Endothelial Glycocalyx

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Abstract

Endothelial glycocalyx is the luminal layer of blood vessels which grows on the walls of blood vessels. It is very thin and can only be seen by using certain techniques. It has several functions, such as to regulate vascular permeability, regulate the interaction of blood cells and endothelial cells, as well as to regulate the endothelial environment. Under certain condition, endothelial glycocalyx can be shedded, and the components will enter the plasma. This shedding can disrupt the function of endothelial glycocalyx, and it also has a strong relation with the progress of a disease, where the severity of the damage on endothelial glycocalyx signifies the severity of the disease. Therefore, therapies which can maintain and repair endothelial glycocalyx have been developed. This journal will describe the structure, function, damage and therapy of endothelial glycocalyx layer in detail.

Keywords: Endothelial glycocalyx

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INTRODUCTION

Endothelial glycocalyx is a functional, gel-like layer which exists on every surface of endothelial cells. It consists of an array of components of glycans and glycoconjugates. The former refers to the carbohydrate component of glycocalyx layer, while the latter refers to glycans bound to other chemical compounds, such as lipids and proteins (i.e. proteoglycans, glycoproteins, glycosaminoglycans and plasma protein). Glycocalyx may consist of several glycoconjugates bound together to form a layer and cover the cell surface. This layer can only be seen with an electron microscope.(1,2)

About 70 years ago, this concept is first put forward by Danielli, followed by Chambers and Zweifach. They introduce the concept of a thin non-cellular layer on the surface of vascular endothelial cells. However, this thin layer still cannot be proven due to the limited technology at that time. Then, in 1966, by using electron microscopy and staining rutherium red; Luft is able to prove its presence on the surface of endothelial cells. This layer is then referred to as glycocalyx.(3,4)

This discovery is followed by other researches, which mainly report the role of endothelial glycocalyx (both physiologically and pathologically) on diseases; including atherosclerosis, stroke, hypertension, sepsis, even on the malignancy.(1,2)

Structure And Function Of Endotelial Glycocalix

Structure

Endothelial glycocalyx layer can only be seen with an electron microscope. It is very thin; and the thickness varies depending on the diameter of the blood vessels, organs, as well as on the blood flow velocity, which is around 0.5 - 5.0 µm. Endothelial glycocalyx is directly exposed to mechanical force due to blood flow, making it difficult to measure its exact thickness due to its dynamic nature.(3–5)

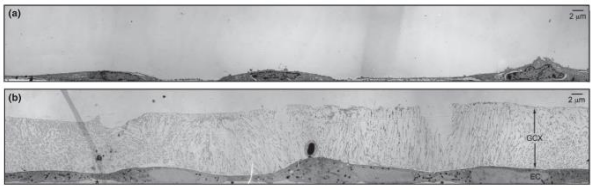

Figure 1. Glycocalyx when seen with an electron microscope.

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(a) Glycocalyx stained with ruthenium red and osmium tetroxide.
(b) Glycocalyx stained with osmium tetroxide.(1)

In general, glycoproteins and proteoglycans (PG) are the main components which make up this layer. Both are the sites of attachment for plasma proteins and glycosaminoglycans (GAGs). They then form a gel-like web which covers the endothelial cells.(1,3,6)

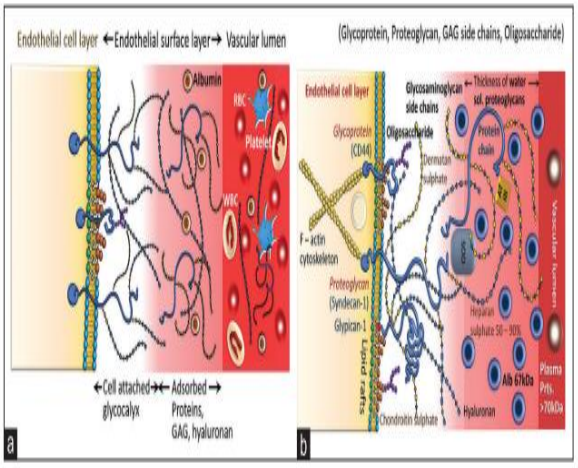

Figure 2. Structure of Endothelial Glycocalyx (7)

Proteoglycan (PG)

