The endothelial surface layer: a new target of research in kidney failure and peritoneal dialysis

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The endothelial surface layer: a new target of research in kidney failure and peritoneal dialysis

This thesis reports investigations aimed at defining the state of the endothelial glycocalyx in chronic kidney failure. More specifically, in part I we investigated the state of the endothelial surface layer in patients with end-stage renal disease, and after successful kidney transplantation. In part II we focused on the role of the endothelial surface layer in peritoneal dialysis. For this, we assessed the peritoneal microvascular endothelial glycoalyx, and investigated the relationships with peritoneal transport. In part III we described the peritoneal alterations induced by long-term exposure to high glucose concentration dialysis solutions in an experimental model of chronic kidney disease.

Chapter 1 gives a summary of the endothelial glycocalyx structure and functions, followed by a description of the assessment methods which are currently available in humans. Then, we give an overview of chronic kidney failure and peritoneal dialysis as method of renal replacement therapy. Patients with chronic kidney failure form a high risk patient group because of the high prevalence of cardiovascular disease which is associated with their condition. The uremic milieu is associated with increased oxidative stress, a chronic inflammatory state, decreased availability of antioxidant systems and hypervolemia, which all contribute to the vascular endothelial dysfunction and may impact the endothelial glycoalyx. Through its numerous vasculoprotective functions, the endothelial glycoalyx is essential in maintaining vascular integrity, and its alteration will increase endothelial vulnerability. The gap in the current knowledge on the state of the endothelial surface layer in kidney disease has led to the investigations performed in chapter 2. In patients with ESRD treated with hemodialysis or peritoneal dialysis, we used Sidestream Darkfield imaging of the sublingual microcirculation, to measure the perfused boundary region (PBR), which reflects the erythrocyte-permeable part of the endothelial surface layer. The results were compared with those from healthy individuals. Both HD and PD patients had a thicker PBR and larger perfused diameters in the sublingual microcirculation, indicating a change in the RBC-excluding properties of the endothelial surface layer. The results were compared with those from healthy individuals. Both HD and PD patients had a thicker PBR and larger perfused diameters in the sublingual microcirculation, indicating a change in the RBC-excluding properties of the endothelial surface layer. In addition, dialysis patients had higher plasma concentrations of glycoalyx constituents hyaluronan, syndecan-1, and increased hyaluronidase activity compared with healthy individuals. Higher plasma concentrations of C-reactive protein in dialysis patients were associated with a thicker erythrocyte-permeable part of the endothelial surface layer. Anuric patients had higher plasma hyaluronan concentrations and reduced hyaluronidase activity compared with patients with residual renal function. Interestingly, the presence of cardiovascular disease was not associated with additional changes in any of the study parameters. In conclusion, by analyzing the dynamic variations of erythrocyte column width in the sublingual microcirculation, we showed that dialysis patients, treated with either HD or PD, have changes in glycoalyx barrier properties.
These patients have high concentrations of hyaluronan and syndecan-1 in blood, but the extent to which these are caused by shedding from the endothelial surface layer, has not been determined. The role of kidney function in their removal from circulation should be considered as well, and should be carefully investigated.

In chapter 3, we used similar methods to assess the endothelial surface layer in renal transplant recipients with stable graft function. Successful kidney transplantation is associated with a survival benefit compared to patients treated with dialysis. Transplantation diminishes inflammation, oxidative stress and uremia-related complications, and therefore might impact the endothelial glycocalyx. In renal transplant recipients, the erythrocyte-permeable region of the endothelial surface layer was thicker compared with healthy controls, and the magnitude of this alteration was dependent on graft function. In addition, plasma hyaluronan concentrations were similar to those in controls, and only modest increases in plasma syndecan-1 and hyaluronidase activity were found in patients. In conclusion, successful kidney transplantation may be beneficial and induce normalization of the turnover within the endothelial glycocalyx. The restoration of the endothelial surface layer barrier properties is dependent on graft function.

