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Review Article

Endothelial glycocalyx in health and kidney disease: Rising star or false Dawn?

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ABSTRACT:

The endothelial glycocalyx is a layer comprised of proteins and carbohydrates on the luminal surface of vascular endothelial cells, thought to have an important role in the health and function of the endothelium. Disrupted by various pathophysiological conditions and linked with clinical outcomes, it is increasingly recognized as an early indicator of endothelial injury and a potential marker of vascular injury. In this review, we discuss current methods of assessment (including novel optical approaches), evidence for its use as a marker of vascular disease and its potential role in relation to microalbuminuria and glomerular endothelial dysfunction. Therapeutic strategies for restoration of the glycocalyx following injury are also explored.

SUMMARY AT A GLANCE

The endothelial glycocalyx on the surface of vascular endothelial cells, an early indicator of endothelial injury and a potential marker of vascular injury, could be demonstrated by a variety of methods of assessment, including novel optical approaches and might be a potential therapeutic target.

Conventional vascular risk factors such as hypertension, hypercholesterolaemia, diabetes mellitus and smoking only partially explain the high prevalence of cardiovascular disease in patients with chronic kidney disease (CKD).[1,2] Non-traditional risk factors such as uraemia, inflammation and endothelial dysfunction are increasingly recognized as key contributors to cardiovascular disease, the last of which is characterized by an imbalance between small-vessel vasodilation and vasoconstriction.[3,4] Endothelial function can be assessed by various techniques, including flow-mediated vasodilation, carotid intima-medial thickness and through measurement of serum biomarkers. Another potential assessment target discovered in recent years is the endothelial glycocalyx (EG), increasingly recognized as a novel biomarker of vascular damage. In vascular disease, both risk and event rates have been shown to correlate with measures of the EG[5,6] which is affected at different stages of CKD, including haemodialysis and transplant.[7–9] Some studies have also linked it with microalbuminuria.[10] Here, we outline the structure of the EG and current available methods of its assessment. We discuss its potential as a marker of endothelial health and vascular disease, and whether glycocalyx studies performed in kidney disease and microalbuminuria can be correlated with endothelial dysfunction. We also provide a brief overview of therapeutic strategies for EG preservation.

STRUCTURE OF THE ENDOTHELIAL GLYCOCALYX

The EG is a mesh of proteoglycans and glycoproteins lining the endothelial lumen of all blood vessels.[11] The EG lines both fenestrated and non-fenestrated capillaries, some more densely and uniformly than others – for example, the EG in sinusoidal capillaries is sparse, whereas it is continuous in brain tight junctions. In glomerular capillaries, the EG layer itself is fenestrated, although it also extends into and lines

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the endothelial fenestrae.[12–14] There is some evidence that the EG layer is thicker in larger vessels.[15]

Proteoglycans make up the 'backbone' of the EG. They consist of several core proteins such as transmembrane syndecans and surface-bound glypicans, which are attached to the endothelial cell membrane via a glycosylphosphatidylinositol anchor (Fig. 1). Proteoglycans are structurally linked by five types of glycosaminoglycan (GAG) side-chains. These primarily consist of heparan sulphate which accounts for 50–90% of the mass. The rest of the GAGs is made up of chondroitin, dermatan and keratin sulphates, and hyaluronic acid. Hyaluronic acid, or hyaluronan, is the only non-sulphated GAG and is not attached to a core protein. It provides the EG with an overall negative charge owing to the carboxyl groups that bind with water to give the EG its gel-like property.[15–17]

In contrast to proteoglycans, glycoproteins are comprised of cell adhesion molecules from the selectin, integrin and immunoglobulin families, which assist in coagulation, fibrinolytic and haemostatic pathways.[15] In addition, dynamic interactions between the EG and adsorped plasma proteins such as albumin and orosomucoid also influence the composition, permeability and charge properties of the EG (Fig. 1).[18]

The EG acts as an interface between the blood and the vascular wall. It is a dynamic structure; playing a key dynamic role in vascular homeostasis by transmitting shear stress forces to endothelial cells[16] and regulating vessel wall permeability due to its mesh-like structure and charge- and size-selectivity. Under physiological conditions, the overall net negative charge of the EG acts as a barrier to inhibit leukocyte adhesion by acting as an electrorepulsive shield, but also aids with leukocyte recruitment when necessary, through chemokine presentation and adhesion molecule activity.[19] Additionally, it acts as a sodium buffer system and a reservoir for sodium storage.[20]