Proteoglycans are one of the main components which make up endothelial glycocalyx. In PG, there is a core protein which can bound to one or more GAGs. The core protein consists of syndecan, glypican, perlecan, versican, decorin, biglycan and mimecan. Each of them has characteristics based on size, type of GAG bindings, number of GAG bonds, as well as on the number of bonds to cell membranes.(3,4,6)
Around 50-90% of GAGs in blood vessels is composed of heparan sulfate, while the rest is hyaluronic acid, chondroitin sulfate, dermatan sulfate and keratan sulfate. Heparan sulfate and chondroitin sulfate can both be found bound to syndecan, glypican and perlecan. As for chondroitin sulfate and dermatan sulfate; they are bound to versican, decorin and biglycan. Meanwhile, keratan sulfate is bound to mimecan. Finally, hyaluronic acid is the only GAG which does not bound to any core protein.(3,4,6)
Syndecan core protein has a transmembrane protein, therefore it can bound tightly to endothelial cell membrane. In case a damage occurs (either due to infection or trauma), syndecan can morph into a soluble form, which will then diffuse to the surface of blood vessels and circulates in the circulation.(3,4,6)
Glypican has a glycosylphosphatidylinositol (GPI) anchor on its surface. Due to the presence of GPI anchors, it can also bound strongly to endothelial cell membrane. Perlekan core proteins can be found on the surface of endothelial cell membrane. Syndecan, glypican, and perlekan are all bound to GAG heparan sulfate and chondroitin sulfate.(3,4,6)

Meanwhile; versican, decorin, biglycan and mimecan will be formed in the cell and later secreted to the cell surface; becoming a part of endothelial glycocalyx. The first three are bound to chondroitin sulfate and dermatan sulfate, while the last one is bound to keratan sulfate.(3,4,6)
The core proteins which have been mentioned will be formed based on the given stimulus (e.g. syndecan protein). Syndecan will provide different expression patterns based on the stimulation from endothelial cells and chemokines.(4,6)

Table 1. Characteristics of proteoglycan core protein (3,4)

Core protein	Size (kDa)	Number of sub-types	Number of GAG bonds	Structural characteristics with cell membrane	GAG bound
Syndecan	19-35	4	5	Transmembrane protein	HS, CS
Glypican	57-69	6	3	*GPI-anchored* protein	HS, CS
Perlecan	400	1	3	Secreted	HS, CS
Versican	370	1	10-30	Secreted	CS, DS
Decorin	40	1	1	Secreted	CS, DS
Biglycan	40	1	2	Secreted	CS, DS
Minecan	35	1	2-3	Secreted	KS

HS: heparan sulfate, CS: chondroitin sulfate, DS: dermatian sulfate, KS: keratan sulfate

Glycoprotein

Glycoprotein is also one of the main components of glycocalyx. In contrast to proteoglycans, it has fewer bonds. There are two known groups of glycoprotein, which are cell adhesion molecules and coagulation components – fibrinolysis.(4,6)

Cell adhesion molecules, which play a role in cell recruitment from blood vessels and inter-cellular signaling, consist of selectins, integrins and immunoglobulin superfamily. Furthermore, selectin consists of a cytoplasmic tail compo-nent, a transmembrane component, an epider-mal growth factor-like component and a lectin

component. Selectins which can be found in blood vessels are selectin-P and selectin-E. The former is produced on Weibel-Palade bodies of endothelial cells, and will be released when there is a stimulus (e.g. from thrombin and histamine); whereas the latter will be released when there is a stimulus from interleukin (IL)-1 and tumor necrosis factor (TNF)-α. This process normally takes 2-6 hours.(4,6)

Meanwhile, integrin consists of α and β sub-units, where each has a cytoplasmic tail component and a transmembrane component which will make up a protein membrane. There are 18 α sub-units and 8 β sub-units, where each integrin is composed of specific α and β sub-units.(4)
As for immunoglobulin superfamily, it has a cytoplasmic tail, a transmembrane component, and an immunoglobulin-like component. Examples of this group are intercellar adhesion molecule (ICAM) 1 and 2, vascular cell adhesion molecule (VCAM) 1, as well as platelet/ endothelial cell adhesion molecule (PECAM) 1. They act as ligands for integrins, help blood vessel penetration, and serve as leukocyte mediator for endothelium.(4,6)
Glycoprotein also has components which play a role in homeostasis. These components can bound to von Willebrand factor (vWF) and P-selectin, which play a role in homeostasis.(4)

