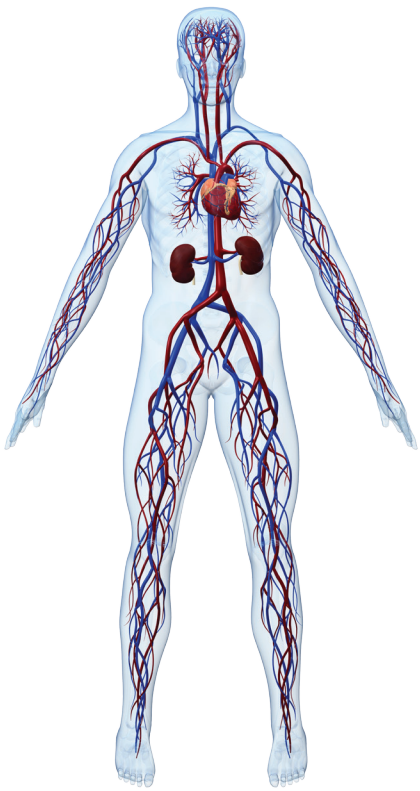
GLYCOCHECK

GLYCOCALYX MEASUREMENT SOFTWARE



Clinical assessment of vascular health with GlycoCheck™ Glycocalyx Measurement Software

A GlycoCheck™ Whitepaper

Problem

The microcirculation is in recent years increasingly recognized at the bedside as the center of several pathophysiological processes. However, accessible insight into microvascular function in a patient has been limited by a current lack of real-time quantification tools.

Abstract

The current white paper describes a novel solution for on-line quantification of the glycocalyx barrier properties of the microcirculation in a person using GlycoCheck™ Glycocalyx Measurement Software.

The glycocalyx is the gel-like layer lining the luminal side of the endothelium in blood vessels which acts as a protective barrier for the vascular wall; impairment of the glycocalyx barrier is regarded as a primary step in microvascular dysfunction. The GlycoCheck™ software has been developed for automated analysis of video recordings of the sublingual microcirculation. These videos can be provided by a range of commercially available clinical videomicroscopic cameras that can be positioned non-invasively under the tongue in a patient friendly manner.

The software automatically controls the quality of the videorecordings with respect to tissue motion, light intensity and image focus, resulting in an acceptable recording time of 1 – 2 minutes. Next, the software automatically identifies all available microvessels in the recording and calculates in them the perfused boundary region (PBR) as a measure of glycocalyx barrier properties; the analysis of over 3000 vascular segments takes about 3 min per patient, allowing for online monitoring of a patient's glycocalyx quality. Results using this methodology so far indicate excellent reproducibility of the PBR measurement in healthy young subjects.

Moreover, robust increases in PBR, indicative of a compromised glycocalyx barrier, have been observed in small groups of patients with diabetes and during septic shock, providing a tool for online glycocalyx monitoring and assessment of acute vascular risk in individual patients.

Background

The microcirculation serves major functions in the body: it regulates adequate organ perfusion and the distribution and exchange of oxygen, nutrients, and hormones within tissues. Furthermore, it controls tissue hydration and organizes the defense against pathogens. The endothelial glycocalyx has been demonstrated to be central in orchestrating these functions. The glycocalyx, a gel-like layer lining the endothelial cells at the luminal side, provides a micro-environment for many important vascular processes. The glycocalyx has been shown to limit the accessibility of lipids and proteins and to form a barrier for adhesion of platelets and inflammatory cells to the vascular wall; further, the glycocalyx is involved in mechanosensing and transduction of hemodynamic stimuli to the endothelium, thereby