Measurements of plasma concentrations of glycocalyx constituents provide an indirect tool for the assessment of the endothelial glycocalyx. Because of its presumed vasculo-protective effects within this layer, hyaluronan, together with the regulating enzyme hyaluronidase, have been described in literature, but almost never from a clinical point of view and without much focus on renal function. In chapter 4, we addressed the utility of plasma hyaluronan levels and hyaluronidase activity as potential markers for endothelial glycocalyx damage in patients with chronic kidney failure. Hyaluronic acid is an important constituent of the extracellular matrix and the endothelial glycocalyx. It is a negatively charged, unsulfated GAG, synthesized at the cytosolic side of the cell membrane by hyaluronan synthases. The major part of hyaluronan has a molecular weight exceeding 1000 kD, but small amounts of low molecular weight HA with pro-inflammatory and pro-angiogenic effects, can also be found. Hyaluronidase-2 activity may be important for the composition of the endothelial glycocalyx due to its localization at the outer cell membrane. In patients with kidney failure, plasma levels of hyaluronan are increased. However, the precise mechanism is unknown, and also, mutual relationships with hyaluronidase have not been investigated. Consequently, correct interpretation of a high plasma hyaluronan concentration without other determinations is impossible.

The second part of the thesis focuses on the importance of endothelial glycocalyx in peritoneal dialysis. During PD various solutes and water are transported from the circulation into the peritoneal cavity through the peritoneum. The peritoneal membrane
Summary and conclusions

consists of three main anatomical layers: the capillary wall, the interstitium and the mesothelium, the former creating the main resistance to transport. Endothelial permeability is partly dependent on the negatively charged glycocalyx that extends into the interendothelial clefts. In chapter 5 we investigated the relation between the state of the endothelial surface layer assessed in the sublingual microcirculation, and parameters of peritoneal transport in stable PD patients. A fast solute transport status was defined based on glucose absorption and the mass transfer coefficient of creatinine and urate. This condition is associated with rapid disappearance of the osmotic gradient, thereby impairing efficient dialysis. We found no relation between the imaging parameters and peritoneal transport parameters neither in the group as a whole, nor in patients with a fast transport status. However, in patients with non-fast transport status, peritoneal transport parameters were related to the perfused boundary region: the thicker the erythrocyte-permeable part of the endothelial surface layer in the sublingual vasculature, the lower the transport rates of small solutes and the higher the net ultrafiltration through the peritoneal membrane. The interpretation of these findings is challenging. Besides the relationships between perfused boundary region, blood vessel density and solute transport, which can explain the results, an increased diffusion distance is one of the possibilities. Unlike the systemic endothelial glycocalyx, peritoneal endothelial cells are exposed to high glucose concentrations, which may result in specific alterations of the peritoneal endothelial glycocalyx. Therefore, the measurements performed in the sublingual microvasculature may not reflect the peritoneal microvasculature, and subsequently, the relationships between peritoneal transport and the peritoneal endothelial glycocalyx may be different. SDF imaging of the peritoneal microcirculation in PD patients is currently impossible.

In chapter 6 we therefore attempted to address this question in a rat model of chronic kidney failure and exposure to dialysis solutions. In contrast to the data in patients, the PBR thickness in the peritoneal microcirculation was similar in rats with chronic kidney disease (CKD), rats with CKD exposed to dialysis solutions, and rats with normal kidney function. The exposure to dialysis fluids was associated with higher peritoneal microvascular densities. Relationships were present between small solute transport and peritoneal microvascular density, and PBR. A thicker erythrocyte-permeable region of the endothelial surface layer was associated with higher transport rates for small solutes. Taken together, our findings confirm our hypothesis that specific changes occur in the peritoneal microcirculation during PD, that are not captured by the assessment of the sublingual vasculature. Plasma concentrations of syndecan-1 were increased in rats with CKD and also in PDF exposed rats, and were associated with renal function. In order to gain insight into the biochemical alterations that occur within the endothelial glycocalyx, we performed immunostaining of heparan sulfate (HS) 10E4 epitope and syndecan-1, a proteoglycan to which both HS and chondroitin sulfate attach. HS 10E4 had predomi-
nantely a basolateral distribution and syndecan-1 was expressed in the interendothelial junction, nucleus, and the cytoplasmic compartment. Syndecan-1 expression in interendothelial junctions was decreased in rats with CKD, whereas in rats exposed to peritoneal dialysis fluids, the expression was similar to that in rats with normal kidney function. No relationships with peritoneal solute transport were present. Taken together, our findings suggest that the endothelial surface layer may be important in peritoneal transport during PD. The localization of syndecan-1 and HS 10E4 epitope precludes an essential role for these molecules in the establishment of the peritoneal endothelial glycocalyx at the luminal side.

