Endothelial glycocalyx layer and interdependence of lymphatic and integumentary systems


Authors:
Robyn Bjork and Heather Hettrick

The endothelial glycocalyx layer controls movement of proteins and fluid across the blood capillary wall and there is no reabsorption back into the venous side of blood capillaries. Reabsorption occurs through lymphatic capillaries alone; consequently, a new paradigm of all oedemas falling into a continuum of relative lymphatic dysfunction is embraced. Additionally, areas of lymphatic failure produce areas of integumentary vulnerability subject to infection, inflammation and carcinogenesis; essentially, skin barrier failure. These combined findings highlight interdependence of the lymphatic and integumentary systems and the need for a more unified clinical approach.

***Robyn Bjork** is Founder, CEO, Executive Director of Education, International Lymphedema & Wound Training Institute (ILWTI), Independent Wound & Lymphedema Consultant, Sigvaris, Inc; **Heather Hettrick** is Associate Professor, Department of Physical Therapy, Nova Southeastern University, Director of Wound Education, ILWTI, Fort Lauderdale, FL, USA*

Today's rapid advancements in chronic wound management and lymphatic medicine are exciting, bringing together branches of clinical practice that have been largely segmented in the past. As parallel interests in the treatment of chronic wounds and lymphoedema grows, it is essential that physicians, wound specialists and certified lymphoedema therapists have the same understanding of capillary fluid exchange.

Unfortunately, even some of the most current textbooks fail to recognise the importance of the endothelial glycocalyx layer (EGL), and its implications in a new lymphoedema paradigm. Additionally, it is important for physicians and clinicians alike to appreciate the interconnectedness of the lymphatic and integumentary systems to improve outcomes and patient quality of life. This review aims to summarise the historical training on capillary fluid exchange, simplify the current EGL evidence regarding its key functions, explain how the EGL changes the existing lymphedema paradigm, and highlight the relationship between lymphatic and integumentary impairment.

Starling's Law

In the late 1800s, Ernest Starling introduced a model of capillary fluid exchange, based on hydrostatic and oncotic pressures in the blood capillaries and interstitium (Starling, 1894). The capillary was viewed as a semi-porous membrane through which fluid could freely move in and out. Dilation of pre-capillary sphincters, increasing capillary hydrostatic fluid pressure, permitted fluid egress into the interstitium. Conversely, lower capillary hydrostatic pressure, coupled with higher capillary oncotic pressure of blood proteins, was thought to pull fluid back into the venous end of the blood capillaries. This became the basis of training in wound and lymphoedema certification programmes as the mechanism of fluid homeostasis.

Endothelial glycocalyx layer discovery

In 1940, Danielli introduced the concept of a protein-based lining of vessels that played a vital role in fluid filtration. In 1966, Luft succeeded in visualising this layer through electron microscopy. This led to a flurry of biomedical research, which was eloquently summarised by Reitsma et al (2007), and Weinbaum et al (2007). Each review cited over 135 research studies as supporting evidence. The "endothelial glycocalyx layer" gained recognition as controlling the movement of proteins and fluid across the blood capillary wall, through dynamic and complex processes.

New "no capillary reabsorption" rule

In 2010, Levick and Michel mathematically demonstrated that there is no net reabsorption of fluid back into the venous side of the blood capillaries, and that there is only diminishing

net filtration across the capillary bed. Only in extreme situations can capillaries and venules resorb fluid. In 2012, Woodcock and Woodcock stated that an acute reduction of transendothelial pressure (pre-capillary vasoconstriction, post-capillary vasodilation, hemorrhage or hypovolemia) will allow for transient venous absorption ('autotransfusion' up to 500 ml) to preserve blood volume. Mortimer and Rockson (2014) echoed this statement when they stated disturbances in Starling pressures will create a transient response (venous reabsorption) that is short lived as Starling forces rapidly readjust to a state of filtration.