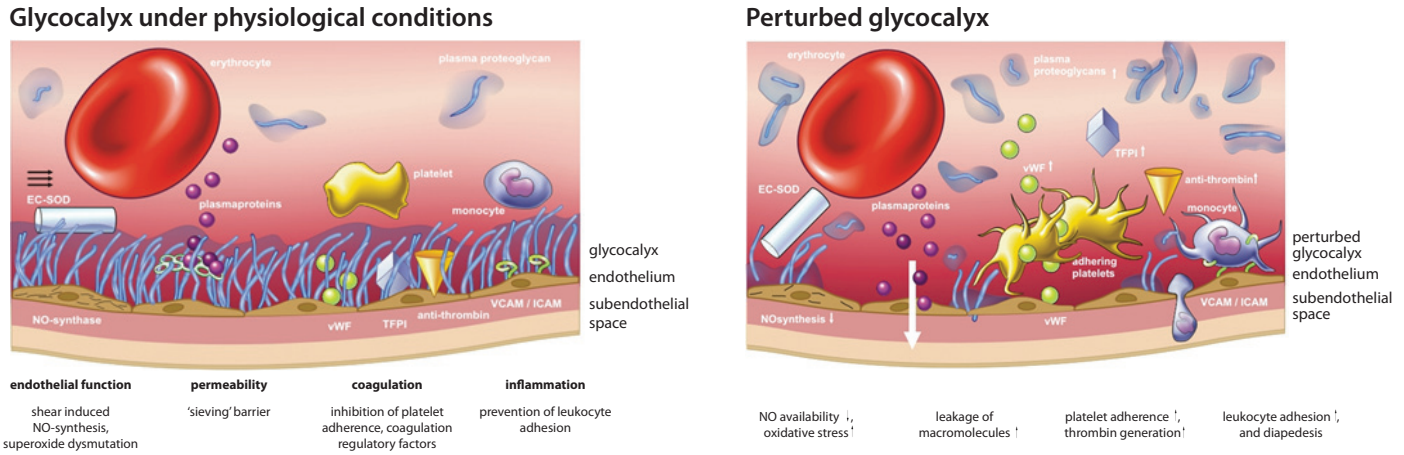


Figure 1. The endothelial glycocalyx is central to microvascular function. GlycoCheck™ Glycocalyx Measurement Software is developed for identifying glycocalyx perturbation in a patient at risk.

regulating the production of amongst others nitric oxide (Figure 1, left panel).

Consequently, damage to the glycocalyx has been associated with impaired endothelial function, including inflammatory and coagulatory endothelial activation, vascular leakage of fluid and proteins and a diminished NO bioavailability (Figure 1, right panel).

In line with its important functions, the microcirculation is in recent years increasingly recognized as locus of early pathophysiological processes associated with the onset of cardiovascular disease in e.g. diabetes and hyperlipidemia, as well as with circulatory failure in critically ill patients in the ICU.

Noticeably, microvascular failure in critically ill patients is nowadays regarded as the most sensitive indicator of circulatory failure associated with adverse outcome.

While novel bedside techniques allowing evaluation of the microcirculation within a patient have been introduced in recent years, their potential applicability for functional hemodynamic monitoring in daily clinical practice has been hindered by the current lack of robust and on-line quantification of microvascular function.

To meet this need, GlycoCheck™ has been dedicated in developing a software solution which permits on-line assessment of the glycocalyx barrier properties of the microcirculation in an individual patient; this solution is described here.

Solution: on-line monitoring of the perfused boundary region (PBR)

The perfused boundary region (PBR) is the main readout parameter calculated by GlycoCheck™ Glycocalyx Measurement Software. The PBR in microvessels is the cell-poor layer which results from the phase separation between the flowing red blood