In the third part of this thesis, we made an extensive analysis of the peritoneal membrane alterations induced by long term exposure to either a conventional or a biocompatible dialysis solution, both with the same high glucose concentration. For this, we used the above mentioned model of rats with chronic failure exposed to dialysis solutions for 16 weeks. In contrast to the current literature on this topic, we used specific endothelial markers and applied new methodologies, such as unbiased stereology, for the analysis. This is currently considered to be the best-practice method for quantitative histology as it enables high accuracy of the results. In chapter 7, we report that chronic kidney failure itself induces mesothelial alterations, fibrosis, angiogenesis, lymphangiogenesis and these are partially augmented by exposure to dialysis solutions. Interestingly, the biocompatible dialysis solution seemed to induce less fibrosis than the conventional one, and different mesothelial cell phenotypes were associated with the two solutions. However, contrary to published data from various experimental models of peritoneal exposure, we could not show a beneficial effect of the biocompatible solutions with regard to neither morphologic parameters such as neoangiogenesis, nor peritoneal transport. Our findings are consistent with the results of clinical studies, which showed no definite advantage of biocompatible dialysis solutions on transport parameters. Lymphangiogenesis developed during CKD and in rats with CKD exposed to dialysis solutions, and was associated with fibrosis. In chapter 8, we showed that new lymph vessel formation and the effective lymphatic reabsorption rate, a functional parameter measured during a standard peritoneal permeability analysis, are both increased in CKD, and are positively related.
Conclusions

The endothelial glycocalyx is an important regulator of vascular homeostasis, and damage to this complex structure results in increased vascular vulnerability. Because of its vasculoprotective effects, the endothelial glycocalyx is highly relevant in the context of high vascular risk conditions, such as chronic kidney failure. Patients with end-stage renal disease have endothelial dysfunction and are at high risk to develop cardiovascular disease. The changes in the erythrocyte-excluding properties of the endothelial surface layer in the sublingual microcirculation together with the high plasma concentrations of glycocalyx constituents indicate that alterations of the endothelial glycocalyx occur in dialysis patients, regardless of the type of dialysis treatment they receive. Successful renal transplantation may have a beneficial effect on the endothelial glycocalyx and the restoration of the endothelial surface layer RBC-excluding properties is dependent upon graft function. Future studies should also address the effects of various immunosuppressive therapies on the ESL in this condition.

A critical appraisal of changes in plasma concentrations of glycocalyx constituents should be done to establish their value as markers for the endothelial glycocalyx, taking the role of the kidneys in their clearance from circulation into account.

The exposure of the peritoneal vasculature to high glucose concentration during peritoneal dialysis results in specific changes in the endothelial surface layer in the peritoneal microcirculation that are not reflected in the sublingual vasculature. Relationships are present between peritoneal small solute transport parameters and the thickness of the erythrocyte-permeable region of the endothelial surface layer, supporting a role for the endothelial glycocalyx in peritoneal transport. Because of their localization, the relevance of both syndecan-1 and HS10E4 for luminal endothelial glycocalyx seems to be limited. The expression of syndecan-1 in the peritoneal microvasculature changes in chronic kidney disease and after long-term exposure to glucose-based dialysis solutions and its role in peritoneal neoangiogenesis and fibrosis should be addressed in future studies. The expression of different glycocalyx constituents, like glypicans and other glycocalyx constituents, such as other glycosaminoglycans or additional heparan sulfate epitopes, may offer more insight into the biochemical composition of the peritoneal endothelial glycocalyx during peritoneal dialysis.

