

# Circulation Research

JOURNAL OF THE AMERICAN HEART ASSOCIATION

American Heart  
Association®   
*Learn and Live*™

## **The Endothelial Glycocalyx Protects Against Myocardial Edema**

Bernard M. van den Berg, Hans Vink and Jos A.E. Spaan

*Circ. Res.* 2003;92;592-594; originally published online Mar 13, 2003;

DOI: 10.1161/01.RES.0000065917.53950.75

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75214

Copyright © 2003 American Heart Association. All rights reserved. Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:

<http://circres.ahajournals.org/cgi/content/full/92/6/592>

Subscriptions: Information about subscribing to Circulation Research is online at  
<http://circres.ahajournals.org/subscriptions/>

Permissions: Permissions & Rights Desk, Lippincott Williams & Wilkins, a division of Wolters Kluwer Health, 351 West Camden Street, Baltimore, MD 21202-2436. Phone: 410-528-4050. Fax: 410-528-8550. E-mail:  
[journalpermissions@lww.com](mailto:journalpermissions@lww.com)

Reprints: Information about reprints can be found online at  
<http://www.lww.com/reprints>

## The Endothelial Glycocalyx Protects Against Myocardial Edema

Bernard M. van den Berg, Hans Vink,  
Jos A.E. Spaan

**Myocardial tissue edema attributable to increased microvascular fluid loss contributes to cardiac dysfunction after myocardial ischemia, cardiopulmonary bypass, hypertension, and sepsis. Recent studies suggest that carbohydrate structures on the luminal surface of microvascular endothelium are essential to prevent tissue edema. We carefully preserved these structures for visualization with electron microscopy, revealing that the rat myocardial capillary endothelial surface is coated with a 0.2- to 0.5- $\mu$ m-thick carbohydrate layer and that its degradation instantly results in notable myocardial tissue edema.**

Myocardial tissue edema attributable to increased microvascular fluid loss contributes to cardiac dysfunction after myocardial ischemia, cardiopulmonary bypass, hypertension, and sepsis.<sup>1</sup> This loss of microvascular integrity is associated with a reduction of the number of negatively charged molecules at the endothelial cell surface.<sup>2</sup>

Vascular endothelial cells are shielded from direct exposure to flowing blood by a highly hydrated mesh of membrane-associated proteoglycans, glycosaminoglycans, glycoproteins, and glycolipids. Recent intravital microscopic studies provide evidence for an *in vivo* endothelial surface layer, or glycocalyx, up to 0.5  $\mu$ m thick.<sup>3-5</sup> An endothelial surface layer of this dimension would reduce perfused microvascular volume by 20% to 50% and may explain the discrepancy between *in vivo* and *in vitro* estimates of microvascular resistance to blood flow.<sup>6</sup> Moreover, it is suggested that protein concentration gradients across this layer are essential for control of transvascular fluid exchange.<sup>7,8</sup>

Using a new approach to stabilize the anionic carbohydrate structures in rat myocardial capillaries by Alcian blue 8GX, we carefully preserved these structures for visualization with electron microscopy. Perfusion of the coronary circulation with hyaluronidase was performed to confirm the hyaluronate nature of the stained structures and to determine whether glycocalyx hyaluronan protects against myocardial tissue edema.

