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Prolonged shear stress modifies the composition of the endothelial glycocalyx

In preparation

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Abstract

Throughout the vasculature a gel-like layer consisting of proteoglycans, glycosaminoglycans, glycoproteins and adsorbed plasma molecules covers the endothelium. Within the endothelial glycocalyx (EG) heparan sulfates and hyaluronan have been proposed to be the most important functional components. The EG has been demonstrated to regulate vascular permeability, transduce shear stress and bind chemokines and growth factors. Glycocalyx dimension and composition both have been shown to be crucial for endothelial function. We hypothesized that long-term continuous laminar shear stress induces changes in the EG composition. For this purpose, human umbilical vein endothelial cells (HUVECs) were cultured under flow for 7 days. Using wheat germ agglutinin (WGA) and the carbohydrate specific antibodies 10E4 and JM403, we observed an increased density and thickness of carbohydrates on top of the endothelium and a change in heparan sulfate composition within the endothelial glycocalyx. Furthermore, by blocking the JM403 epitope within HS in static conditions, we observed a reduction of THP-1 monocyte adhesion upon TNF-α stimulation, confirming the predicted pro-inflammatory role of the JM403 binding epitope. Consequently, the observed reduction in JM403 epitope expression within heparan sulfate suggests that long-term shear stress leads to a glycocalyx with anti-inflammatory properties.

Introduction

Throughout the vasculature, the endothelium is covered by a membrane-bound glycocalyx with adsorbed plasma proteins [1,2]. The membrane bound part of the glycocalyx contains glycoproteins, glycosaminoglycans and proteoglycans (PG), i.e. core proteins with long side-branches that consist of repeated disaccharide glycosaminoglycans (GAGs). Examples of GAGs present in the endothelial glycocalyx are heparan sulfate (HS), hyaluronan (HA) and chondroitin sulfate (CS) [2].

Heparan sulfate is attached to membrane bound core-proteins such as syndecans 1 and -4, glypican-1 and versican, forming proteoglycans. Starting with glycosylation at serine residues on a core protein, HS and CS GAGs are produced in the Golgi apparatus. Here, especially HS is extensively modified. N-Deacetylase/N-Sulfotransferase (NDST) modifies the N-acetylglucosamine by N-deacetylation or subsequent N-sulfation, while some glucuronic acids are epimerized to L-iduronic acid (IdoA). Hereafter, the sulfation pattern of the HS chain is modified by several sulfotransferases, which sulfates C2 of the uronic acids and C6 (and rarely C3) of the glucosamine residues. After modification, the HSPG is transported to the membrane where it can be modified again by endosulfatases (SULF1 and SULF2) and heparanases. In contrast to heparan sulfates, hyaluronan is not directly attached to the membrane via a core-protein, but can be bound to hyaluronan synthase, CD44, or hyaluronidase. Hyaluronan can also be cross-linked by various hyaluronan binding proteins, such as versican, aggrecan, neurocan and tumor necrosis factor-stimulated gene 6 (TSG-6), some of which are also able to bind HS [3,4]. This interconnected matrix of carbohydrates has been proposed to be involved in almost all functions of the endothelial layer, such as inflammation, coagulation, permeability, shear sensing and regulation of perfusion [2,5,6]. Perturbation of this layer has been associated with cardiovascular disease, sepsis, renal failure and diabetes [5].

While HS functions as an interactive matrix for protein interaction, the GAG composition is critical for the function of the EG and the underlying endothelium. In particular, variation in HS disaccharide sequence and sulfation patterns determines binding properties for circulating proteins [7,8]. Binding of growth factors, such as FGF-2 and VEGF, or cytokines have been shown to be critically dependent on binding to specific sulfated HS moieties, generating gradients (e.g. chemokines) over the endothelium to guide cells towards the underlying tissue or concentrate factors near their receptor (e.g. growth factors) [9-11]. HS are made up of up to 150 modified disaccharide units, which allow for a high variety in binding sites for different proteins [7,9,12]. Consequently, HS are involved in many cellular processes such as attachment, migration, differentiation, blood coagulation, lipid metabolism, and inflammation [7]. Because the production of HS is continuously ongoing and the sulfation pattern can be rapidly modified by enzymes such as sulfotransferases and sulfatases, protein binding specificity can be rapidly adjusted upon changes in the environment [13,14].