Function

Endothelial glycocalyx has several functions, such as to regulate vascular permeability, regulate the interaction of blood cells and endothelial cells, as well as to regulate the environment of endothelium.(6–8)

Endothelial Glycocalyx In Vascular Permeability

Endothelial glycocalyx has the role of protecting blood vessels by regulating fluid permeability. Normally, it has a negative charge - hence negatively charged molecules cannot pass. Moreover, molecules measuring >70kDa also cannot pass through this layer.(6,7)
Glycocalyx plays a role in regulating vascular permeability through the role of albumin. According to Starling's law, there are four pressures regulating fluid homeostasis, which are oncotic pressure, hydrostatic pressure, arterial capillaries pressure, and venous capillary pressure. Their balance keeps fluid in the blood vessels. However, this theory is unable to explain the role of venous reabsorption, capillary filtration rate and pressure which are struggling against capillary filtration.(8)
Therefore, Starling law needs to be revised (where endothelial glycocalyx theory is able to explain fluid homeostasis). Endothelial glycocalyx contains dissolved albumin molecules which account for 60% of oncotic pressure. Beneath it, lies the subglycocalyx space. Due to the fact that albumin exists in endothelial glycocalyx but is absent in the subglycocalyx space; an osmotic gradient has aroused between the two (instead of between interstitial and intravascular tissues) - as Starling suggests.(8)
With this albumin gradient, fluid will be drawn from the interstitial; which is called ultrafiltration fluid. In that particular space, the hydrostatic and oncotic pressures are so low that there are no occurrence of absorption on venous return. Moreover, lymphatic system also plays a role in absorbing excess fluid in the interstitial space and bringing it back into the circulation.(8)

Therefore, based on the revised Starling law, there are three changes, which are: (8)

1. Intravascular volume not only consists of plasma volume and erythrocyte volume, instead it also consists of endothelial glycocalyx volume
2. Ultrafiltration fluid is formed as a result of fluid filtration through endothelial glycocalyx, and that there is no venous uptake in the venous capillaries
3. Lymphatic fluid is the main route of return for fluid from the interstitial into the circulation.

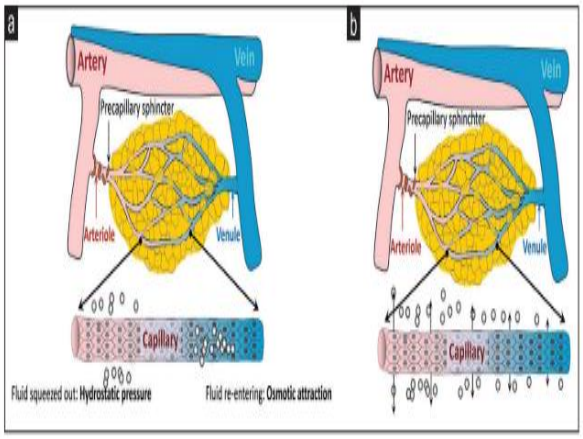

Figure 3. (a) Starling Law. (b) Revised Starling Law (7)

Endothelial Glycocalyx In The Interaction Of Blood Cell And Endothelial

Endothelial glycocalyx covers endothelial surface, hence interactions with blood cells are limited. Normally, glycocalyx will prevent the attachment of blood cells to the endothelial layer so as to protect the endothelial layer from any damage. However, under certain conditions (e.g. inflammation), the adhesion molecules present in this layer will be activated to help blood cells to stick and pass through the endothelial layer.(6,7)

Glycocalyx also serves to protect endothelial layer from mechanical stress due to friction with blood flow. If there is an increase in mechanical stress on the endothelial layer, it may trigger an increase in the oxidative stress. Glycocalyx will release extracellular superoxide dismutase (SOD) and increase nitric oxide (NO). This increase will trigger vasodilation of blood vessels, which may reduce the mechanical stress - therefore endothelium can be protected. However, this mechanism cannot be activated if the endothelial glycocalyx layer is damaged.(4,6)