## Materials and Methods

### Perfusion and Tissue Preparation

In accordance with institutional guidelines, hearts of anesthetized male Wistar rats (body weight 250 to 350 g) were exposed, and after transecting the vena cava, the aorta was cannulated retrogradely at the aorta/brachiocephalic trunk bifurcation. Perfusion started with the removal of blood by oxygenated calcium-free cardioplegic solution containing 0.1% BSA (CCS-BSA) (pH 7.4, 37°C) at 8 mL/min for 3 minutes, 54 $\pm$ 1.9 mm Hg (mean $\pm$ SE). Next, hearts were perfused with a phosphate-buffered fixative (pH 7.4) containing 30 mmol/L MgCl<sub>2</sub> at 8 mL/min (62 $\pm$ 1.4 mm Hg) in which after 2 minutes Alcian blue 8GX (Sigma Chemical Co.) was added (final concentration, 0.05%) and continued for the next 30 minutes at room temperature (79 $\pm$ 5.3 mm Hg). Tissue segments were postfixed with 1% osmium tetroxide (Electron Microscopy Sciences) and 1% lanthanum nitrate (Sigma) in water for 1 hour at room temperature followed by 1% aqueous Uranyl acetate for 1 hour and processed additionally for electron microscopy. Digital pictures of ultra-thin sections of capillaries were obtained with a built-in Megaview II CCD camera and processed with analySIS image-analytical software (both from Soft Imaging Systems GmbH).

### Hyaluronidase Treatment of Rat Heart

Before fixation and staining, hearts were perfused with oxygenated CCS-BSA (37°C) containing 25 IU/mL of hyaluronidase (bovine testis, fraction IV-S, Sigma), benzamidine-HCL (1 mmol/L, Sigma), and 6-amino-n-caproic acid (5 mmol/L, Sigma) for 1 hour at 8 mL/min. Control hearts were perfused with the preceding solution, except hyaluronidase, as shown above.

### Data Analysis

Pictures were analyzed using Image-Pro Plus software version 3.0 (Media Cybernetics). Mean glycocalyx thickness of 429 normal and 196 hyaluronidase-treated capillaries was determined to be the distance between luminal membrane and the optical background density plus 2 $\times$ SD, representing  $\approx$ 95% of detectable stained structures. Results are given in box plots with 5th and 95th percentiles shown, and difference in glycocalyx thickness of normal and hyaluronidase-treated capillaries was assessed by means of Mann-Whitney *U* nonparametric test.

Endothelial cell thickness was determined by subtracting the inner from the outer capillary diameter, with diameters calculated from measured perimeters of endothelial cells without a nucleus shown. Endothelial cell thickness was expressed as mean $\pm$ SD.

Pericapillary space was determined by subtracting the outer capillary diameter from the inner diameter of surrounding myocardial tissue. Distributions of calculated pericapillary spaces given in 0.2- $\mu$ m intervals are expressed as a median value, and difference between normal and hyaluronidase-treated capillaries was assessed by means of Mann-Whitney *U* nonparametric test.

A discussion of detailed methods of perfusion and tissue preparation and gold-labeled lectin binding can be found in the online data supplement, available at <http://www.circresaha.org>.

## Results

Luminal endothelial surfaces of stained myocardial capillaries were coated with evenly distributed discrete hairy-like bushes (Figures 1A and 1C, left). Colocalization of lectins *Canavalia ensiformis* and *Solanum tuberosum* to these extended stained structures confirmed its saccharine nature (see the online data supplement and online Figure 1). Enzymatic degradation with hyaluronidase before fixation and staining resulted in a condensed endothelial cell surface staining (Figures 1B and 1C, right), confirming the presence of surface-bound hyaluronan on normal myocardial capillaries.

Original received January 13, 2003; revision received February 25, 2003; accepted February 27, 2003.

From the Department of Medical Physics, Academic Medical Center, University of Amsterdam, The Netherlands.