Chronic kidney disease itself induces peritoneal alterations, and these are partly augmented by exposure to dialysis solutions with high concentrations of glucose. The use of a biocompatible dialysis solution is likely beneficial with regard to the development of fibrosis, despite identical glucose concentrations compared to the conventional one. Specific endothelial markers and new methodologies, such as stereological methods that allow for unbiased and accurate morphological data, are currently available and should be used for quantitative histology.
General discussion

The endothelial glycocalyx in chronic kidney failure

The aim of this thesis was to gain insight in the alterations of the endothelial glycocalyx that occur in chronic kidney failure. Damage to the endothelial glycocalyx results in increased vascular vulnerability and therefore, this layer is of major importance in the setting of high vascular risk conditions such as chronic kidney failure. Non-invasive assessment of the endothelial surface layer in humans is currently possible by using Side-stream darkfield imaging of the sublingual microcirculation. In normal conditions, the presence of the glycocalyx limits the access of erythrocytes towards the endothelial cells. Based on experimental studies, the concept was formulated that alterations of the glycocalyx are associated with perturbation of its erythrocyte-excluding properties, reflected by the variation in the RBC column width, from which the perfused boundary region (PBR) is calculated. This parameter reflects the part of the endothelial surface layer that is permeable to red blood cells and therefore, it is not a measure of the anatomic thickness of this structure. The ability to restrict red blood cells depends on the complex organization and interactions between glycocalyx components. Importantly a normal thickness of the perfused boundary region does not exclude changes in the endothelial glycocalyx structure or permeability to various solutes. This was demonstrated by Henry et al who showed that treatment of hamster cremaster muscle with hyaluronidase resulted in increased permeability for various macromolecules but did not affect the access of the red blood cells into the glycocalyx. Both dialysis patients and renal transplant recipients have a thicker perfused boundary region in the sublingual microcirculation, suggesting that changes in the erythrocyte excluding properties of the glycocalyx occur in these patients. In a given condition, the changes in the endothelial surface layer reflect the combination of damage to the endothelial glycocalyx and the adaptive response of the endothelium. In the setting of chronic kidney failure, the oxidative stress, proinflammatory mediators and overhydration may all induce damage to the glycocalyx. The endothelial response may translate into increased expression of certain glycocalyx constituents. This is probably the case for hyaluronan (HA) in chronic kidney failure. Chronic kidney disease stage 4 and 5 is associated with increased plasma concentrations of hyaluronan and adhesion molecules, and relationships are present between these parameters. It is unknown if an increased plasma HA reflects a higher hyaluronan content of the microvascular glycocalyx or shedding from this structure into circulation. The endothelial synthesis of HA is triggered by pro-inflammatory cytokines which also increase expression of various adhesion molecules. High molecular weight hyaluronan is synthesized and will interact with CD44 present on both endothelium and immune cells, contributing to inflammation. The precursor availability is also of importance for HA synthesis, especially in hyperglycemic conditions. Renal failure is also associated with increased plasma concentrations of proinflammatory mediators and adhesion molecules, which makes it likely that in this condition hyaluronan synthesis is upregulated.
Hyaluronan is very hydrophilic and attracts water. Because of its length of several microns and its ability to bind water, hyaluronan is thought to have an essential contribution to the glycocalyx volume. Goa et al. investigated the composition of the endothelial glycocalyx and its relation to its thickness and diffusion of small solutes, in rat mesentery. The authors used techniques of macromolecule exclusion to measure the glycocalyx thickness, and the diffusion of the fluorescent dye FITC to assess the permeability of the glycocalyx to small solutes. It appeared that various GAGs are unevenly distributed throughout the endothelial glycocalyx. Hyaluronan and chondroitin sulfate (CS) are distributed towards the endothelial cell membrane whereas heparan sulfate is mainly present in the most luminal part of the glycocalyx. The measurements of diffusion coefficients of FITC indicated the presence of a more compact, denser sublayer adjacent to the endothelial cell membrane, in which the diffusion of small solutes is hindered compared to the more luminal part of the glycocalyx where loss of distal GAGs into the circulation occur. HA seems to contribute to the formation of this denser sublayer near the endothelial cell surface. Treatment with hyaluronidase decreased the thickness of the barrier to dextran 70 and increased the diffusion coefficient of small solutes in both the luminal part of the glycocalyx and in the more compact part adjacent to the cell membrane. The contribution of each GAG to the permeability of the endothelial glycocalyx varies. Heparan sulfate seems to provide the structural support of the upper part of the glycocalyx, whereas CS and HA contribute significantly to glycocalyx permeability to solutes.