In vitro, the endothelial surface layer lacks most of its barrier function in cultured HUVECs when compared to the in vivo situation [15,16]. In addition, the endothelial glycocalyx dimensions observed in vitro are reduced [17]. One of the explanations for these differences between in vivo and in vitro is lack of a proper endothelial environment during culture [18-20]. Especially, shear stress has been shown to be involved in glycocalyx production and function. For example, shear stress has been shown to result in incorporation of hyaluronan within the endothelial glycocalyx [21]. In vivo, perturbation of the endothelial glycocalyx was observed in areas of disturbed flow, areas that are also vulnerable for development of atherosclerosis [22-24]. These findings have been also demonstrated in cultured endothelial cells through the exposure to either atheroprone or atheroprotective waveforms during prolonged culture and suggest that uniform laminar shear stress is beneficial for presence of a healthy endothelial glycocalyx [25].

In this study we hypothesized that prolonged laminar shear stress leads to compositional, dimensional and consequently also functional changes within the endothelial glycocalyx. To study this, primary isolated human umbilical vein endothelial cells (HUVECs) were cultured under continuous laminar flow (shear stress is 10 dyne/cm^2) for 7 days. Wheat germ agglutinin (WGA) was used to quantify the total amount of carbohydrates on top of the endothelial cell. Compositional changes were studied using antibodies against different HS moieties (domains). For this we used HS antibodies 10E4 and JM403, since the cell-associated HS-epitope necessary for binding is known for both of these antibodies: 10E4 mainly binds to mixed HS domains, containing both N-acetylated and N-sulfated disaccharide units [26], whereas JM403 binding depends on the presence of N-unsubstituted glucosamine [27]. Functional changes within the glycocalyx were examined by analyzing the role of the JM403 binding epitope in monocytic THP-1 cell adhesion.

Material and methods

Cells
HUVECs were isolated from umbilical cords by perfusion, and 20 minutes incubation, with trypsin at 37°C. Freshly isolated HUVECs were cultured on 0.5% gelatin coated 75 cm^2 flasks (Greiner Bio-one) in EGM2 medium (Lonza, Basel, Switzerland) supplemented with antibiotics/antimycotics (Life technology, Carlsbad, California, USA) and used for experiments at passage 1-3.

Flow experiments
Flow experiments were performed using an ibidi flow system (Ibidi, Martinsried, Germany). HUVECs were seeded into closed perfusion chambers (ibiTreat 0.4 µ-Slide I or VI, Luer) at a concentration of $1.5x10^6$ cells per mL. Cells were allowed to adhere for 3 hours. Hereafter, the chamber was connected to a computer-controlled air pressure pump and a fluidic unit with a two-way switching valve. The pump setup allowed pumping of 16 mL cell culture medium from two reservoirs in a unidirectional way through the flow channel over the monolayer of endothelial cells at a constant shear stress of 10 dyne/cm^2. Medium was refreshed after 1 and 4 days of culture. The chamber and the reservoirs containing the medium were kept in an incubator at 37°C and 5% CO_2. RNA was isolated from cells subjected to shear stress in a 0.4 µ-Slide I Luer flow chamber, while the 6 lanes of a 0.4 µ-Slide VI Luer were used for immunofluorescent staining experiments.

RNA Isolation and qRT-PCR
Total RNA was isolated from HUVECs cultured in a µ-Slide using Trizol reagent (Life Technologies, Carlsbad, California, USA) and isolation kit (Qiagen, Hilden, Germany). Reverse transcription was performed using a 5 minute 65°C incubation of 500 ng total RNA with deoxyribonucleotide triphosphates (Life Technologies) and oligo(dT) (Life Technologies), cDNA was synthesized using M-MLV First-Strand Synthesis (Life Technologies), and detection was carried out using SYBR Green Master Mix (Life technologies). Levels of expression were determined by normalizing to GAPDH levels.