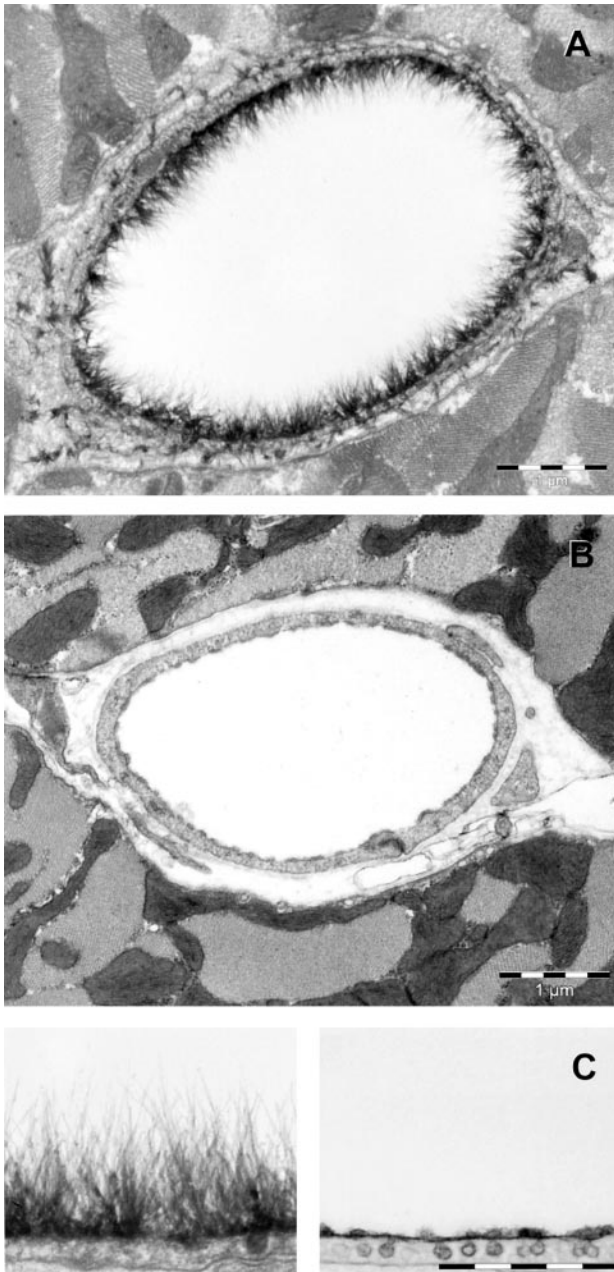
Correspondence to H. Vink, Department of Medical Physics, Academic Medical Center, University of Amsterdam, PO Box 22700, 1100 DE Amsterdam, The Netherlands. E-mail [h.vink@amc.uva.nl](mailto:h.vink@amc.uva.nl)

(*Circ Res.* 2003;92:592-594.)

© 2003 American Heart Association, Inc.

*Circulation Research* is available at <http://www.circresaha.org>

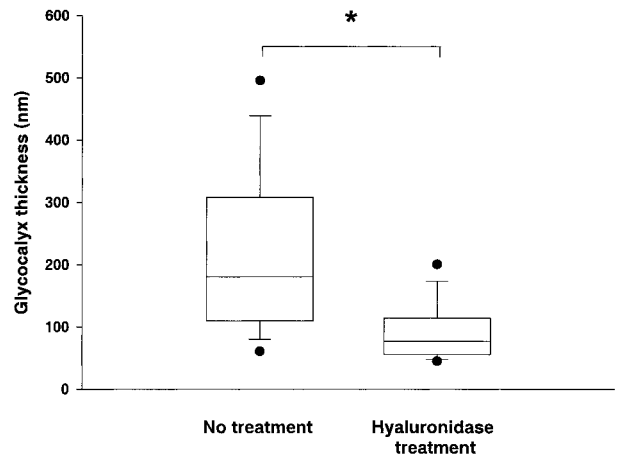
DOI: 10.1161/01.RES.0000065917.53950.75



**Figure 1.** A, Electron microscopic overview of an Alcian blue 8GX-stained rat left ventricular myocardial capillary (bar=1  $\mu\text{m}$ ). B, After hyaluronidase treatment, before Alcian blue staining (bar=1  $\mu\text{m}$ ). C, Detailed pictures of glycocalyx on normal (left) and of hyaluronidase-treated (right) capillaries (bar=0.5  $\mu\text{m}$ ).

