© Wydawnictwo UR 2021
ISSN 2544-1361 (online); ISSN 2544-2406
doi: 10.15584/ejcem.2021.3.6

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2021; 19 (3): 246–250

REVIEW PAPER

Jakub Wawrzkowicz (ABCDFG), Marcin Witek (ABCDFG), Izabela Winiarczyk (ABCDFG), Michał Wyleciał (ABCDFG), Agata Drożdżyk (ABCDFG), Klaudia Szelengiewicz (ABCDFG)

The significance of glycocalyx in medicine

Student's Scientific Club "URCell" at the Medical College of Rzeszow University, Rzeszow, Poland
Supervisors: Dorota Bartusik-Aebisher, Sabina Galiniak

ABSTRACT

Introduction. The glycocalyx is a gel-like layer covering the membrane of many cells, especially cells of epithelial tissue. It consists of membrane-bound proteoglycans, glycosaminoglycan chains, glycoproteins, and adjacent proteins. Glycocalyx is necessary in maintaining the permeability of vessels, modulation of inflammatory responses and interactions between cells. It is also involved in cell adherence, mobility, mechanotransduction, regulation of the cell cycle and cell. Abnormalities in the structure and function of the glycocalyx underlie many diseases and disorders such as dry eyes disease, diabetes and its complications as well as sepsis.

Aim. In this review, we present the current view on the role of glycocalyx in human diseases.

Material and methods. This review was performed according to latest literature from the following databases: EBSCO, PubMed, Science Direct, and Springer Link.

Analysis of the literature. Pathological mechanisms such as disruption of the glycocalyx barrier and decreased hydration of the ocular epithelial surface cause dry eye disease. During hyperglycaemia, glycocalyx dysfunction occurs, which leads to its dysfunction and activation of the prothrombotic system. Moreover, the increase in the concentration of hyaluronidase leads to increase in the plasma hyaluronan levels and promotion of endothelial dysfunction. Additionally, degradation of glycocalyx in sepsis prevails over increased synthesis of its components strongly favors its enhanced enzymatic degradation.

Conclusion. A better understanding of glycocalyx impairment in disease could alter therapeutic strategies to improve patient outcomes.

Keywords. disease, glycocalyx degradation, glycosaminoglycans, hyperglycemia, medicine

Introduction

The glycocalyx is a carbohydrate-rich layer that covers the membrane of many cells, especially cells of epithelial tissue. It was first described on the surface of endothelial cells by Danielli in 1940.[1] Glycocalyx is necessary in maintaining the proper permeability of blood vessels and modulates interactions between blood cells and endothelium, and is also involved in cell adherence, mobility, mechanotransduction, regulation of the cell cycle and proliferation of normal cells.[2] Moreover, glycocalyx

Corresponding author: Jakub Wawrzkowicz, e-mail: jakubpiotr.wawrzkowicz@gmail.com

Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

Received: 24.06.2020 | Accepted: 5.08.2021
Publication date: September 2021

Wawrzkowicz J, Witek M, Winiarczyk I, Wyleciał M, Drożdżyk A, Szelengiewicz K. *The significance of glycocalyx in medicine*. Eur J Clin Exp Med. 2021;19(3):246–250. doi: 10.15584/ejcem.2021.3.6

participates in immunological processes by modulating inflammatory responses by binding cytokines and weakening their binding to cell membrane receptors. In addition, it is known to be involved in the process of angiogenesis and metastasis.[3]

The biochemical composition of glycocalyx depends on the type of cell, type of tissue and the prevailing mechanical and physicochemical conditions, but its main building blocks are proteoglycans and glycoproteins. Proteoglycans have long and unbranched carbohydrate chains consisting of core proteins that covalently link to at least one molecule of glycosaminoglycans (GAG). GAGs are linear polysaccharides made up of repeating disaccharide units with N-acetylated or N-sulfated hexosamine or uronic acid (glucuronic acid or iduronic acid) or galactose. The dominant GAG group is heparan sulfate, which has numerous sulfate groups, which accounts for 50–90% of GAG. The second most common GAG in the glycocalyx of endothelial cell is chondroitin sulfate/dermatan sulfate.[4] The presence of heparan sulfate and chondroitin sulfate is estimated to have a typical ratio of 4:1 for the endothelium.[5] Proteoglycans show great structural diversity as they can contain GAGs of various numbers and chain lengths, which are further modified by repeating disaccharides forming a complex pattern of sulfate groups and a different core protein structure. The core proteins of proteoglycans, mainly syndecan and glypican, are linked to the cell membrane by a glycosylphosphatidylinositol anchor or a transmembrane protein domain.[6] On the other hand, glycoproteins are composed of a protein to which usually straight-chain oligosaccharides are covalently attached, usually consisting of several monosaccharide residues (N-acetylhexosamine, galactose or mannose). The major glycoproteins of the glycocalyx include selectins, integrins, and other adhesion molecules such as intercellular adhesion molecules 1 and 2, and thrombocyte/endothelial adhesives.[7,8] Additionally, other molecules are attached to the glycocalyx, including growth factors and their receptors, coagulation inhibitors, lipoprotein lipase and low-density lipoproteins, and plasma and extracellular matrix proteins.[6] Research by Kabedev and Lobaskin shows that changes in glycocalyx glycan density determine its resistance to slight deformation, which also prevents stress transfer to the cell membrane.[9]