The EG has also changed the paradigm of fluid physiology. Where the original Starling principle of transcapillary flow is based on the pressure difference between the capillary and interstitium, the proposed revision to this principle incorporates the EG and hypothesizes that the key pressure difference is generated within the capillaries across the plasma and protein-free sub-EG region. This osmotic pressure difference creates a net filtration effect, maintaining a one-way flow across the capillaries (no absorption rule), which explains the discrepancies observed in clinical studies where colloids used as resuscitation fluid do not improve outcomes compared to isotonic solutions.[14]

The EG is damaged when it is exposed to turbulent shear stress and oxidative stress, conditions that are clinically observed in diabetes and at times of inflammation, ischaemia-reperfusion injury, and hypernatraemia.[21–23] It

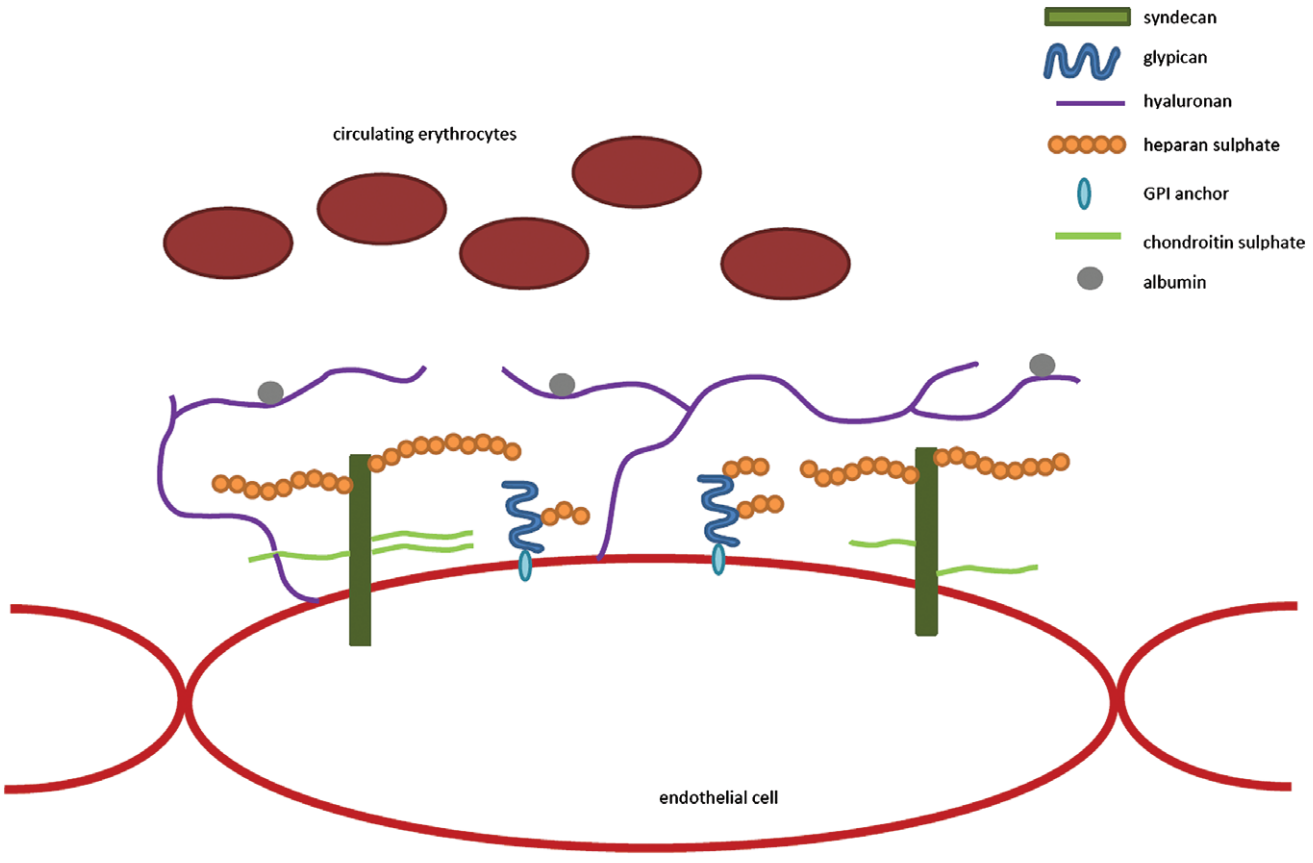

Fig. 1 Structure of the endothelial glycocalyx (EG).