It follows from the above that renal failure probably leads to an increase of the hyaluronan content of the microvascular glycocalyx. This would contribute to the formation of the denser sublayer on the microvascular luminal surface, which hinders the diffusion of small solutes. This is of interest especially in the setting of peritoneal dialysis where small solutes diffuse from the circulation into the interstitial space and peritoneal cavity.

**The endothelial surface layer in the peritoneal microcirculation**

The endothelial glycocalyx in peritoneal dialysis may be of importance for peritoneal transport, but also for the development of peritoneal membrane alterations, such as fibrosis and angiogenesis. The current knowledge on the state of the peritoneal endothelial glycocalyx is limited and needs to be expanded. In contrast to the situation in the sublingual vasculature, the endothelial glycocalyx in the peritoneal microvasculature is exposed not only to the uremic milieu present at the luminal side, but also to the high glucose concentration present in the peritoneal cavity. Whereas physiologic glucose concentrations may provide a substrate for glycocalyx synthesis, extremely high glucose concentrations are associated with generation of reactive oxygen species, which would add to the uremia-associated harmful stimuli and may result in additional glycocalyx damage. It is possible that the increased HA content in the endothelial glycocalyx
induced by chronic kidney failure is overruled by the harmful effect of high glucose concentrations to which the peritoneal vasculature is exposed. In contrast to the thicker PBR measured in the sublingual vasculature of PD patients, the PBR in the peritoneal microcirculation of rats with kidney failure exposed to dialysis solutions was not different from the control group. Our results suggest that specific changes occur in the peritoneal endothelial glycocalyx. However, the peritoneal measurements were done in rats and therefore the difference in species as cause of this dissimilarity, cannot be ruled out. Importantly, as mentioned previously, the unaltered thickness of the erythrocyte-permeable region of the endothelial surface layer does not exclude the presence of glycocalyx alterations in the peritoneal microcirculation. The experiments that we report here were aimed to gain insight into the biochemical composition of the glycocalyx, and revealed that syndecan-1 and HS 10E4 are probably not major constituents of the luminal endothelial glycocalyx. Syndecan-1 may be important with regard to the development of peritoneal alterations such as fibrosis or neoangiogenesis during PD, and future studies should address these aspects. Heparan sulfate is the main glycosaminoglycan within the endothelial glycocalyx. Its structure is complex and highly variable, and antibodies directed various epitopes are currently available. The HS 10E4 epitope occurs in native heparan sulfate chains, is nitric oxide-sensitive and is partly inaccessible in the HS chains attached to glypican-1 proteoglycan. N-acetyl groups are essential for 10E4 binding. In our study HS 10E4 had predominantly basolateral distribution. Antibodies against different HS epitopes should be used in order to assess the HS alterations in PD.