Cell culture and confocal immunofluorescence microscopy
After static culture (24 hours) or exposure to flow (24 hours or 7 days), HUVECs were fixed in freshly made 4% paraformaldehyde in HBSS for 10 minutes, washed twice with HBSS (life technologies) and blocked for 30 minutes with 3% normal goat serum (NGS) in HBSS. Cells were incubated overnight (16 hours) at 4°C with 10 µg/mL TRITC-labeled wheat germ agglutinin (WGA) (Sigma-Aldrich, St Louis, MA, USA), Hoechst 33528 (Sigma-Aldrich, 1:5000) and one of the primary antibodies (at 10 µg/mL): 10E4 (Amsbio), JM403 (kind gift of dr. van der Vlag, Radboud University Medical Centre, Nijmegen, The Netherlands), eNOS (BD bioscience), VE-cadherin (BD bioscience), phalloidin-rhodamine (Sigma-Aldrich), or the appropriate control IgM or IgG isotype antibodies diluted in HBSS. After washing three times with HBSS, cells were incubated with a secondary antibody for 1 hour (2 µg/mL of anti-mouse IgM-Alexa488 or anti-mouse IgG-

Alexa488). Cells were again washed for three times with HBSS and imaged using a Leica SP5 confocal microscope and a 63x (N.A. 1.4) objective.

Mononuclear cell adhesion assays

Mononuclear THP-1 cell adhesion assay was performed as published previously with some modifications [28]. In short, HUVECs ($1.5x10^6$ cells/mL in EGM2 medium) were seeded into closed perfusion chambers (ibiTreat 0.4 µ-Slide VI Luer). After 24 hours of static culture the monolayer of endothelial cells was stimulated with TNF-α (10 ng/ml) for 5 hours. Cells were washed with HBSS containing 1% BSA cells and incubated with either JM403 or IgM isotype control (both 10µg/mL) for 1 hour at room temperature. Next, flow chambers were positioned on an inverted microscope (Leica AF6000) and attached to a syringe pump to perfuse the endothelial monolayer with THP-1 monocytes ($5x10^5$ cells/mL in HBSS + 1% BSA). THP-1 cells were perfused over the EC layer for 5 minutes at 0.5 dyne/cm^2 after which the monolayer was washed with HBSS + 1% BSA for 5 minutes. Flow chambers were imaged at 10 different locations. All images were taken at the centerline of the flow chamber. The number of adherent cells per field of view was quantified using the public domain National Institutes of Health IMAGE program (ImageJ, available at http://rsb.info.nih.gov/nih-image).

CLSM image analysis and quantification

Confocal 12-bit gray-scale axial image stacks (xyz dimensions, 0.08×0.08×0.13 µm) that covered 6724 $µm^2$ of surface area per image and a height of 5 to 10 µm above the EC nuclear plane were recorded using LAS-AF image software (Leica). The image stacks were analyzed using ImageJ software. For the luminal endothelial glycocalyx quantification, first the nuclear and peri-nuclear area was selected, to exclude the cell area in which the cell thickness was too thin to differentiate between luminal and abluminal staining. Thickness of the WGA stained layer was estimated within this area of interest, between the peak nuclear (Hoechst) signal and the luminal end of WGA fluorescence, designated as twice the background signal. Next, total luminal WGA signal of the 3 dimensional area of interest was calculated from the sum of average fluorescence in every z-plane within this area. In addition, heterogeneity in WGA distribution was determined by calculating the coefficient of variance between these planes. To quantify specific staining for the HS moiety antibodies, total isotype control (IgM) signal was subtracted from the total 10E4 or JM403 signal. A visualization of this method is shown in **figure 1**.

Statistical analyses

Data are presented as mean ± SEM, unless stated otherwise. To exclude variations due to technical differences between the experiments, fold change of staining intensity and coefficient of variances were shown relative to the static control group. Differences between the groups were tested by analysis of variance (ANOVA). Differences were considered statistically significant if $p<0.05$. Data analysis was performed using SPSS version 20.0 (SPSS Inc, Chicago, IL) and GraphPad Prism, version 5.0 (GraphPad Prism Software Inc, San Diego, CA).