Realising this concept challenged over 100 years of dogma surrounding Starling's Law, Levick and Michel wrote: "In making these forceful statements, we are mindful of William Harvey's remark in his classic, *De Motu Cordis* (1628): 'I tremble lest I have mankind as my enemies, so much has wont and custom become second nature. Doctrine once sown strikes deep its root, and respect for antiquity influences all men.'"

In 2013, the American Association of Nurse Anesthetists included continuing education training on the glycocalyx and its functions, through a pragmatic review by Biddle, who used the slime that coats fish as an analogy for the EGL. Yet, the new paradigm of the EGL eluded wound and lymphoedema specialists. Then, in 2014, Peter Mortimer and Stanley Rockson integrated this new understanding of no net reabsorption into their review 'New developments in clinical aspects of lymphatic disease', published in the Journal of Clinical Investigation. This set the stage for a new paradigm in wound care and lymphoedema management.

Questions raised

For healthcare professionals who treat patients with chronic wounds and oedema, and certified lymphoedema therapists trained in traditional theories, several questions arise. How exactly does the glycocalyx regulate fluid and protein movement through the capillary wall? What other functions does it have, and why can't fluid move back into the venous side of the capillaries, even with compression? Further, according to Levick and Michel (2010), the entire plasma fluid volume of ~3L leaves the circulation approximately once every 9 hours. With an estimated 8 L of total fluid leaving the capillaries per day, how do we reconcile that only ~ 4 L per day has been reported to re-enter

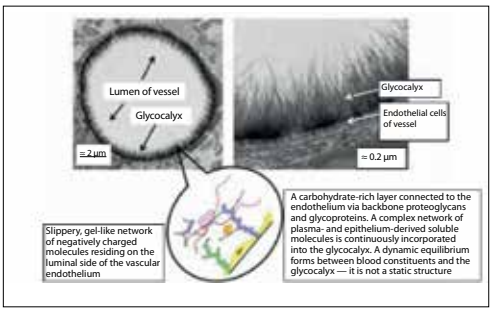

Figure 1. Vascular endothelial glycocalyx (Biddle, 2013). This figure was used with permission from the author.

the venous system at the angles of the internal jugular and subclavian veins? These questions can be answered through a closer look at the structure and function of the EGL.

Structure and functions of the endothelial glycocalyx layer

A healthy EGL is approximately o.5 um thick in the blood capillaries; it is progressively thicker in larger vessels, up to 4.5 um in carotid arteries (Reitsma et al, 2007; Weinbaum et al, 2007). The EGL is made up of two continuous layers. The base is a slimy layer coating the endothelial cells of the vessel wall. This gel matrix is made up of chains of glycoproteins and proteoglycans that attach directly into the membranes of the endothelial cells, creating 'backbones' (Reitsma et al, 2007; Weinbaum et al, 2007; Biddle, 2013)*[Figure 1]*. These backbones are linked together by a web of glycosaminoglycans that can absorb 10,000 times their weight in water (Biddle, 2013), thus creating a slimy gel layer. Within this base layer are curvy clefts, or channels, with tight junctions that control fluid and protein movement through the EGL (Weinbaum et al, 2007) *[Figure 2]*. These can be conceptualised as locks that are able to control the movement of ships through the Panama Canal.

The second layer of the EGL is made up of hair-like projections attached to the backbone proteins and matrix, which extend into the lumen of the blood vessel (Reitsma et al, 2007; Weinbaum et al, 2007) *[Figure 1]*. These hair-like projections are organised like bushes, with roots communicating with the gel base layer, all organised into a hexagonal matrix (Weinbaum et al, 2007) *[Figure 3]*. Since the backbone proteins are tethered into the endothelial cell membranes of the capillary wall, and crosslinked in the matrix, blood flow shear forces acting on the hair-like projections

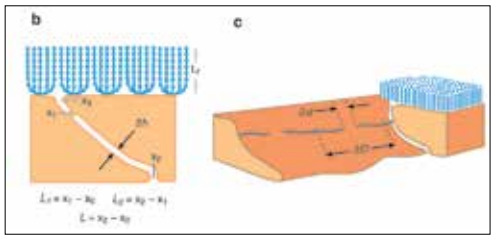

Figure 2. Schematic of EGL bush-like organisation of the hair-like projections, and curvy clefts in EGL gel-like base layer which control fluid and protein movement through the blood capillary wall (Weinbaum et al, 2007). This figure was used with permission from the Annual Review of Biomedical Engineering.

