1997) Electron microscopic demonstrations of filamentous molecular sieve plugs in capillary fenestrae. Microvasc Res 53: 1-13.
25. Friden V, Oveland E, Tenstad O, Ebefors K, Nystrom J, et al. (2011) The glomerular endothelial cell coat is essential for glomerular filtration. Kidney Int 79: 1322-1330.
26. Jeansson M, Haraldsson B (2003) Glomerular size and charge selectivity in the mouse after exposure to glucosaminoglycan-degrading enzymes. J Am Soc Nephrol 14: 1756-1765.
27. Jeansson M, Haraldsson B (2006) Morphological and functional evidence for an important role of the endothelial cell glycocalyx in the glomerular barrier. Am J Physiol Renal Physiol 290: F111-116.
28. Ikegami-Kawai M, Suzuki A, Karita I, Takahashi T (2003) Increased hyaluronidase activity in the kidney of streptozotocin-induced diabetic rats. J Biochem 134: 875-880.
29. Gil N, Goldberg R, Neuman T, Garsen M, Zcharia E, et al. (2012) Heparanase is essential for the development of diabetic nephropathy in mice. Diabetes 61: 208-216.
30. Wijnhoven TJ, van den Hoven MJ, Ding H, van Kuppevelt TH, van der Vlag J, et al. (2008) Heparanase induces a differential loss of heparan sulphate domains in overt diabetic nephropathy. Diabetologia 51: 372-382.
31. Garsen M, Rops AL, Rabelink TJ, Berden JH, van der Vlag J (2014) The role of heparanase and the endothelial glycocalyx in the development of proteinuria. Nephrol Dial Transplant 29: 49-55.
32. Nieuwdorp M, van Haeften TW, Gouverneur MC, Mooij HL, van Lieshout MH, et al. (2006) Loss of endothelial glycocalyx during acute hyperglycemia coincides with endothelial dysfunction and coagulation activation in vivo. Diabetes 55: 480-486.
33. Nieuwdorp M, Mooij HL, Kroon J, Atasever B, Spaan JA, et al. (2006) Endothelial glycocalyx damage coincides with microalbuminuria in type 1 diabetes. Diabetes 55: 1127-1132.
34. Morigi M, Buelli S, Angioletti S, Zanchi C, Longaretti L, et al. (2005) In response

to protein load podocytes reorganize cytoskeleton and modulate endothelin-1 gene: implication for permselective dysfunction of chronic nephropathies. Am J Pathol 166: 1309-1320.

35. Pavenstadt H, Kriz W, Kretzler M (2003) Cell biology of the glomerular podocyte. Physiol Rev 83: 253-307.
36. Salmon AH, Satchell SC (2012) Endothelial glycocalyx dysfunction in disease: albuminuria and increased microvascular permeability. J Pathol 226: 562-574.
37. Deckert T, Feldt-Rasmussen B, Borch-Johnsen K, Jensen T, Kofoed-Enevoldsen A (1989) Albuminuria reflects widespread vascular damage. The Steno hypothesis. Diabetologia 32:219-226.
38. Bartlett AH, Hayashida K, Park PW (2007) Molecular and cellular mechanisms of syndecan in tissue injury and inflammation. Mol Cells 24: 153-166.
39. Martens RJ, Vink H, van Oostenbrugge RJ, Staals J (2013) Sublingual microvascular glycocalyx dimensions in lacunar stroke patients. Cerebrovasc Dis 35: 451-454.
40. Iwashima Y, Sato T, Watanabe K, Ooshima E, Hiraishi S, et al. (1990) Elevation of plasma thrombomodulin level in diabetic patients with early diabetic nephropathy. Diabetes 39:983-988.
41. Oida K, Takai H, Maeda H, Takahashi S, Tamai T, et al. (1990) Plasma thrombomodulin concentration in diabetes mellitus. Diabetes Res Clin Pract 10: 193-196.
42. Dane MJ, Khairoun M, Lee DH, van den Berg BM, Eskens BJ, et al. (2014) Association of kidney function with changes in the endothelial surface layer. Clin J Am Soc Nephrol 9: 698-704.
43. Nagy N, Freudenberger T, Melchior-Becker A, Rock K, Ter Braak M, et al. (2010) Inhibition of hyaluronan synthesis accelerates murine atherosclerosis: novel insights into the role of hyaluronan synthesis. Circulation 122: 2313-2322.
44. Dane MJC, van den Berg BM, Avramut MC, Faas FGA, van der Vlag J, et al. (2013) Glomerular Endothelial Surface Layer Acts as a Barrier against Albumin Filtration. American Journal of Pathology 182: 1532-1540.
45. Lee DH, Dane MJ, van den Berg BM, Boels MG, van Teeffelen JW, et al. (2014) Deeper penetration of erythrocytes into the endothelial glycocalyx is associated with impaired microvascular perfusion. PLoS One 9: e96477.


7