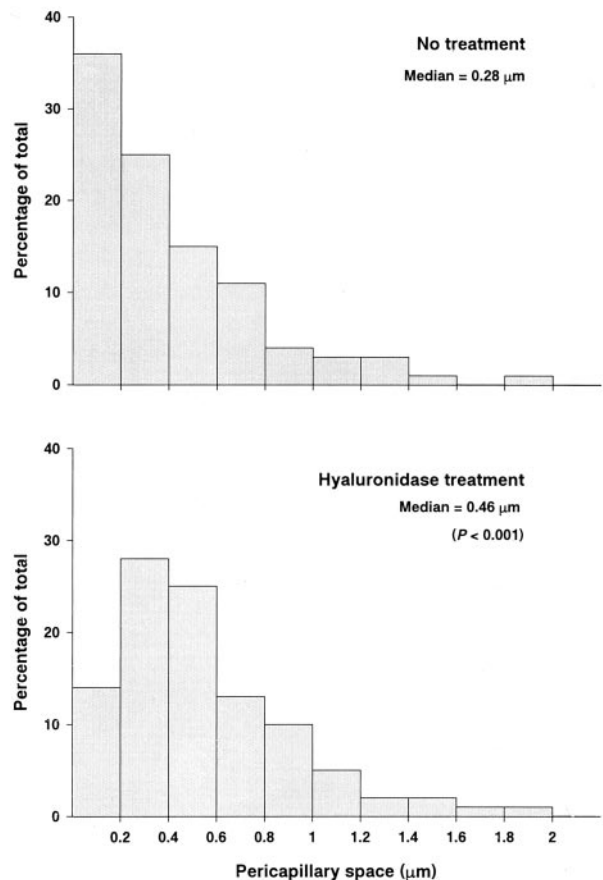
Normal myocardial capillary glycocalyx, with a median overall surface coat of 182 to 512 nm thick (95th percentile), was significantly greater ( $P < 0.001$ ) than the glycocalyx of 77 to 201 nm thick found on hyaluronidase-treated capillaries (Figure 2).

Enzymatic degradation of the endothelial glycocalyx had no effect on endothelial cell thickness. With a mean endothelial cell thickness of  $0.18 \pm 0.04 \mu\text{m}$  ( $n=270$ ) and  $0.16 \pm 0.03 \mu\text{m}$  ( $n=124$ ) in normal and hyaluronidase-treated capillaries, respectively, these cells were well within the normal range.<sup>9</sup> However, the interstitial space between capillaries and their



**Figure 2.** Distribution of glycocalyx thickness within normal capillaries (no treatment) and hyaluronidase-treated capillaries. Box plots indicate median values with 5th and 95th percentiles. \* $P < 0.001$  by means of Mann-Whitney  $U$  nonparametric test.

surrounding tissue was significantly ( $P < 0.001$ ) affected on treatment with hyaluronidase (Figures 3A and 3B). Although most of the normal vessels were surrounded by an interstitium with a median dimension of 0.28  $\mu\text{m}$  (Figure 3A),



**Figure 3.** Normalized frequency distribution of pericapillary spaces of normal (no treatment) and hyaluronidase-treated capillaries. Difference in pericapillary space between normal and hyaluronidase-treated capillaries was assessed by means of Mann-Whitney  $U$  nonparametric test. ( $P < 0.001$ )

distribution of the interstitial space in hyaluronidase-treated capillaries was clearly shifted to the right and resulted in a median value of 0.46  $\mu\text{m}$  (Figure 3B).

### Discussion

The observed dimension of the stained structures displayed a median thickness of 0.2 to 0.5  $\mu\text{m}$  (95th percentile) that is consistent with previous, indirect estimates of 0.3 to 1.0  $\mu\text{m}$  in mammalian skeletal muscle tissues.<sup>3–5</sup> Hyaluronidase treatment altered the hairy-like structures into a condensed stained layer with a median thickness of 0.08 to 0.2  $\mu\text{m}$ , resembling earlier electron-microscopic visualizations of the glycocalyx.<sup>10–12</sup> Moreover, hyaluronidase treatment significantly increased the pericapillary space, indicating a protective role for hyaluronan in preventing tissue edema.