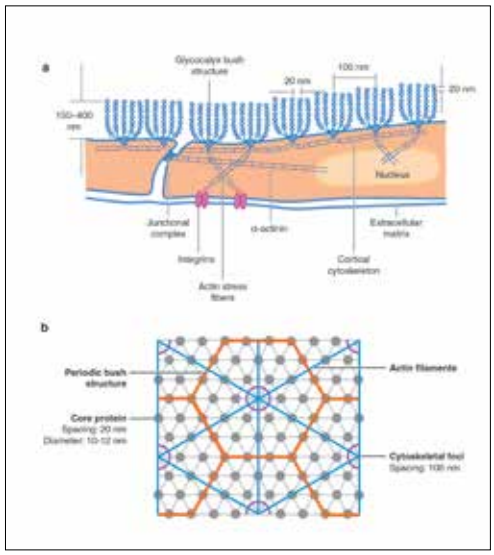

Figure 3. Schematic of the hexagonal organisation of the EGL (Weinbaum et al, 2007). This figure was used with permission from the Annual Review of Biomedical Engineering.

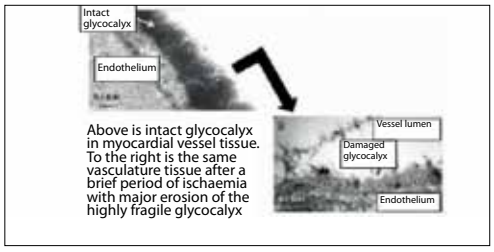

Figure 4. Shedding of the EGL in response to ischemia (Biddle, 2013). This figure was used with permission from the author.

mechanically transmit this information into the endothelial cells themselves. The endothelial cells then respond to the mechanical signals, such as producing and releasing nitric oxide that dilates the vessel (Biddle, 2013).

Compositively, the EGL layers plus the soluble proteins and other components that bind to it, have a negative charge that repels red blood cells (RBCs) and platelets so they do not touch the vessel wall. This space between the RBCs and the EGL is called the "exclusion zone" (Reitsma et al, 2007). The EGL is dynamic and can 'shed' in response to stimuli, such as during inflammation or disease states *[Figure 4]*. Shedding is conceptualised as a dog shedding its fur, or a person receiving a short haircut. During inflammation, this shedding allows more fluid to escape through the EGL. Shedding also exposes adhesion molecules (Reitsma et al, 2007) to which platelets or white blood cells (WBCs) attach. WBCs are squeezed in the blood capillary where they enter the venule and are known to crush the EGL temporarily by 20% (Weinbaum et al, 2007). It is here that WBCs tether to exposed adhesion molecules and then remain tethered as they roll across the venule wall to exit into the tissues, known as diapedesis.

The EGL is particularly sensitive to ischaemia, which can result in rapid shedding. A high fat, high cholesterol diet, oxidative low-density lipoproteins, and hyperglycemia all cause shedding of the EGL, and the EGL has been found to be thinner in areas prone to atherosclerosis (Reitsma et al, 2007). The EGL plays a key role in diabetes mellitus, peripheral arterial disease, reperfusion injury, intravenous fluid mismanagement, renal disease and dialysis (Biddle, 2013). It also has an antithrombotic effect due to "enzyme docking" and plays a significant role in reducing oxidative stress (Biddle, 2013).