**Peritoneal alterations associated with peritoneal dialysis**

Another aim of this thesis was to do an extensive analysis of the peritoneal alterations induced by chronic kidney failure and long term-exposure to dialysis solutions with high glucose-concentrations. In addition, we specifically addressed the changes induced by a conventional and a biocompatible dialysis solution. Some of our findings deserve special attention and raise additional questions. An important limitation to long-term treatment with PD is the development of structural and functional membrane alterations, which may lead to technique failure. The ‘so-called’ biocompatible solutions were developed in order to reduce the peritoneal alterations induced by treatment with peritoneal dialysis. Numerous experimental studies have shown better morphological parameters associated with the biocompatible solution. Contrary to expectations, the biocompatible solutions were associated with no obvious advantage when the peritoneal transport characteristics were investigated in patients, making the benefit of biocompatible solutions on outcomes uncertain. Here, we combined the functional, morphologic and ultrastructural analysis of the peritoneal alterations to address this question.
In our study, both the conventional and the biocompatible solutions induced in general similar peritoneal alterations: neoangiogenesis, lymphangiogenesis, mesothelial cell alterations, vascular alterations. However, the extensive analysis of peritoneal fibrosis showed that biocompatible solutions may have a beneficial effect with regard to the development of fibrosis. Our findings may be of interest also with regard to the development of encapsulating peritoneal sclerosis (EPS). EPS is a rare but very severe complication of treatment with peritoneal dialysis, and is characterised by loss of mesothelial cells and progressive fibrotic thickening of the peritoneum. It has been argued that the use of poorly biocompatible acidic glucose-based dialysis solutions may be of importance for the development of this complication. Studies evaluating the incidence of EPS and factors related to EPS occurrence are currently being performed in PD patients using dialysis solutions with a low content of glucose degradation products. This is in line with observations from our center and other centers in the Netherlands that experienced a decrease in EPS incidence in the last years, as orally reported by the EPS registry. This coincided with the increased availability and use of biocompatible solutions.

We also showed that the biocompatible and the conventional solutions were associated with a different mesothelial cell phenotype. To our knowledge this is the first report to address this point. The mesothelium represents the cellular source of effluent cancer antigen 125 (CA125). CA125 is a large glycoprotein mainly located at the cell membrane and, regardless of the glucose concentration used, it follows a linear course in the effluent during a PD exchange. Several studies have suggested that CA125 may be a good marker for mesothelial cell mass, but this hypothesis has been questioned by others. To date, a combination of biochemical determinations of CA125 with morphometric analysis has not been done. Therefore it has not been established with certainty whether the effluent CA125 is a marker for mesothelial cell viability or damage. Effluent CA125 increases in patients after switch to a biocompatible solution, suggesting a different effect of the relatively new solutions on the mesothelium. Our findings are consistent with these data in patients. Rats treated with a biocompatible solution have an enlarged cell membrane area compared to the conventional group. Further research should investigate whether this could be the source for higher effluent levels of CA125, as found in patients. Unfortunately, we have not been able to analyze the level of CA125 in our rats, since the antibody against CA125 is generated in mice and has cross-reactivity with rats. Studies on mesothelium as source for CA125 in combination with morphometric analysis are required to solve the issue of mesothelial cell turn-over or mesothelial cell mass.

Overall, it can be concluded that chronic renal failure leads to alterations in the systemic glycocalyx, that are partially adaptive. Peritoneal dialysis leads to damage of the peritoneal endothelial glycocalyx, which may be caused by the high glucose content of the dialysis solutions. Biocompatible solutions are useful to limit the formation of peritoneal fibrosis.
References


Nederlandse samenvatting

De binnenkant van alle bloedvaten is bekleed met een laag van suikerstructuren, de endotheliale glycocalyx genaamd. Deze laag bestaat uit proteoglycanen en glycosaminoglycanen, en deze beschermt de vaatwand tegen de schadelijke invloeden vanuit de bloedstroom. De glycocalyx kan onder verschillende omstandigheden worden beschadigd, waardoor de kwetsbaarheid van het endotheel toeneemt. In dit proefschrift wordt ons onderzoek naar de endotheliale glycocalyx bij chronische nierinsufficiëntie beschreven. Hierbij ligt de focus op peritoneale dialyse en het belang van de endotheliale glycocalyx in de peritoneale bloedvaten.

Hoofdstuk 1 geeft een inleiding over de structuur en functies van de endotheliale glycocalyx en hoe deze bij patiënten gemeten kan worden. Daarna volgt een overzicht van chronische nierinsufficiëntie en peritoneale dialyse. Tevens beschrijven wij de functie van het peritoneum wat betreft het transport van water en deeltjes en de veranderingen van het peritoneale membraan tijdens lang-duurige PD.