The thickness of the glycocalyx layer also depends on the type of cell and the diameter of the vessels, but measurements with an atomic force microscope revealed that on the surface of the endothelium the structure is about 0.3–1 μm.[10] Nevertheless, Ebong et al. using transmission electron microscopy observed that the glycocalyx on the surface of the endothelium lining the bovine aorta can be as much as 11 μm thick.[11]

Pathological breakdown of glycocalyx occurs in response to many factors including mechanical cell stress, endotoxins, pro-inflammatory cytokines such as tumor necrosis factor alpha, reactive oxygen species, and hyperglycemia.[12,13] Abnormalities in the structure and functioning of the glycocalyx underlie many diseases and disorders such as dry eyes disease, diabetes and its complications as well as sepsis.

Aim

In this review, we present the current view on the role of glycocalyx in human diseases

Material and methods

This review was performed according to latest literature from the following databases: EBSCO, PubMed, Science Direct, and Springer Link.

Analysis of the literature

Dry eyes disease

Dry eye disease is a common and multifactorial disease with a high prevalence worldwide. In the apical part of the eye epithelium there are microfolds covered with glycocalyx, whose main function is to increase the surface area. Glycocalyx protects the surface of epithelial cells against chemical and mechanical damage, and also prevents the penetration of pathogens into the eye, reduces friction during blinking and maintains the hydrophilic surface of the eye. Pathological mechanisms such as disruption of the glycocalyx barrier and decreased hydration of the ocular epithelial surface cause dry eye disease.[14]

Research indicates that transmembrane mucins and galectin-3 play a key role in the glycocalyx epithelial barrier and are essential for maintaining proper hydration of the eye epithelium. The very large glycans in transmembrane mucus are necessary to keep the eye surface wettable due to their very high water-holding capacity. Among them, three main mucins can be distinguished – MUC1, MUC4 and MUC16. *In vitro* studies show that MUC1 serves as an anti-adhesion and signaling molecule. In turn, MUC4 is involved in proliferation signaling by activating the ErbB2 tyrosine kinase receptor. MUC16, which is the largest membrane-bound mucin, plays a key role in the formation of the protective shell.[15,16] It has also been shown that the reduction or alteration of the chemical composition of the glycocalyx on the surface of the eye may be one of the factors contributing to the dryness of the eyes associated with the use of soft contact lenses.[17]

Diabetes and its complications

One of the diseases in which the glycocalyx plays an important and confirmed role is diabetes. With this disease, there is a marked increase in the risk of developing vascular complications. Vascular complications developing on a micro and macro scale lead to a deterioration of the comfort and quality of life as well as a shortened life expectancy of patients.[18] An early symptom of dam-

age to blood vessels is an increase in their permeability, which results in the disruption of their physiological functions.[19,20] This leads to the onset of albuminuria and the development of cardiac complications.[12] Under physiological conditions, the glycocalyx protects cells from direct contact with blood. During hyperglycaemia, glycocalyx dysfunction occurs, which leads to its dysfunction and activation of the prothrombotic system.[21] In patients with type 1 diabetes, the volume of glycocalyx is reduced by 50% within 6 h after induction of acute hyperglycaemia.[22] The loss of glycocalyx is directly related to the increase in the concentration of hyaluronidase what leads to increase in the plasma hyaluronan levels and promotion of endothelial dysfunction.[23] Moreover, hyperglycemia changes the phenotype of fibroblasts which are more elongated but less motile and less contractile than healthy dermal fibroblasts. It is caused by formation of larger focal adhesions stabilized by a glycocalyx as well as associated with increased expression of the cell surface glycoprotein MUC16.[24] Additionally, recent study by Wadowski et al. indicated that the loss of glycocalyx dimensions is correlated with increasing level of glycated hemoglobin.[25]