New lymphoedema paradigm

Acting as a complex molecular sieve, the EGL precisely regulates fluid and protein movement through the capillary wall into the tissues (Reitsma et al, 2007; Weinbaum et al, 2007; Woodcock and Woodcock, 2012). Conversely, the EGL prevents movement of proteins and fluid back into the venous side of the capillaries, even when interstitial tissue hydrostatic pressure is increased, or capillary oncotic pressure is higher than the tissue oncotic pressure. Thus, all fluid and proteins exiting the blood capillaries must be removed from the interstitium by the lymphatic capillaries alone.

For healthcare providers, wound specialists and certified lymphedema therapists, this is of paramount importance. All oedemas are on a lymphedema continuum. As noted by Rockson and Mortimer (2014): "Arguably, it may be better to consider the presence of chronic oedema as synonymous with the presence of lymphoedema, inasmuch all

oedema represents relative lymph drainage failure". They go on to further state that "all chronic oedema indicates an inadequacy or failure of lymph drainage; therefore, a clinical approach to peripheral (i.e., subcutaneous) oedema should begin with a consideration of lymphatic function to assess whether this is a primary impairment or whether a normal lymphatic circulation is simply overloaded by high microvascular filtration." Complete Decongestive Therapy (CDT) can be integrated into all aspects of rehabilitation to manage chronic oedemas to prevent progression to the inflammatory tissue changes that have been associated with the traditional view of lymphoedema diagnosis in the past. The new lymphoedema paradigm opens doors to useful applications of CDT for a variety of oedemas, and as a possible preventative measure against various skin impairments associated with inflammatory tissue changes.

For an astute observer, the Stemmer's testing method can be used to assess any oedema, at any location, to determine the progression in this lymphoedema continuum. For example, retro-malleolar swelling post ankle fracture, or chronic oedema surrounding a venous leg ulcer, can lead to localised protein accumulation and degradation, resulting in localised inflammation and connective tissue proliferation. These integumentary changes associated with lymphoedema will be discussed below as the health of skin in regional areas of lymphatic dysfunction are subject to skin barrier failure and compromised integrity. As such, wounds and impaired cutaneous function are highly associated with inflammation and fibrosis associated with lymphatic dysfunction. CDT, including compression and manual therapy, can be utilised as an intervention to influence soft tissue remodeling to a more normalised state. Further, CDT can assist with improving skin barrier function and wound resolution.

Reabsorption exception in lymph nodes

Once the interstitial fluid enters the lymphatic capillary, the lymph is funneled through pre-collectors and into collectors that propel the lymph toward lymph nodes, via sequenced contraction of lymphangions, coupled with one-way valves. As previously described, under normal conditions, ~4 L of lymph re-enters the venous system at the venous angles in the neck. However, a sum of ~8 L/day of fluid moves out of the blood capillaries and into the tissues. The structure and function of the lymph nodes is key to reconciling this apparent discrepancy.

In 1983, Knox et al found that ~50% of the fluid portion of lymph is reabsorbed into the venous circulation via the blood capillaries in canine lymph nodes. Further, Adair and Guyton (1983) also demonstrated that increasing the venous pressure in canine lymph nodes resulted in movement of fluid back into the node, reducing the concentration of proteins in the efferent lymph vessels. This elevates the role of the lymphatics and lymph nodes in fluid homeostasis, as well as the impact of chronic venous hypertension. Elevated venous pressure not only results in ultrafiltration from the blood capillaries, but also slows reabsorption of fluid from the lymph nodes back into the venous circulation. The dense, capsular design of the lymph nodes, their placement in joint areas that are mechanically compressed by movement, and the presumed absence of EGL, all work synergistically to facilitate fluid reabsorption back into the venous system. Conversely, immobility and decreased joint movement through the full range of motion, lymph node removal, or venous hypertension, can have a significant impact on fluid retention in the dermis and subcutaneous tissues.

Relationship between lymphatic and integumentary systems

It is well established that there is a paucity and lack of standardisation with respect to wound and lymphoedema-related education in traditional medical and healthcare profession education. A study published in 2008 by Patel et al, compared wound education in medical school curricula between the United States, Germany and the United Kingdom. The results of this retrospective indicated that the "total hours of required wound education received in the United States was 9.2 hours in the 4 years of medical school. In the United Kingdom, the total time devoted to wound-related issues equaled 4.9 hours over 5 years. In Germany, a total of 9 hours of wound education was provided over 6 years." This study concluded that there is a deficiency with respect to wound education in preparing future physicians to manage wounds.