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has been linked to increased mortality in trauma and sepsis.[24] During inflammation, activated neutrophils produce reactive oxygen species and proteases and mast cells release heparanase, all of which degrade the EG. Damage to this layer is dramatic and rapid, with patchy loss occurring within 5 min and progressing to over 70% destruction within 30 min in sepsis models.[25,26]

When the EG is destroyed, there is increased platelet aggregation, leukocyte recruitment and adherence of monocytes to endothelial cells, especially in a high sodium milieu.[27–29] It loses its function as a vascular barrier with a resultant increased permeability to sodium, albumin and microvascular fluid.[10,30,31] Loss of the EG also contributes to myocardial oedema[31] and the no-reflow phenomenon seen after myocardial ischaemia, due to local occlusion from degraded EG components and the reduction of functional capillary density.[32]

It appears to take 5–7 days for the physiological glycocalyx layer to regenerate *in vivo* after degradation.[33] When restored, the vessel wall re-establishes its ability to maintain its barrier properties and nonadhesion qualities.[34,35]

ASSESSMENT OF THE ENDOTHELIAL GLYCOCALYX

Fragile, and unable to be visualized or its complex mesh easily measured, the properties of the EG have traditionally proven difficult to study. Investigative techniques include *in vivo*, *ex vivo* and *in vitro* studies, all with limited success and utility. *In vivo* studies such as systemic EG volume estimates provide an indirect method of assessing the EG by comparing the distribution volume of an EG-permeable tracer to an EG-impermeable tracer.[36] Endothelial cell culture has the potential advantage of manipulating the culture milieu and assessing the impact on the EG, but *ex vivo* experiments may not accurately replicate in vivo conditions in the absence of laminar flow.[37,38] Electron microscopy visualization offers a direct method of assessing the EG thickness but is limited by handling and staining methods. Atomic force microscopy measures the stiffness of the EG by using a cantilever which estimates the pressure required to cause an indentation – a healthy endothelial cell with an EG layer is relatively soft compared to one that is denuded.[23] Estimates of its breakdown products however, including heparan sulphate, syndecan-1 and hyaluronan, have proven more readily assessable and reliable through serum assays.

New techniques to visualize the EG have also emerged in the field of optics, using a handheld video microscope by means of a probe placed sublingually to capture recordings of the microcirculation. The technique incorporates commercially-available orthogonal polarised spectral (OPS) imaging or its successor, sidestream darkfield (SDF) imaging. The EG width is calculated by measuring the perfused boundary region (PBR), which is the distance between the median red blood cell column width and the red blood cell-impermeable EG. When the EG is damaged, circulating red cells are able to verge closer to the endothelium, veering further away from the column's median width (Fig. 2). A larger PBR value reflects a damaged EG.[39] This technique has been used in numerous clinical scenarios with variable interpretative success; however, there currently is no established PBR normal range. Additional complexities with this technique include the fact that the sublingual circulation may not be representative of other microcirculatory beds, and that the technique is operator-dependent and requires considerable training.