Research was conducted to confirm the hypothesis of possible beneficial effects that restoration of glycocalyx volume may have in the context of preventing the development of diabetes complications. It was examined whether there was a reduction in the amount of glycocalyx in patients with type 2 diabetes and whether oral administration of glycosaminoglycan precursors improved the activity of glycocalyx. Ten men with type 2 diabetes and ten control group were included in the study, in whom the level of glycocalyx was determined before and 2 months after the administration of one of GAGs – sulodexide at a dose of 200 mg/day. The conducted research has shown that in the case of patients with type 2 diabetes, the activity of glycocalyx in relation to healthy people was impaired. This group of patients also experienced an increase in vascular permeability (increased albuminuria).[26] GAGs such as sulodexide act at multiple levels: they promote glycocalyx reconstitution, control glycocalyx degrading enzymes, exert anti-inflammatory effects and have anti-apoptotic and anti-senescence effects on endothelial cells.[27]

The kidneys of people with diabetes are characterized by glomerular hypertrophy, thickening of the basement membrane, and damage to endothelial cells and glycocalyx. Even in the case of microalbuminuria, a reduced amount of glycocalyx and the enzyme heparinidase, responsible for the breakdown of GAGs, were engraved.[28,29]

The hyperglycemia present in diabetes causes the production of reactive oxygen species as well as vascular endothelial growth factor and angiotensin II.[30,31] These substances reduce the selectivity of the barrier as a result of the weakening of the action of superoxide dismutase, the function of which is the decomposition of the superoxide anion. Such oxidative stress damages the glycocalyx and impairs filtration.[32] As the disease progresses, the amount of protein in the urine increases – podocytes are damaged, and angiotensin II, along with increased glucose levels, weakens the production of proteoglycans by podocytes.[33-35] More and more active nephrons are lost, causing the remaining nephrons to become overloaded and hypertrophied, followed by interstitial tissue fibrosis and renal dysfunction.[33] As a result, anemia, secondary hyperparathyroidism, bone mineralization disorders, and soft tissue calcification appear.[36]

In improving the health of people with cardiovascular diseases, related to type II diabetes, exercise and a low-salt diet can help. This helps to maintain a "thick layer" of the endothelial glycocalyx which is important in preventing clotting.[37] With the increase of physical activity, increases heart rate, and the rate of blood flow in blood vessels. This favors the transport of Na^+ ions from the endothelial glycocalyx. Due to the action of electrostatic forces, the space between red blood cells, the glycocalyx of the endothelium, increases.[38,39] The charge of erythrocytes is negative, and along with the removal of sodium cations (supplied, among others, with NaCl salt) from the glycocalyx, the total charge is more negative and repulsion increases.[40]

Sepsis

Sepsis is an ominous clinical manifestation of a severe generalized infection leading to systemic multiorgan sequelae such as hypotension, acute respiratory distress syndrome, renal failure, and lactic acidosis, among others. Glycocalyx is also used effectively as a biomarker for sepsis. During the initial development of sepsis, it is damaged.[41,42] The fact that the degradation of glycocalyx in sepsis prevails over increased synthesis of at least one of its components strongly favors its enhanced enzymatic degradation. It is carried out by sheddases, a disintegrin and metalloproteinases, matrix metalloproteinase, heparanase-1, and hyaluronidases.[43-45] As a result, the level of circulating glycocalyx components such as syndecans, heparan sulfates and hyaluronic acid increases. Their level is measured, on the basis of which the developing pathogenic process can be determined. Despite the accumulated knowledge regarding the important roles of the glycocalyx, the relationship between derangement of the endothelial glycocalyx and severity of sepsis has not been adequately elucidated.[46]

Conclusion

Over the past two decades, glycocalyx has attracted more and more attention from scientists, and its role in health and disease has been intensively studied. However, despite the well-described pathological sequelae that follow glycocalyx damage, there are few reports of therapeutic strategies targeting impairment of the glycocalyx structure and function.