Deel I is gericht op het meten van de endotheliale glycocalyx in dialyse patiënten, niertransplantatie patiënten en gezonde vrijwilligers. Patiënten met chronische nierinsufficiëntie hebben een verhoogd risico op het ontwikkelen van hart- en vaatziekten. De niertransplantatie vermindert de uremische complicaties en verbetert de overleving van deze groep patiënten. In hoofdstukken 2 en 3 gebruiken wij een niet-invasieve beeldvormingstechniek, Sidestream Darkfield imaging, om de perfused boundary region (PBR) te meten in de bloedvaten onder de tong. De PBR is de dikte van het luminale deel van de glycocalyx dat permeabel is voor erytrocyten. Bij dialyse patiënten hebben wij een dikkere PBR gevonden (er treedt een verandering op in de erytrocyten-excluderende eigenschap van de glycocalyx), samen met hogere plasma concentraties van glycocalyx producten hyaluronzuur, syndecan-1 en toegenomen hyaluronidase activiteit. Dit suggereert dat bij dialyse patiënten veranderingen in de endotheliale glycocalyx optreden. Bij getransplanteerde patiënten was alleen de PBR dikker, en gerelateerd aan de nierfunctie. In hoofdstuk 4 bespreken wij het nut van plasma concentraties van hyaluronan en hyaluronidase als markers voor de kwaliteit van de endotheliale glycocalyx bij nierinsufficiëntie. Hyaluronzuur is een belangrijk bestanddeel van de extracellulaire matrix en de endotheliale glycocalyx. Deze is heel belangrijk voor de beschermende rol van deze laag in het vaatstelsel, en tevens een aantrekkelijk molecuul als marker voor de endotheliale glycocalyx. Het merendeel van het hyaluronzuur in het bloed bestaat uit een hoog moleculair gewicht hyaluronan (boven 1000 kDa), maar een laag moleculair gewicht hyaluronan is ook te vinden en heeft pro-inflammatoire en pro-angiogene eigenschappen. Naast de lever, is ook de nier belangrijk voor de verwijdering van hyaluronzuur vanuit de bloedstroom. Hyaluronidase-2 kan aanwezig zijn op de apicale celmembraan en kan daarom belangrijk zijn voor de endotheliale glycocalyx.
Patiënten met nierinsufficiëntie hebben hogere hyaluronzuur concentraties in het bloed maar zonder aanvullende bepalingen moet dit voorzichtig geïnterpreteerd worden.

**Deel II** van dit proefschrift is gericht op de endotheliale glycocalyx tijdens peritoneale dialyse. In hoofdstuk 5 beschrijf ik de relaties tussen de sublinguale glycocalyx metingen en het transport van deeltjes en water tijdens peritoneale dialyse in patiënten. In het algemeen was er geen relatie aanwezig tussen de parameters. Niettemin, bij patiënten met niet-snel peritoneaal transport bleek dat een dikkere PBR was geassocieerd met een trager transport van kleine deeltjes. In hoofdstuk 6 tonen wij aan dat er een tegenovergestelde relatie is tussen de peritoneale endotheliale glycocalyx metingen en peritoneaal transport. Om de glycocalyx in de peritoneale bloedvaten te bestuderen, gebruiken wij een rattenmodel met chronische nierinsufficiëntie en langdurige blootstelling aan dialyse vloeistoffen met hoge glucose concentraties. In tegenstelling tot het sublinguale vaatstelsel, wordt het peritoneum tijdens peritoneale dialyse blootgesteld aan heel hoge glucose concentraties en hierdoor kunnen verschillende veranderingen in de peritoneale endotheliale glycocalyx optreden. De dikte van het voor rode bloedcellen toegankelijke deel van de glycocalyx was hetzelfde in ratten met chronische nierinsufficiëntie, ratten met chronische nierinsufficiëntie blootgesteld aan dialyse vloeistoffen en in ratten met normale nierfunctie. Om inzicht te krijgen in de veranderingen van de peritoneale endotheliale glycocalyx hebben we kleuringen voor syndecan-1 proteoglycan en het 10E4 epitoot van heparansulfaat glycosaminoglycaan toegepast. Syndecan-1 was aanwezig met name in de interendotheliale verbindingen en niet op de apicale membraan van het endotheel. De expressie van syndecan-1 neemt af bij chronische nierinsufficiëntie maar wordt na langdurige blootstelling aan dialyse vloeistoffen gelijk aan die bij ratten met normale nierfunctie. Ook, het heparansulfaat 10E4 epitoot bevond zich in die condities vooral aan de abluminale zijde van het endotheel.