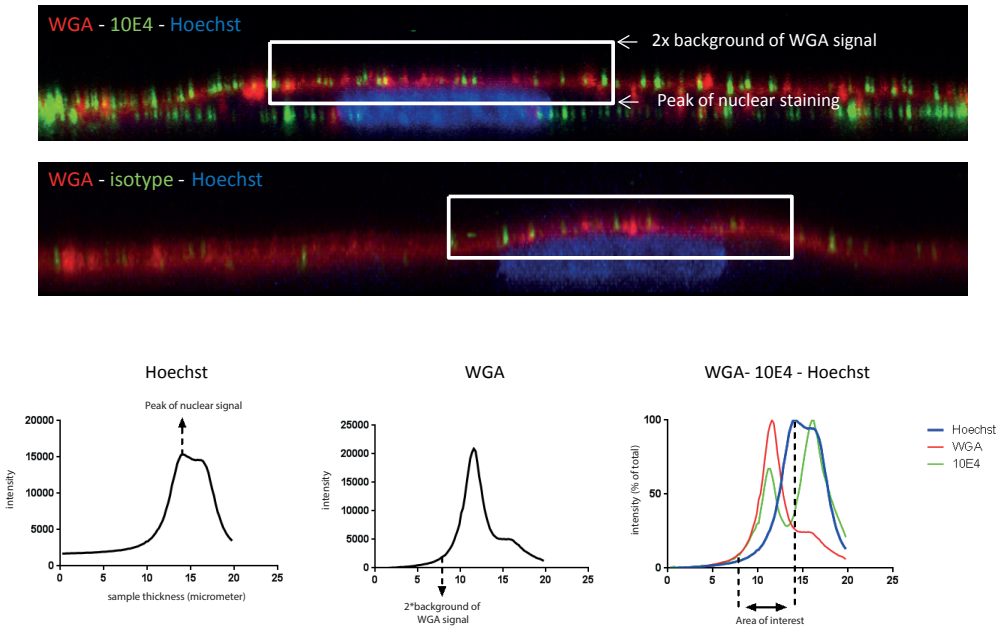

Figure 1: Methods to quantify the luminal endothelial glycocalyx stainings. *A) Method to quantify the luminal glycocalyx. Example of endothelial glycocalyx stained with wheat germ agglutinin (WGA), 10E4 and Hoechst. White box shows the area of interest which should be quantified (upper). B) Example of endothelial glycocalyx stained with wheat germ agglutinin (WGA), IgM isotype control and Hoechst. Total signal within the white box was subtracted from total signal of the HS staining to control for non-specific staining. C) Example of quantification method. Peak of Hoechst signal determines the lower border of the area of interest (left). 2x WGA background signal is used to determine the upper border of the area of interest (middle). Total area under the curve of the area of interest is used to determine the total luminal signal. Thickness of area of interest is used to determine dimensions of WGA signal.*

Results

Prolonged laminar flow leads to a quiescent monolayer of HUVECs

HUVECs were cultured under a laminar flow of 10 dyne/cm^2 for 7 days to confirm the ability to induce a quiescent endothelial phenotype.(20) First, the effect of prolonged shear stress on endothelium was tested by analyzing cell morphology and expression of shear responsive genes and proteins. After 7 days at 10 dyne/cm^2, shear stress sensitive genes Krüppel-like Factor 2 (KLF2) and endothelial nitric oxide synthase (NOS3) were up regulated compared to static cultured cells (14.1% to 1.7%, $p<0.05$ for KLF2 and 8.9% to 2.3%, $p<0.01$ for eNOS, relative to GAPDH expression respectively). In contrast, gene expression of the inflammatory markers E-selectin and IL-8 decreased after 7 days of flow culture (**figure 2**). The inflammatory marker IL-8, however, dramatically increased after 24 hours of culture under flow (0.2% to 24.2% of GAPDH expression levels, static vs. flow 24hrs, respectively), which confirms previous data showing an acute inflammatory response upon short-term exposure to shear stress [29].