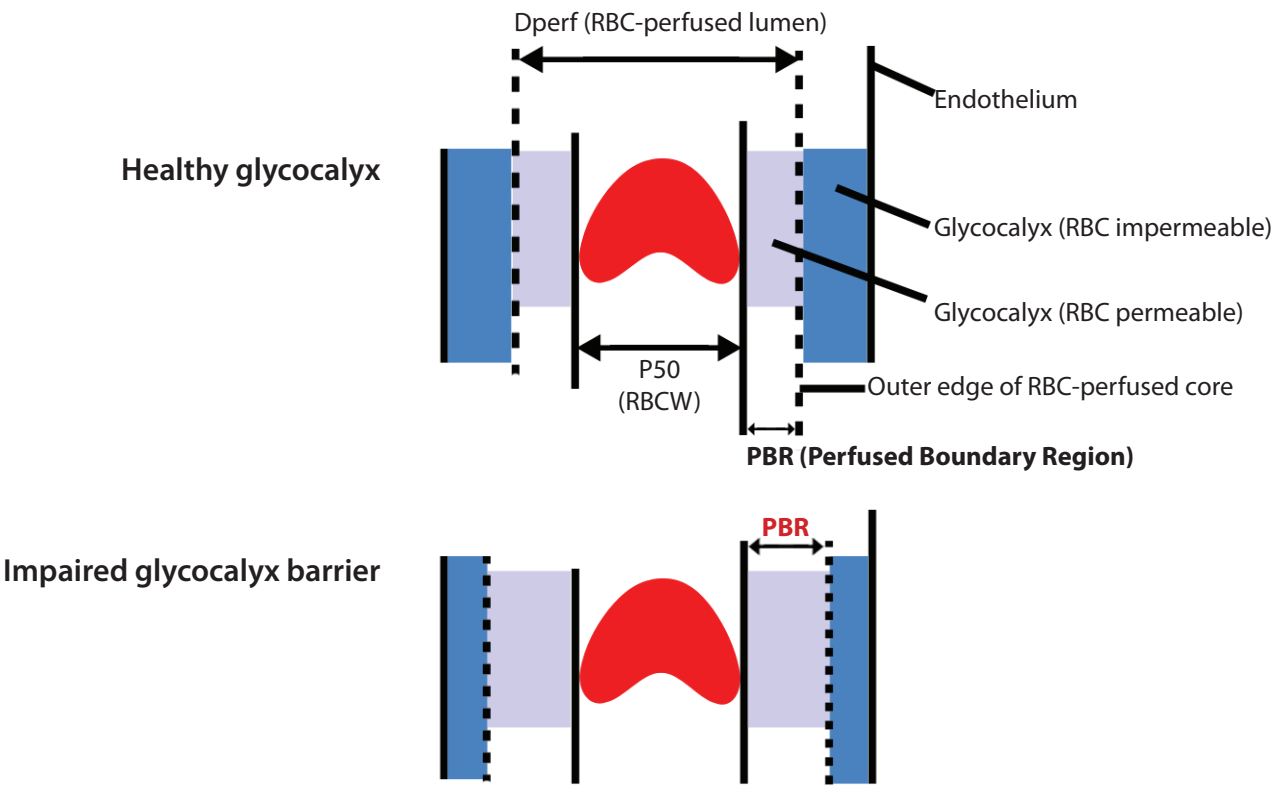


Figure 2. Portrayal of the perfused boundary region (PBR) in a healthy microvessel (top) and in a microvessel at risk (bottom). Deterioration of the cell-impermeable glycocalyx barrier in a vessel results in an outward movement of the outer edge of the RBC-perfused lumen resulting in an increased PBR in that vessel. The PBR is the main readout parameter of GlycoCheck™ Glycocalyx Measurement Software.

cells (RBC) and plasma. The PBR includes the most luminal part of glycocalyx that does allow cell penetration (Figure 2).

The outer edge of PBR is defined by the protective part of the glycocalyx that does not allow cell penetration and which shields the endothelial surface from direct contact with circulating cells. Loss of glycocalyx integrity allows for deeper penetration by the outer edge of the RBC-perfused lumen and thereby increases PBR, resulting in increased vulnerability of the endothelium.

GlycoCheck™ Glycocalyx Measurement Software

GlycoCheck™ Glycocalyx Measurement Software complies with current commercially available cameras made for on-line microscopic observations of the sublingual microcirculation. The actual measurement covers two stages:

1) recording of an adequate number of high-quality movie frames.

The software guarantees the recording of a suffcient number of high-quality videos by online monitoring of
a) tissue motion,
b) illumination intensity, and
c) focus level of the sublingual microvasculature.

Video acquisition automatically starts when image quality is within acceptable range and automatically stops when a suffcient number of vascular segments (> 3000) have been collected for analysis (see below). Data acquisition does not take more than 1-2 minutes.

2) image analysis resulting in calculation of the PBR throughout the monitored microcirculation.