EG AS A MARKER OF ENDOTHELIAL DAMAGE AND VASCULAR DISEASE

The EG is a protective lining on the inside of the blood vessel and its damage may be used as a marker of vascular damage. Studies that support the EG as a marker of endothelial damage have used various techniques including cell culture, volume assessment, serological indices, and optical analysis. Studies in hypoxic and ischaemic heart models have shown changes in the EG before any visible damage to the endothelial cells.[41,42] It may also be involved in the initiation and development of atherosclerosis. In one study, damage to the local EG by free oxygen radicals and oxidized lipoproteins resulted in endothelial platelet and erythrocyte adhesion, one of the key initial steps in plaque formation.[29] In murine models, the EG appears to be thinnest in the regions of the carotid arteries most prone to disease and mice fed a high-fat, high-cholesterol diet had a significantly reduced EG structure as assessed by direct visualization of electron micrographs.[29,43] In addition, when compared to control rats, spontaneously-hypertensive rats have markedly damaged EG in the blood–brain-barrier, as well as retinal and choroidal vessels.[44,45]

Clinically, a modest correlation between the EG PBR and traditional CVD risk factors such as glucose, cholesterol, and BMI has been shown using OPS imaging in healthy volunteers, and a reduction in systemic EG volume during hyperglycaemia coincided with reduced flow-mediated dilation.[6,36] At least one other study has shown a correlation between serum markers of EG and serum markers of endothelial dysfunction.[7] Patients with chest pain from an acute coronary syndrome had higher levels of syndecan-1 compared to those with non-coronary chest pain and controls.[5] However, clinical studies assessing the correlation between the EG and CVD risk using SDF imaging are conflicting.[46,47] This may be explained by methodological issues. For example, a negative study in stroke patients may have related both to the limited numbers and the inclusion of patients taking medications that may have affected results.[48]

The questionable validity of some EG measurements in these clinical studies may have been improved by incorporating more than a single, cross-sectional measure of the EG. However, notwithstanding the study limitations, the

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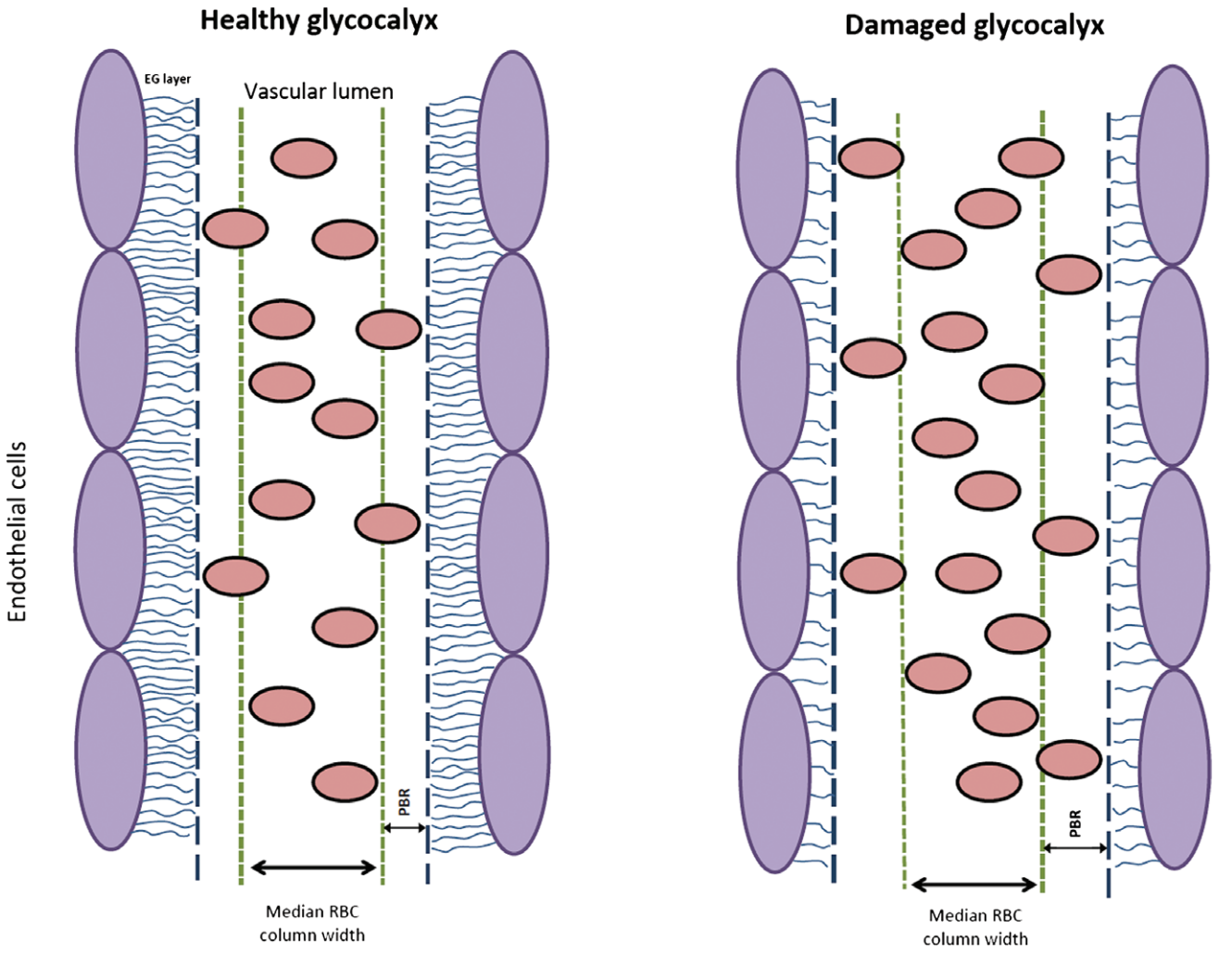