Although these electron micrographs show for the first time hairy-like stained structures of a hyaluronate nature on the luminal endothelial cell surface, the presence of luminal bound hyaluronan is suggested previously by others using colloidal gold-labeled hyaluronan binding proteins.<sup>13</sup> Furthermore, elongated structures of a hyaluronate nature have been demonstrated within filamentous plugs from fenestrated capillaries<sup>14</sup> and as part of a pericellular matrix from smooth muscle cells.<sup>15</sup>

The capillary endothelial cell thickness was not affected on hyaluronidase treatment of the endothelial glycocalyx and remained well within the normal range between 0.17 and 0.23  $\mu\text{m}$ .<sup>9</sup> Intravital measurements revealed that local hyaluronidase treatment only increased glycocalyx permeability for dextran molecules of 70 and 145 kDa, but larger ones still remained excluded from the glycocalyx and did not move across the capillary wall.<sup>16</sup> These functional data support the possibility that hyaluronidase can affect glycocalyx permeability in the absence of evident endothelial membrane damage and associated gross increases in capillary wall permeability.

With regard to the observed heterogeneity in stained structures, different local conditions within the coronary vascular bed itself may account for the observed variety in glycocalyx staining. Besides the topological structure of the coronary arterial tree as a major determinant of a heterogeneous blood flow distribution,<sup>17,18</sup> other factors of influence on the distribution of thickness might be the wide range of wall shear stresses (15 to 70 dynes/cm<sup>2</sup>) within capillary networks,<sup>19</sup> which result in greater red cell deformation, and reduced glycocalyx compression at high flow rates.<sup>5,20</sup> Heterogeneously distributed shear forces might cause a natural heterogeneity in glycocalyx thickness or result in differences in Alcian blue staining and in turn loss of carbohydrate structures.

Numerous factors are described that may modulate the integrity of the endothelial cell glycocalyx. Enzymatic degradation of carbohydrate bushes clearly increases the dimension of the pericapillary interstitial space, indicating that microvascular endothelial carbohydrate damage during ischemia reperfusion,<sup>11</sup> during hypoxia,<sup>12</sup> and after exposure to atherogenic plasma levels of oxidized low-density lipoprotein<sup>21,22</sup> most likely contributes to the associated tissue edema. Present results give additional evidence for the importance of a thick glycocalyx in the control of vascular integrity.<sup>7,8</sup>

### Conclusion

Electron micrographs demonstrated that surface-bound hyaluronan is a major determinant of the endothelial glycocalyx and that its degradation from the coronary endothelial surface

results in myocardial tissue edema. Additional discussion paragraphs can be found in the online data supplement, available at <http://www.circresaha.org>.

### Acknowledgments

This work was supported by the Netherlands Organization for Scientific Research (NWO No. 902-16-192) and a research fellowship from the Royal Netherlands Academy of Arts and Sciences (KNAW) to H.V. We are grateful for the expert technical assistance provided by T.M. Rolf, and the support in using the electron microscope given by J. van Marle and H.A. van Veen is appreciated.