In **deel III** worden in hetzelfde rattenmodel de veranderingen beschreven die optreden in het peritoneale membraan bij chronische nierinsufficiëntie en na langdurige blootstelling aan dialyse vloeistoffen. Tevens hebben wij de effecten van een conventionele met die van een biocompatible (minder afwijkend van de samenstelling van plasma) dialyse vloeistof vergeleken. Chronische nierinsufficiëntie induceert veranderingen in het mesotheel, fibrose, het verdikken en duplicatie van de basale lamina in de peritoneale bloedvaten, lymphangiogenese en neoangiogenese. Deze veranderingen nemen deels toe door de blootstelling aan dialyse vloeistoffen met hoge glucose concentratie. In het algemeen hadden de twee dialyse vloeistoffen dezelfde schadelijk effecten op het peritoneum. Bovendien waren de transport karakteristieken vergelijkbaar. Niettemin lijkt de biocompatible vloeistof geassocieerd te zijn met minder fibrose dan de conventionele vloeistof. Tevens was in de twee groepen een verschillend mesotheelcel fenotype te zien.
**Conclusies**

De endotheliale glycocalyx is heel belangrijk voor de bescherming van de vaatwand tegen schadelijke prikkels vanuit de bloedstroom. Daardoor is de glycocalyx van belang in condities met endotheliale dysfunctie en een verhoogd risico op het ontwikkelen van cardiovasculaire aandoeningen, zoals chronische nierinsufficiëntie. Bij dialyse patiënten treden veranderingen in de systemische endotheliale glycocalyx op, onafhankelijk van het soort dialyse. Een geslaagde niertransplantatie lijkt een positief effect te hebben op de endotheliale glycocalyx. Echter, dit effect is afhankelijk van de functie van de getransplanteerde nier. Nader onderzoek moet worden gedaan naar de effecten van de immunsuppressiva op de endotheliale glycocalyx.

Een kritische evaluatie moet plaatsvinden van de veranderingen in de plasma concentraties van glycocalyx bestanddelen om hun waarde als markers voor de endotheliale glycocalyx te bepalen, rekening houdend met de rol van de nier bij hun verwijdering vanuit het bloed.

De blootstelling van de peritoneale bloedvaten aan dialyse vloeistoffen met hoge glucose concentratie leidt tot specifieke veranderingen die niet in de sublinguale bloedvaten te zien zijn. Er zijn relaties aanwezig tussen het transport van kleine deeltjes en de dikte van het deel van de endotheliale glycocalyx dat toegankelijk is voor erytrocyten. Dit suggereert dat de endotheliale glycocalyx belangrijk kan zijn voor peritoneaal transport. Nader onderzoek moet worden gedaan naar de veranderingen die in de endotheliale glycocalyx optreden in relatie tot de veranderingen in de peritoneale membraan. Syndecan-1 en het 10E4 epitoot van heparan sulfaat lijken niet essentieel te zijn voor de samenstelling van de glycocalyx aan de luminale kant van het endotheel. Om inzicht te krijgen in de biochemische structuur van de endotheliale glycocalyx, moeten andere bestanddelen zoals glypicanen, andere glycosaminoglycanen en heparan sulfat epitopen bestudeerd worden.

Chronische nierinsufficiëntie leidt tot veranderingen in de peritoneale membraan. Deze nemen deels toe na de blootstelling aan dialyse vloeistoffen met hoge glucose concentraties. De biocompatibele vloeistof lijkt een positief effect te hebben op de ontwikkeling van peritoneale fibrose.

Specifieke endotheliale markers en nieuwe methodologie zoals stereologische methoden waarmee onbevooroordeelde en accurate morfologische data verkregen kunnen worden, zijn momenteel beschikbaar en moeten gebruikt worden voor kwantitatieve histologie.