Endothelial Glycocalyx In Regulating Endotelal Environment

The proteoglycans of endothelial glycocalyx layer play an important role in this function. The GAG chain of proteoglycans contains adhesion receptors - to which other molecules attach. The molecules can only work when attached to GAG. Moreover, the GAG chain also serves to protect the receptors. The two-most common adhesion receptors are selectins and immuno-globulins.(4,7)

Table 2. Molecules Which Must Interact With Glycocalyx To Be Active (4)

Molecules	Function
Antitrombin III	Potent inhibition of procoagulants - such as thrombin and factor Xa. Bound to heparan sulfate
Heparin ko-faktor II	Inhibition of thrombin. Bound to dermatan sulfate
TFPI	Anticoagulant, delays the activation of factor VII and factor X
LPL	Enzyme, helps to destroy low density lipoprotein
LDL	Brings cholesterol and triglycerides into circulation
VEGF	Helps angiogenesis and active in hypoxic condition
TGFβ1/2	Growth factor on smooth muscle and blood vessels
FGF(r)	Growth factor receptors, endothelial proliferation and angiogenesis
Ec-SOD	Binds extracellular oxidative stress
IL 2, 3, 4, 5, 7, 8, 12, RANTES	Leukocyte chemotaxis to sub-endothelium

TFPI: Tissue factor pathway inhibitor, LPL: lipoprotein lipase, LDL: low density lipoprotein, VEGF: vascular endothelial growth factor, TGFβ1/2: transforming growth factor β1 or β2, FGF(r): fibroblast growth factor (receptor), ec-SOD: extracellular superoxide dismutase, IL: interleukin, RANTES: Regulated on Activation.

Clinical Implications Of Endothelial Glycocalix

Under certain conditions, endothelial glycocalyx can be damaged, and the damaged components will enter the plasma. This damage may disrupt the function of endothelial glycocalyx. Normally, glycocalyx regeneration takes 6-8 hours. However, in pathological conditions, it requires varying regeneration times, ranging from hours to days (depending on the severity of the damage).(7)

Fluid Therapy

Intravenous fluid therapy is given when oral therapy is deemed impossible. There are two types of fluids which can be administered, which are crystalloids and colloids. Both have their own advantages and disadvantages. Rapid and excessive fluid administration may damage endothelial glycocalyx layer and disrupt vascular permeability, causing interstitial edema and acute kidney injury (AKI).(8)

Crystalloid fluid is one of the fluids which can be given as fluid therapy. Rapid and excessive administration of normal saline (NS) crystalloid solution with high chloride levels may cause hyperchloremic metabolic acidosis and damage

the endothelial glycocalyx layer. Meanwhile, lactated Ringer's fluid or Ringer's acetate contains chloride which is physiologically similar to blood plasma, therefore it is safer for endothelial glycocalyx. However, these two fluids are still not completely safe, since they are hypotonic.(8)

The other fluid is colloid, which has a large molecular size, therefore it can stay longer in blood vessels and increase oncotic pressure. Colloidal albumin (for 5%) can protect endothelial glycocalyx. Colloid is recommended in trauma (except for head trauma). Synthetic colloids; such as gelatin, dextran and hydroxy ethyl starch (HES) can also be given - although they are not as safe as albumin (5%).(8)

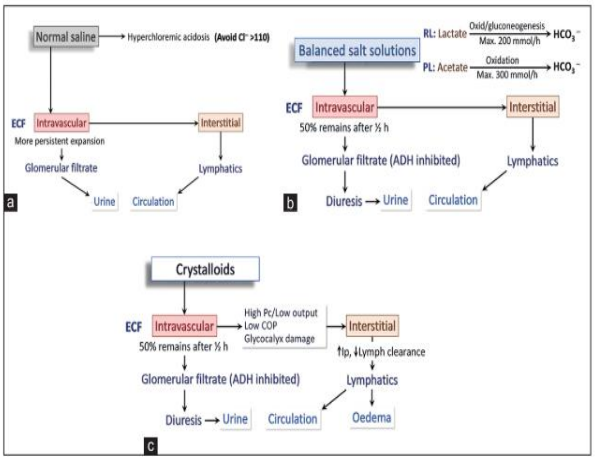

Figure 5. (a) Normal Distribution and Excretion of Intravenous Saline. (b) Distribution, Metabolism and Excretion of Intravenous Ringer's Lactate and Ringer's Acetate. (c) Distribution and Excretion of Ringer's Lactate and Ringer's Acetate in Damaged Endothelial Glycocalyx