With respect to lymphoedema, the education is even more sparse. A survey study in 2011 by Vuong, Nguyen and Piller found that the level of lymphatic education provided in medical schools around the United States, indicated that most programmes dedicated 30 minutes

or less to teaching lymphatic function in the first two years of medical school. Further, nearly 40% of respondents indicated that 1–3 hours of time was devoted to the lymphatic system, while 25% indicated that 15 minutes or less was spent on the topic. The apparent lack of dedicated time in traditional medical education is further compounded by the fact that these two systems are highly inter-dependent; meaning, impairment in one system directly impacts the other.

Published work by Carlson and Földi and Földi highlight this inter-dependence. Carlson describes in his 2014 review article how lymphatic failure produces a cutaneous region susceptible to infection, inflammation and carcinogenesis. What he describes as a "locus minoris resistentiae", or path of least resistance. He describes in his article how lymphatic failure causes a disruption of adaptive immunity by "decreasing or obstructing immune trafficking by antigen, lymphocytes, macrophages and dendritic/antigen presenting cells (Langerhans cells) to the lymph node, creating a cutaneous region of immunosuppression ... or a condition called lymphatic dermopathy, which is failure of the skin as an immune organ." Carlson's findings are amplified when applied to the work undertaken by Földi and Földi.

Lymphoedema is caused by dynamic or mechanical insufficiency of the lymphatic system, resulting in a high-protein oedema in the dermis and subcutaneous tissues. According to Földi (2012): "[...] stagnating high protein oedema develops a pathohistological state of chronic inflammation, with infiltration of the tissue by mononuclear cells, angiogenesis, proliferation of connective tissue, fibrosis and fibrosclerosis." He further goes on to describe how oxidation and degradation of interstitial proteins attracts monocytes (macrophages) that, in turn, ingest proteins and activate fibroblasts and adipocytes. This results in connective tissue and adipose proliferation causing enlargement of the body part with thickened, fibrotic dermis and subcutaneous tissues.

These sequelae also damage lymphatic capillaries and vessels (Lee, 2018), further exacerbating the lymphoedema, and compromising the essential immune function of the skin. Chronic wounds in these locations are rendered vulnerable to high bioburden, chronic infections, and recurrent cellulitis, particularly in the presence of underlying comorbidities, such as diabetes and peripheral arterial disease, to name a few. Lymphatic impairment leads to integumentary dysfunction and integumentary dysfunction can exacerbate lymphatic dysfunction. For clinicians, it is important to recognise the inter-dependence these systems have on one another so proper diagnosis and interventions can be delivered.

A key example of this relationship is highlighted with chronic venous insufficiency (CVI). Venous hypertension increases fluid in the tissues secondary to high filtration pressure. This increased fluid load exceeds the lymphatic transport capacity. Over time, this can lead to lymphatic hypertension which can set off a cascade of deleterious tissue changes further damaging the lymphatics and the integument. Clinically, this manifests as phlebolymphedema, (particularly at the CEAP 3 classification level) and contributes to the development of venous ulceration.

Conclusion

In conclusion, the role of the vascular endothelial glycocalyx layer as the gate-keeper for capillary fluid exchange is extensively supported in the literature. It is now well established that there is only diminishing net fluid filtration, and no reabsorption, across the blood capillaries of the dermis and subcutaneous tissues, except in extreme instances, such as hemorrhage or hypovolemia. All fluid and blood proteins moving into the interstitium each day must be removed via reabsorption through the lymphatic capillaries alone. Thus, all oedemas fall on a continuum of lymphoedema, and can lead to chronic inflammation and tissue thickening caused by accumulation and degradation of proteins.