References

1. Danielli JF. Capillary permeability and oedema in the perfused frog. *J Physiol.* 1940;98:109-129.
2. Kapellos GE, Alexiou TS. Chapter 1 - Modeling Momentum and Mass Transport in Cellular Biological Media: From the Molecular to the Tissue Scale. *Transport in Biological Media* 2013:1-40.
3. Kang H, Wu Q, Sun A, Liu X, Fan Y, Deng X. Cancer Cell Glycocalyx and Its Significance in Cancer Progression. *Int J Mol Sci.* 2018;19(9):2484.
4. Bar P, Galiniak S, Bartusik-Aebisher D, et al. Glycosaminoglycan concentration in cancer tissue. *Eur J Clin Exp Med.* 2019;17(2):175-178.
5. Mulivor AW, Lipowsky HH. Inflammation- and ischemia-induced shedding of venular glycocalyx. *Am J Physiol Heart Circ Physiol.* 2004;286(5):H1672-H1680.
6. Kolářová H, Ambrůzová B, Svihálková Šindlerová L, Klinke A, Kubala L. Modulation of endothelial glycocalyx structure under inflammatory conditions. *Mediators Inflamm.* 2014;2014:694312.
7. Yilmaz O, Afsar B, Ortiz A, Kanbay M. The role of endothelial glycocalyx in health and disease. *Clin Kidney J.* 2019;12(5):611-619.
8. Tarbell JM, Cancel LM. The glycocalyx and its significance in human medicine. *J Intern Med.* 2016;280(1):97-113.
9. Kabedev A, Lobaskin V. Structure and elasticity of bush and brush-like models of the endothelial glycocalyx. *Sci Rep.* 2018;8(1):240.
10. Bai K, Wang W. Spatio-temporal development of the endothelial glycocalyx layer and its mechanical property in vitro. *J R Soc Interface.* 2012;9(74):2290-2298.
11. Ebong EE, Macaluso FP, Spray DC, Tarbell JM. Imaging the endothelial glycocalyx in vitro by rapid freezing/freeze substitution transmission electron microscopy. *Arterioscler Thromb Vasc Biol.* 2011;31(8):1908-1915.
12. Butler MJ, Down CJ, Foster RR, Satchell SC. The Pathological Relevance of Increased Endothelial Glycocalyx Permeability. *Am J Pathol.* 2020;190(4):742-751.
13. Hahn RG, Patel V, Dull RO. Human glycocalyx shedding: Systematic review and critical appraisal. *Acta Anaesthesiol Scand.* 2021;65(5):590-606.
14. Uchino Y. The Ocular Surface Glycocalyx and its Alteration in Dry Eye Disease: A Review. *Invest Ophthalmol Vis Sci.* 2018;59(14):DES157-DES162.
15. Ablamowicz AF, Nichols JJ. Ocular Surface Membrane-Associated Mucins. *Ocul Surf.* 2016;14(3):331-341.
16. Portal C, Gouyer V, Gottrand F, Desseyn JL. Ocular mucins in dry eye disease. *Exp Eye Res.* 2019;186:107724.
17. Fukui M, Yamada M, Akune Y, Shigeyasu C, Tsubota K. Fluorophotometric Analysis of the Ocular Surface Glycocalyx in Soft Contact Lens Wearers. *Curr Eye Res.* 2016;41(1):9-14.
18. Dogné S, Flamion B, Caron N. Endothelial Glycocalyx as a Shield Against Diabetic Vascular Complications: Involvement of Hyaluronan and Hyaluronidases. *Arterioscler Thromb Vasc Biol.* 2018;38(7):1427-1439.
19. Parsons JT, Horwitz AR, Schwartz MA. Cell adhesion: integrating cytoskeletal dynamics and cellular tension. *Nat Rev Mol Cell Biol.* 2010;11(9):633-643.
20. Weinbaum S, Cancel LM, Fu BM, Tarbell JM. The Glycocalyx and Its Role in Vascular Physiology and Vascular Related Diseases. *Cardiovasc Eng Technol.* 2021;12(1):37-71.
21. Cao RN, Tang L, Xia ZY, Xia R. Endothelial glycocalyx as a potential theriapeutic target in organ injuries. *Chin Med J (Engl).* 2019;132(8):963-975.
22. Nieuwdorp M, van Haeften TW, Gouverneur MCLG, et al. Loss of endothelial glycocalyx during acute hyperglycemia coincides with endothelial dysfunction and coagulation activation in vivo. *Diabetes.* 2006;55:480-486.
23. Nieuwdorp M, Mooij HL, Kroon J, et al. Endothelial glycocalyx damage coincides with microalbuminuria in type 1 diabetes. *Diabetes.* 2006;55(4):1127-1132.
24. Jahan I, Pandya J, Munshi R, Sen S. Glycocalyx disruption enhances motility, proliferation and collagen synthesis in diabetic fibroblasts. *Biochim Biophys Acta Mol Cell Res.* 2021;1868(4):118955.
25. Wadowski PP, Kautzky-Willer A, Gremmel T, et al. Sublingual microvasculature in diabetic patients. *Microvasc Res.* 2020;129:103971.
26. Broekhuizen LN, Lemkes BA, Mooij HL, et al. Effect of sulodexide on endothelial glycocalyx and vascular permeability in patients with type 2 diabetes mellitus. *Diabetologia.* 2010;53(12):2646-2655.
27. Masola V, Zaza G, Onisto M, Lupo A, Gambaro G. Glycosaminoglycans, proteoglycans and sulodexide and the endothelium: biological roles and pharmacological effects. *Int Angiol.* 2014;33(3):243-254.
28. Mahtal N, Lenoir O, Tharaux PL. Glomerular Endothelial Cell Crosstalk With Podocytes in Diabetic Kidney Disease. *Front Med (Lausanne).* 2021;8:659013.
29. van der Vlag J, Buijsers B. Heparanase in Kidney Disease. *Adv Exp Med Biol.* 2020;1221:647-667.
30. Meza CA, La Favor JD, Kim DH, Hickner RC. Endothelial Dysfunction: Is There a Hyperglycemia-Induced Imbalance of NOX and NOS? *Int J Mol Sci.* 2019;20(15):3775.
31. Fakhruddin S, Alanazi W, Jackson KE. Diabetes-Induced Reactive Oxygen Species: Mechanism of Their Generation and Role in Renal Injury. *J Diabetes Res.* 2017;2017:8379327.
32. Jourde-Chiche N, Fakhouri F, Dou L, et al. Endothelium structure and function in kidney health and disease. *Nat Rev Nephrol.* 2019;15(2):87-108.
33. Singh A, Ramnath RD, Foster RR, et al. Reactive oxygen species modulate the barrier function of the human glomerular endothelial glycocalyx. *PLoS One.* 2013;8(2):e55852.
34. Fu J, Lee K, Chuang PY, Liu Z, He JC. Glomerular endothelial cell injury and cross talk in diabetic kidney disease. *Am J Physiol Renal Physiol.* 2015;308(4):F287-297.
35. Ebefors K, Lassén E, Anandakrishnan N, Azeloglu EU, Daehn IS. Modeling the Glomerular Filtration Barrier and Intercellular Crosstalk. *Front Physiol.* 2021;12:689083.