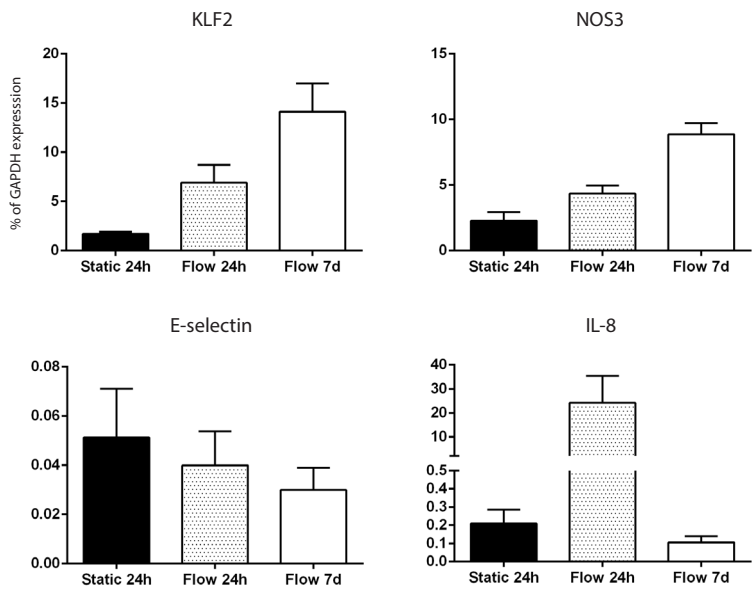

Figure 2: Effects of prolonged shear stress on gene expression of shear responsive genes. *Gene expression as percentage of GAPDH expression after static, 1 day flow and 7 days of flow culture for KLF2, eNOS, E-selectin and IL-8.*

In addition, typical short F-actin shear fibers and alignment to the direction of the flow was observed after the prolonged shear culture [30] and VE-cadherin and eNOS expression both were increased and robustly expressed at the cell-cell contacts (**figure 3**).

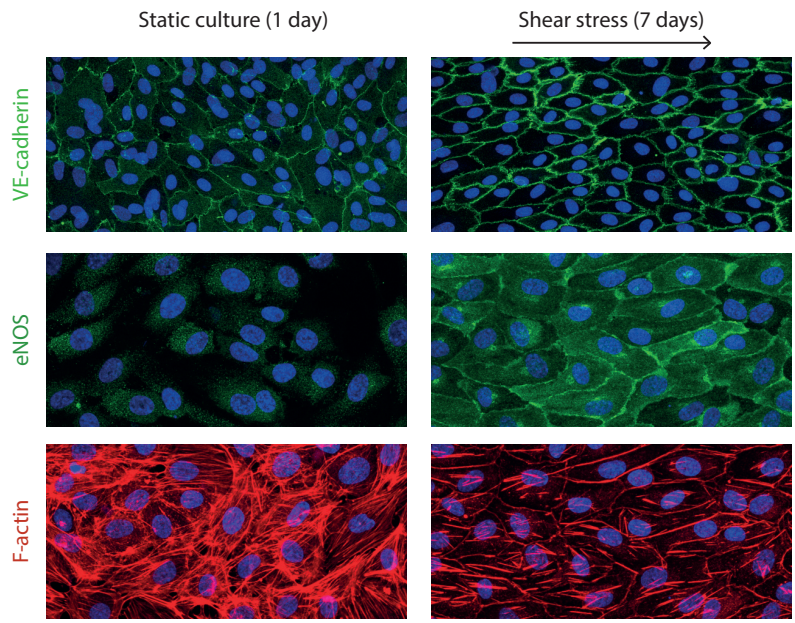

Figure 3: Cells change upon prolonged shear stress culture. *HUVECs are stained with antibodies against VE-cadherin (upper) and eNOS (middle) and the F-actin binding phalloidin-rhodamine (lower) to visualize endothelial phenotypic changes. Static cultured HUVECs (left) were compared to HUVECs cultured for 7 days at a shear stress of 10 dynes/cm2.*

Laminar shear stress induces a thicker glycocalyx

Next, we tested our hypothesis that this prolonged exposure leads to changes in endothelial glycocalyx production and composition. For this, we stained the EC after indicated time periods with the N-acetylneuraminic acid and N-acetyl-β-D-glucosamine binding lectin WGA and the specific HS-binding antibodies 10E4 and JM403. After 7 days of exposure to 10 dyne/cm^2 shear stress, total intensity of WGA staining was significantly increased compared to static conditions (1.49 fold increase, $p<0.05$) (**figure 4**). EG thickness was also increased after 7 days of shear stress compared to both 24hrs of static culture and 24hrs of shear stress (3.10 vs 2.52 and 2.61 μm respectively, $p<0.01$ and $p<0.05$). Besides this notable increase in WGA intensity and thickness, a more homogeneously distributed layer was observed (reflected by the lowest coefficient of variance: 0.84 fold change compared to 24hrs of static culture, $p<0.05$).