Since the lymphatic and integumentary systems are inter-dependent, impairment in one system influences impairment in the other, with varying levels of complexity and clinical presentation. Impaired lymphatics compromise the essential immune functions of the skin, rendering the skin and chronic wounds vulnerable to high bioburden, chronic infections, and recurrent cellulitis. Thus, improved collaboration is needed between physicians, wound specialists and lymphoedema therapists to establish the cohesiveness of paradigms and common language, as well as interdisciplinary care for individuals with lymphoedema and chronic wounds.

Acknowledgements

The authors would like to thank Stanley G. Rockson, Chief of Consultative Cardiology, Allan and Tina Neill Professor of Lymphatic Research and Medicine, Professor of Medicine and Director, Center for Lymphatic and Venous Disorders Stanford University School of Medicine, Stanford, California, USA, for constructive advice relating to this article.

References

Adair TH, Guyton AC (1983) Modification of lymph by lymph nodes. II. Effect of increased lymph node venous blood pressure. *Am J Physiol* 245(4): H616–H622

Biddle C (2013) Like a slippery fish, a little slime is a good thing: the glycocalyx revealed. *AANA J* 81(6): 473–80

Carlson JA (2014) Lymphedema and subclinical lymphostasis (microlymphedema) facilitate cutaneous infection, inflammatory dermatoses, and neoplasia: A locus minoris resistentiae. *Clin Dermatol* 32(5): 599–615

Danielli JF (1940) Capillary permeability and oedema in the perfused frog. *J Physiol* 98(1): 109–29

Foldi M, Foldi E (2012) *Textbook of Lymphology* (3rd edn.). Elsevier GmbH, Munchen, Germany

Knox P, Pflug JJ (1983) The effect of the canine popliteal node on the composition of lymph. *J Physiol* 345: 1–14

Lee BB, Rockson SG, Bergan J (2018) *Lymphedema: A Concise Compendium of Theory and Practice*. Springer-Verlag, London

Levick JR, Michel CC (2010) Microvascular fluid exchange and the revised Starling principle. *Cardiovasc Res* 87(2): 198–210

Lipowsky HH, Gao L, Lescanic A (2011) Shedding of the endothelial glycocalyx in arterioles, capillaries, and venules and its effect on capillary hemodynamics during inflammation. *Am J Physiol Heart Circ Physiol* 301(6): H2235–45

Luft JH (1966) Fine structures of capillary and endocapillary layer as revealed by ruthenium red. *Fed Proc* 25(6): 1773–83

Mortimer PS, Rockson SG (2014) New developments in clinical aspects of lymphatic disease. *J Clin Invest* 124(3): 915–21

Patel N, Granick M, Kanakaris N et al (2008) Comparison of wound education in medical schools in the United States, United Kingdom and Germany. *Eplasty* 8: e8

Reitsma S, Slaaf DW, Vink H (2007) The endothelial glycocalyx: composition, functions, and visualization. Pflügers Arch 454(3): 345–59

Rossi A, Weber E, Sacchi G et al (2007) Mechanotransduction in lymphatic endothelial cells. *Lymphology* 40(3): 102–13

Ushiyama A, Kataoka H Iijima T (2016) Glycocalyx and its involvement in clinical pathophysiologies. *J Intensive Care* 4(1): 59

Vuong D, Nguyen M, Piller N (2011) Medical education: a deficiency or a disgrace. *Journal of Lymphoedema* 6(1): 44–9

Weinbaum S, Tarbell JM, Damiano ER (2007) The structure and function of the endothelial glycocalyx layer. *Annu Rev Biomed Eng* 9: 121–67

Woodcock TE, Woodcock TM (2012) Revised Starling equation and the glycocalyx model of transvascular fluid exchange: an improved paradigm for prescribing intravenous fluid therapy. *Br J Anaesth* 108(3): 384–94

Positive feedback from delegates and speakers from the conference has been overwhelming and has encouraged us to further pursue educational opportunities in Asia. Therefore, we are proud to announce that we will soon be launching a website and journal for the region at www.woundsasia.com.