Fig. 2 To calculate the perfused boundary region (PBR), images of the microvasculature are first captured using sidestream darkfield (SDF) imaging and then submitted into the Glycocheck software (Glycocheck BV, Maastricht, the Netherlands) for analysis. The software then determines the red blood cell (RBC) distribution along the length and width of each vascular segment. From there, it determines the median width of the RBC column, and the furthest lateral edge of the RBC on either side of the lumen. The PBR is the distance between these two measurements, divided by two.[39] When the endothelial glycocalyx (EG) layer is damaged, RBCs are able to move closer to the endothelium, giving a higher PBR reading. Figure adapted from Dane *et al.*[40]

correlation between serum and optical measures in particular does appear to have limitations.

EG IN KIDNEY DISEASE

Using serum ELISAs of syndecan-1 and hyaluronan, and assessing glycocalyx thickness using atomic force microscopy, Padberg and coworkers[7] measured the glycocalyx integrity in 95 patients with an eGFR <60 mL/min (CKD stages 3–5). Serum levels of syndecan-1 and hyaluronan were 4-fold higher in CKD compared to healthy controls, with levels incrementally higher across the different stages of CKD. These markers were also positively correlated with several markers of endothelial dysfunction such as angiopoietin-2, human soluble FMS-like tyrosine kinase-1, soluble vascular cell adhesion molecule-1, and von Willebrand factor. This study also correlated serum EG levels with EG thickness in an animal model of CKD: plasma levels of syndecan-1 were elevated in 5/6-nephrectomized rats, and atomic force microscopy of EG thickness in the aortas was less than with sham-operated rats.

Vlahu *et al.*[8] assessed patients on haemodialysis and peritoneal dialysis by measuring the PBR as well as serum components of the EG. Serum hyaluronan and syndecan-1 levels were higher in dialysis patients compared with controls, and this correlated with dialysis vintage. However, no correlation was found between PBR with E-selectin (a marker of endothelial dysfunction), nor was a correlation analysis reported between PBR and serum EG components, or between serum EG components and serum markers of endothelial dysfunction.

Using optical and serum markers, ESKD patients and patients with interstitial fibrosis and tubular atrophy showed

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a significantly elevated PBR and serum levels of syndecan-1 compared to controls and patients with stable kidney transplants.[9] This study used serum thrombomodulin and angiopoietin levels as markers of endothelial dysfunction. While thrombomodulin correlated with the elevated PBR, serum angiopoietin levels did not. Disappointingly, no correlation was found between PBR or syndecan-1 levels, and no correlation analysis was performed between serum EG components and serum markers of endothelial dysfunction. Additionally, in a study of only peritoneal dialysis patients, no correlation was demonstrated between PBR and peritoneal transport parameters.[49] In kidney transplant surgery, SDF imaging was used to visualize peritubular microcirculation of kidneys donated after cardiac death and kidneys from living donors. EG changes on imaging and levels of syndecan-1 and heparan sulphate were more pronounced in the kidneys from deceased cardiac donors, thought possibly related to a longer ischaemic period.[50]

Thus, correlation studies demonstrate that optical and serum EG markers change in renal disease compared to normal subjects, both at progressive stages of CKD and in dialysis patients. Furthermore, markers of EG health are improved in healthy transplants and decline as the transplant fails.[51–53] However, correlation data between EG markers and markers of endothelial dysfunction in CKD are currently limited. It is also not known whether EG damage in kidney disease correlates with clinical outcomes such as cardiovascular events or mortality.