The analysis automatically starts when images during the recording stage have been collected (Figure 3, step 1). The software automatically identifies all available microvessels and defines small vascular segments every 10 µm along the length of the detected vasculature. For each vascular segment 840 radial intensity profiles are obtained, which are

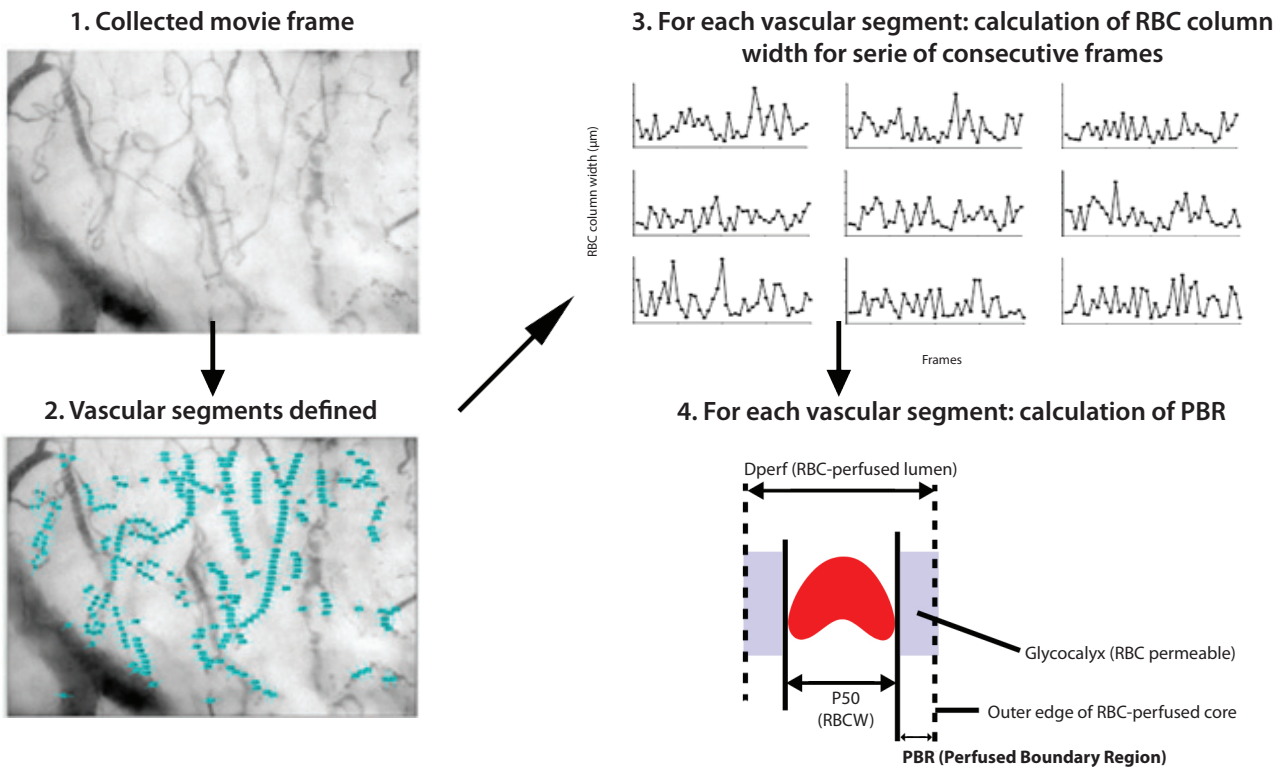


Figure 3: Image analysis involves four steps which in the end result in the determination of the PBR for multiple vascular segments along the microvasculature. Note that in the portrayal of step 3, just 9 representative RBC column width tracings for a particular vascular segment are shown.

tested for the presence of RBCs and signal quality, and red blood cell column widths are determined from these intensity profiles.

This results in a RBC width distribution of each individual vascular segment from which the median RBC width (RBCW) as well as the outer edge of the RBC perfused lumen (Dperf) is determined. The distance of the median RBC width value to the position of the outer edge of the RBC perfused lumen is measured and defined as the RBC Perfused Boundary Region (PBR),