### References

- Mehlhorn U, Geissler HJ, Laine GA, Allen SJ. Myocardial fluid balance. *Eur J Cardiothorac Surg*. 2001;20:1220–1230.
- Gotloib L, Shostak A, Galdi P, Jaichenko J, Fudin R. Loss of microvascular negative charges accompanied by interstitial edema in septic rats' heart. *Circ Shock*. 1992;36:45–56.
- Desjardins C, Duling BR. Heparinase treatment suggests a role for the endothelial cell glycocalyx in regulation of capillary hematocrit. *Am J Physiol*. 1990;258:H647–H654.
- Klitzman B, Duling BR. Microvessel hematocrit and red cell flow in resting and contracting striated muscle. *Am J Physiol*. 1979;237:H481–H490.
- Vink H, Duling BR. Identification of distinct luminal domains for macromolecules, erythrocytes, and leukocytes within mammalian capillaries. *Circ Res*. 1996;79:581–589.
- Pries AR, Secomb TW, Jacobs H, Sperandio M, Osterloh K, Gaehtgens P. Microvascular blood flow resistance: role of endothelial surface layer. *Am J Physiol*. 1997;42:H2272–H2279.
- Hu X, Weinbaum S. A new view of Starling's forces hypothesis at the microstructural level. *Microvasc Res*. 1999;58:281–304.
- Hu X, Adamson RH, Liu B, Curry FE, Weinbaum S. Starling forces that oppose filtration after tissue oncotic pressure is increased. *Am J Physiol*. 2000;279:H1724–H1736.
- Simionescu N, Simionescu M, Palade GE. Structural basis of permeability in sequential segments of the microvasculature, II: pathways followed by microperoxidase across the endothelium. *Microvasc Res*. 1978;15:17–33.
- Luft JH. Fine structure of capillary and endocapillary layer as revealed by ruthenium red. *Fed Proc*. 1966;25:1773–1783.
- Beresewicz A, Czarnowska E, Maczewski M. Ischemic preconditioning and superoxide dismutase protect against endothelial dysfunction and endothelium glycocalyx disruption in the postischemic guinea-pig heart. *Mol Cell Biochem*. 1998;186:87–97.
- Ward BJ, Donnelly JL. Hypoxia induced disruption of the cardiac endothelial glycocalyx: implications for capillary permeability. *Cardiovasc Res*. 1993;27:384–389.
- Eggl PS, Graber W. Association of hyaluronan with rat vascular endothelial and smooth muscle cells. *J Histochem Cytochem*. 1995;43:689–697.
- Rostgaard J, Qvortrup K. Electron microscopic demonstrations of filamentous molecular sieve plugs in capillary fenestrae. *Microvasc Res*. 1997;53:1–13.
- Evanko SP, Angello JC, Wight TN. Formation of hyaluronan- and versican-rich pericellular matrix is required for proliferation and migration of vascular smooth muscle cells. *Arterioscler Vasc Biol*. 1999;19:1004–1013.
- Henry CBS, Duling BR. Permeation of the luminal capillary glycocalyx is determined by hyaluronan. *Am J Physiol*. 1999;277:H508–H514.
- Beard DA, Bassingthwaite JB. The fractal nature of myocardial blood flow emerges from a whole-organ model of arterial network. *J Vasc Res*. 2000;37:282–296.
- Van Bavel E, Spaan JAE. Branching patterns in the porcine coronary arterial tree: estimation of blood flow heterogeneity. *Circ Res*. 1992;71:1200–1212.
- Pries AR, Secomb TW, Gaehtgens P. Design principles of vascular beds. *Circ Res*. 1995;77:1017–1023.
- Secomb TW, Hsu R, Pries AR. Motion of red blood cells in a capillary with an endothelial surface layer: effect of flow velocity. *Am J Physiol*. 2001;281:H629–H636.
- Constantinescu AA, Vink H, Spaan JA. Elevated capillary tube hematocrit reflects degradation of endothelial cell glycocalyx by oxidized LDL. *Am J Physiol*. 2001;280:H1051–H1057.
- Vink H, Constantinescu AA, Spaan JA. Oxidized lipoproteins degrade the endothelial surface layer: implications for platelet-endothelial cell adhesion. *Circulation*. 2000;101:1500–1502.

KEY WORDS: myocardium ■ capillaries ■ glycocalyx ■ hyaluronan ■ edema