EG AND MICROALBUMINURIA

Microalbuminuria in renal disease is associated with the progression of CKD and an increase in all-cause and cardiovascular mortality.[54] The pathophysiology of microalbuminuria is poorly understood, but some reports suggest a possible link with a defect in the EG.[55–58] Glomerular endothelial cells are lined with hyaluronan which anchor the glycocalyx to the glomerular basement membrane and may act as a barrier against albumin filtration.[10,59] Some evidence for this is seen in culture models of glomerular endothelial cells, where removal of the EG resulted in an increase in albumin flux.[10]

Many rat studies have also demonstrated the relevance of the EG in proteinuric disease, showing significant EG damage when they develop proteinuria.[60,61] In humans, the total EG volume is reduced and hyaluronan levels increased in patients with diabetes and microalbuminuria, and patients with nephrotic syndrome have elevated concentrations of syndecan-1 levels. However, correlative data thus far are limited.[22,62]

THERAPEUTIC MODULATION OF THE EG

Numerous studies have examined ways of preserving the EG or restoring it after it has been damaged. In cells, the application of a polyphenol-rich compound increased EG thickness, reduced its stiffness, and improved its barrier function against sodium.[30,63] In another study, the destructive effect of hypernatraemia on the EG was attenuated when spironolactone was added to the culture.[23] In animals, ischaemic pre-conditioning prevented damage to the EG in a guinea pig heart model compared to standard exposure to ischaemia-reperfusion alone.[64] Hydrocortisone also protected against ischaemia-reperfusion injury, prevented EG damage, and reduced EG degradation in rat models of pancreatitis.[65,66] VEGF-A_{165}b injected into diabetic mice for 4 weeks reduced microalbuminuria and improved the glomerular EG on electron microscopy images.[67] Treatment with endothelin receptor antagonists in mice also showed a reduction in microalbuminuria.[68,69] Sulodexide, a glycocalyx-mimetic composed of heparan sulphate and dermatan sulphate, was able to restore the EG in a mouse model of sepsis[70] as well as reduce microalbuminuria in diabetic patients in one study.[71] Disappointingly, this benefit was not replicated in a larger multinational trial which may have been at least partly due to manufacturing issues.[72]

Studies on EG restoration in humans are in fact limited. One study in patients with familial hypercholesterolemia demonstrated lower total EG volumes when subjects were taken off statin therapy, with partial restoration of the EG after statin reintroduction for 8 weeks.[73] Another study showed that pre-treatment with etanercept, a TNF-α inhibitor, reduced the degree of EG damage (measured by sublingual OPS and serum EG components) in healthy subjects who were infused endotoxin.[74] More studies in humans are needed to determine whether EG restoration results in favourable clinical sequelae.

CONCLUSION

The endothelial glycocalyx appears to play an important role in preserving the physiological properties of the endothelium. Its destruction has been demonstrated in various pathological conditions and the implications on its function as a barrier as well as a mediator of vascular homeostasis have been demonstrated in many studies. However, its clinical relevance remains to be fully defined. In particular, clinically-relevant long-term studies relating to both EG damage and restoration are lacking. Part of the reason for this relates to the imperfections and uncertainties associated with the measurement the EG, although recent technological advances should make this more accessible. Studies in kidney disease are as yet also limited, although one potentially important feature of the EG relates to its correlation with microalbuminuria, and thus indirectly to cardiovascular risk. On balance, the EG appears to be a rising star in shaping and refining our understanding of many important aspects of the microcirculation. However, further work is required to determine whether preservation and repair of the EG can protect the microcirculation from endothelial cell

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damage in a clinical context as well as help prevent the associated long-term inflammatory and structural changes.

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DISCLOSURE

The authors have nothing to disclose.

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