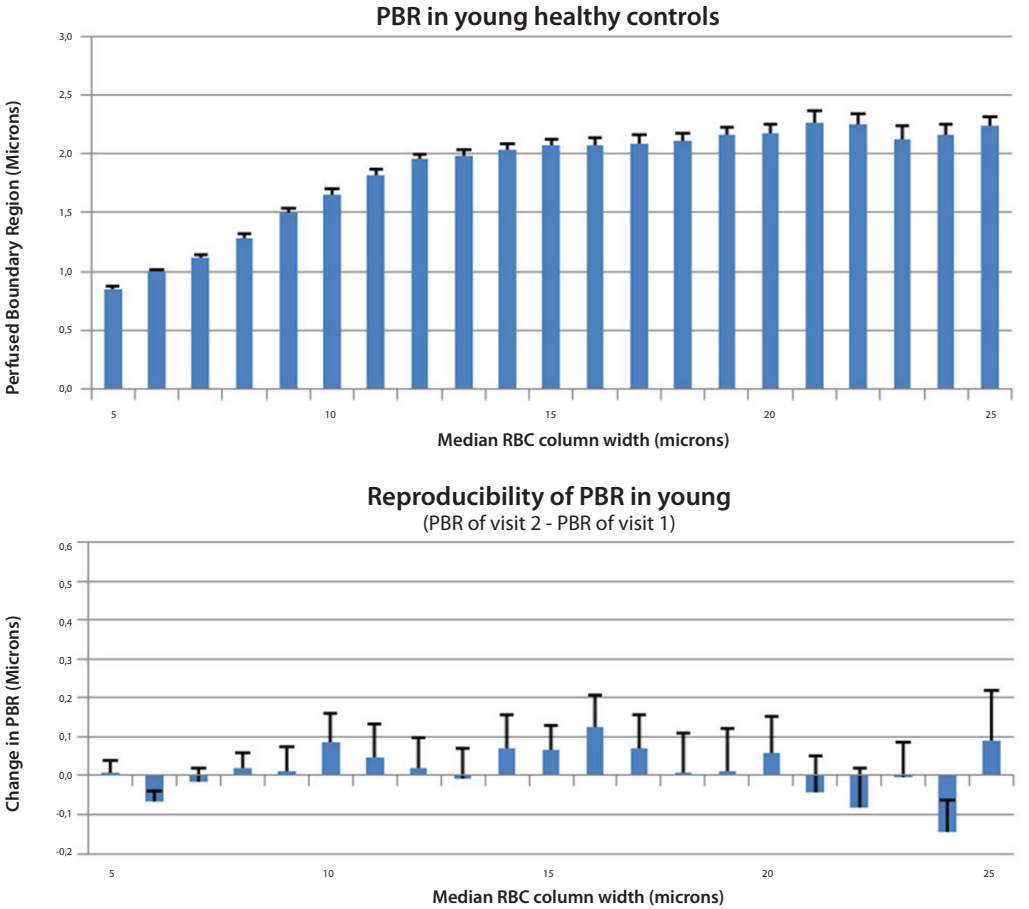


Figure 4: Top; PBR values (mean ± SEM, n=15 subjects) for incremental bin classes of median RBC column width in healthy young male volunteers (age: 20-29 years). Bottom: Difference in PBR between two consecutive measurements in these subjects using GlycoCheck™ Glycocalyx Measurement Software.

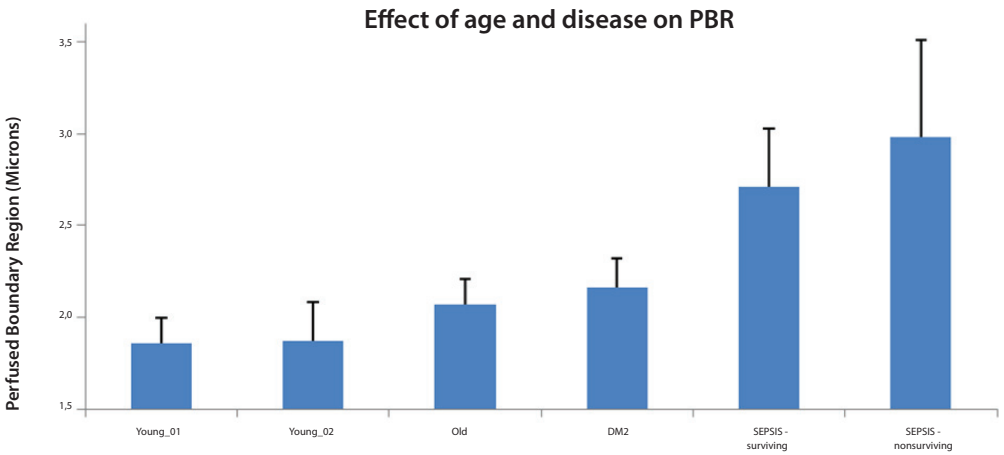


Figure 5: Pilot data of PBR values obtained with GlycoCheck™ Glycocalyx Measurement Software in healthy young volunteers on two consecutive visits (see Figure 4), in healthy old subjects (age: 60-83 years), in type 2 diabetics, and in critically ill patients during septic shock. Groups consist of n=11-16 patients.