36. Shroff R. Reducing the burden of cardiovascular disease in children with chronic kidney disease: prevention vs. damage limitation. *Pediatr Nephrol.* 2021. doi: 10.1007/s00467-021-05102-y.
37. Kröpfl JM, Beltrami FG, Rehm M, Gruber HJ, Stelzer I, Spengler CM. Acute exercise-induced glycocalyx shedding does not differ between exercise modalities, but is associated with total antioxidative capacity. *J Sci Med Sport.* 2021;24(7):689-695.
38. Majerczak J, Grandys M, Duda K, et al. Moderate-intensity endurance training improves endothelial glycocalyx layer integrity in healthy young men. Exp Physiol. 2017;102(1):70-85.
39. Schmitz B, Niehues H, Lenders M, et al. Effects of high-intensity interval training on microvascular glycocalyx and associated microRNAs. *Am J Physiol Heart Circ Physiol.* 2019;316(6):H1538-H1551.
40. Oberleithner H, Peters W, Kusche-Vihrog K, et al. Salt overload damages the glycocalyx sodium barrier of vascular endothelium. *Pflugers Arch.* 2011;462(4):519-528.
41. Wiesinger A, Peters W, Chappell D, et al. Nanomechanics of the endothelial glycocalyx in experimental sepsis. *PLoS One.* 2013;8(11):e80905.
42. Nieuwdorp M, Meuwese MC, Mooij HL, et al. Tumor necrosis factor-alpha inhibition protects against endotoxin-induced endothelial glycocalyx perturbation. *Atherosclerosis.* 2009;202(1):296-303.
43. Uchimido R, Schmidt EP, Shapiro NI. The glycocalyx: a novel diagnostic and therapeutic target in sepsis. *Crit Care*. 2019;23(1):16.
44. Ince C, Mayeux PR, Nguyen T, et al. The endothelium in sepsis. *Shock.* 2016;45(3):259-270.
45. Goligorsky MS, Sun D. Glycocalyx in Endotoxemia and Sepsis. *Am J Pathol.* 2020;190(4):791-798.
46. Iba T, Levy JH. Derangement of the endothelial glycocalyx in sepsis. *J Thromb Haemost.* 2019;17(2):283-294.