Sepsis

Sepsis is a systemic inflammatory syndrome which happens due to a bacterial infection that can lead to multiple organ failure. Bacterial infections will secrete endotoxins, resulting in systemic inflammation and increased proinflammatory cytokines (TNF-α, IL-1β, IL-6, and IL-10). The increase in these cytokines will trigger the degradation of glycocalyx layer, where the layer will become thin and loose; hence resulting in impaired vascular permeability and interstitial edema.(1,5,9)

Because of this damage, the glycocalyx component; such as syndecan-1, heparan sulfate, hyaluronate, and chondroitin sulfate will enter the plasma. Moreover, damage to glycocalyx can also cause an increase in pro-inflammatory cytokines, therefore it further aggravates the damage to glycocalyx. Fluid therapy is one of the main therapies for sepsis. Crystalloid fluid administration of 30cc/kg is recommended for fluid resuscitation. However, rapid and excessive fluid administration may damage endothelial glycocalyx. Therefore, in case of sepsis; a cycle of cause-and-effect will occur - hence worsen the situation.(5,8,9)

hyperglychemic

Hyperglycemia can damage endothelial glycocalyx layer. In a research done by Nieuwdorp et.al., it is found that type 1 diabetes has thinner endothelial glycocalyx and higher plasma hyaluronan levels compared to controls. This condition can be worse if microalbuminuria is present.(6,10)

In another research on 10 Caucasian men regarding the effect of acute hyperglycemia on glycocalyx (which is also done by Nieuwdorp et.al.,), it is found that glycocalyx is damaged and thinner in hyperglycemic conditions compared to controls. Moreover, higher hyaluronan levels are also found in plasma compared to controls, which further supports the fact that damaged glycocalyx will enter the plasma. This damage also disrupts vascular permeability. However, the cause of this condition is not fully understood yet. It is suspected that this condition is caused by oxidative stress.(11)

Hypoalbuminemia

Under normal condition, albumin plays a role in regulating plasma oncotic pressure, protecting glycocalyx, as well as having anti-oxidant and anti-inflammatory effects. It tightens glycocalyx and limits molecules which can penetrate endothelium, therefore glycocalyx is protected and not easily damaged. It can also bind copper ions (Cu2+) in blood which may trigger oxidative stress.(12)

In case hypoalbuminemia occurs (where the albumin level in the plasma is low), the physiological function of albumin will be lost; causing impaired vascular permeability, increased oxidative stress and inflammation to occur. This condition is known to damage endothelial glycocalyx - which will further aggravate the damage caused by hypoalbuminemia.(12)

Disorders Caused By Ischemia And Reperfusion

In case ischemia and reperfusion disorders occur (e.g. cardiac arrest, hemorrhagic shock, cardiogenic shock, embolism, or organ transplantation); it will decrease blood flow and oxygenation. This condition may cause blood vessel damage, inflammation, increased oxidative stress, as well as trigger platelet adhesion to damaged blood vessels. Furthermore, all of those conditions will damage endothelial glycocalyx and enter the plasma.(13)

Endothelial Glycocalix As Therapeutic Target And Biomarker

Damage to endothelial glycolix shows a strong relation with disease progression. The severity of the damage signifies the severity of the disease. Therefore, therapies which can maintain and repair endothelial glycocalyx have been developed as therapeutic targets in several "in vitro" researches. Several therapies used are such as sevoflurane, albumin (5%), hyaluronan sulfate, chondroitin sulfate and glucocorticoids. However, further researches are still necessary to support the therapies.(13,14)

Moreover, the degradation component of endothelial glycocalyx dissolved in plasma can be examined and used as a modality for diagnostic, monitoring and prognostication of a disease. These components include syndecan-1, heparan sulfate, hyaluronate and chondroitin sulfate. The detected level signifies the severity of the disease.(13,14)

CONCLUSION

Endothelial glycocalyx is a gel-like layer which covers the surface of endothelial cells. It is composed of two main components, which are glycoproteins and proteoglycans. Physiologically, glycocalyx functions to regulate vascular permeability, regulate blood cell and endothelial interactions, as well as to regulate endothelial environment. The damage to this layer is related with the disease severity, therefore it has been developed as a therapeutic target and biomarker.

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