i.e. PBR = (Dperf - RBCW) / 2. The calculated PBR values of a given patient are classified according to their corresponding median RBC column width (Figure 4) and an average PBR in the diameter range of 5 – 25 microns is calculated to provide a single PBR value for each patient. PBR values within a subject are demonstrated to be reproducible (Figure 4). Moreover, pilot data show increases in PBR, indicative of an impaired glycocalyx barrier, in diabetics and septic shock patients (Figure 5).

Conclusion and future directions

GlycoCheck™ Glycocalyx Measurement Software allows prompt and easy quantification of the perfused boundary region within the sublingual microvasculature of a person, thereby providing critical information about the properties of the glycocalyx barrier and vascular health.

The measurement is non-invasive and is patient friendly. The measurement is highly reproducible, and preliminary data indicate that the measurement is capable of identifying microvascular glycocalyx deterioration in people with diabetes and in critically ill patients during septic shock conditions allowing identification of individuals that are at acute vascular risk for organ complications.

Furthermore, application of the Glycocalyx Measurement Software in clinical cohort studies will contribute to defining early prognostic markers of microvascular deterioration and early onset of vascular complications.

The Glycocalyx Measurement Software opens new opportunities for online functional hemodynamic monitoring of (micro)vascular health, and allows for personalized monitoring of therapy efficacy which might be particularly useful in critically ill patients.

References

1. Edul VS, Enrico C, Laviolle B, Vazquez AR, Ince C, and Dubin A**.** *Quantitative assessment of the microcirculation in healthy volunteers and in patients with septic shock. Crit Care Med 40: 1443-1448, 2012.*
2. Nieuwdorp M, Meuwese MC, Mooij HL, van Lieshout MH, Hayden A, Levi M, Meijers JC, Ince C, Kastelein JJ, Vink H, and Stroes ES. *Tumor necrosis factor-alpha inhibition protects against endotoxin-induced endothelial glycocalyx perturbation. Atherosclerosis 202: 296-303, 2009.*
3. Nieuwdorp M, Meuwese MC, Vink H, Hoekstra JB, Kastelein JJ, and Stroes ES. *The endothelial glycocalyx: a potential barrier between health and vascular disease. Curr Opin Lipidol 16: 507-511, 2005.*
4. Schmidt EP, Yang Y, Janssen WJ, Gandjeva A, Perez MJ, Barthel L, Zemans RL, Bowman JC, Koyanagi DE, Yunt ZX, Smith LP, Cheng SS, Overdier KH, Thompson KR, Geraci MW, Douglas IS, Pearse DB, and Tuder RM. *The pulmonary endothelial glycocalyx regulates neutrophil adhesion and lung injury during experimental sepsis. Nat Med 2012.*
5. Spronk PE, Zandstra DF, and Ince C. *Bench-to-bedside review: sepsis is a disease of the microcirculation. Crit Care 8: 462-468, 2004.*
6. Van Teeffelen JW, Brands J, Stroes ES, and Vink H. *Endothelial glycocalyx: sweet shield of blood vessels. Trends Cardiovasc Med 17: 101-105, 2007.*
7. VanTeeffelen JW. *How to prevent leaky vessels during reperfusion? Just keep that glycocalyx sealant in place! Crit Care 12: 167, 2008.*

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About GlycoCheck™

GlycoCheck™ is a leading provider of imaging solutions based on an innovative measurement system of the Glycocalyx layer. The Glycocalyx is a natural occurring gel like structure within blood vessels, that plays a decisive role in the early stages of cardiovascular diseases. GlycoCheck™ solutions facilitate the accurate, reliable and non-invasive selection, detection & monitoring of patients in primary care, intensive care, cardiology and internal medicine. Additional information can be found at *www.glycocheck.com*

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