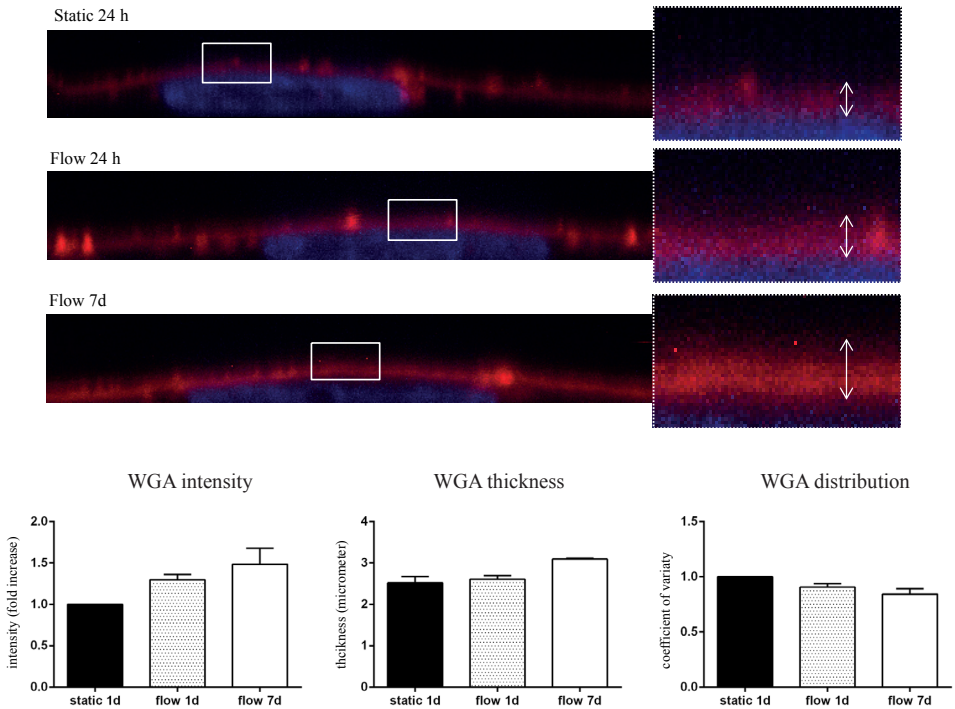

Figure 4: Prolonged shear stress increseses WGA thickness and density on top of HUVECs. *A) Representative examples of WGA staining on top of the endothelial cells. White box shows the zoomed area (right). B) Quantification method described in figure 2 was used for the stainings in A). Within the area of interest the total intensity (left), thickness of the stained area (middle) and distribution on top of the endothelium (right) was measured.*

Laminar shear stress induces a change in enzymes involved in glycocalyx modification.
Production and composition of the endothelial glycocalyx are determined by the presence and activity of several enzymes. Changes in each of these enzymes can results in functional changes of the endothelial glycocalyx. To determine whether the composition of heparan sulfates within the endothelial glycocalyx could be changed by changes in gene expression upon shear stress, gene expression levels of enzymes involved in HS sulfation pattern modification were studied. Seven days of flow exposure did not alter N-deacetylase/N-sulfotransferase-1 and -2 (NDST1, NDST2) expression (data not shown).

Nevertheless, the intracellular HS-chain modifying heparan sulfate sulfotransferases (HS2ST1, HS3ST1 and HS6ST1) were variably changed upon shear stress stimulation. In addition, the extracellular modifying enzymes 6-O-endosulfatase 1 and 2 (SULF1, SULF2) and heparanase (HPSE1) were all increased upon 7 days of shear stress (**figure 5**). Next to enzymes involved in heparan sulfate production, the gene expression of the hyaluronan synthesizing enzyme HAS2 was increased after prolonged shear stress (no changes were observed for HAS1 or HAS3) and was accompanied by an increase in the hyaluronan degrading enzyme hyaluronidase 2 (HYAL2) gene expression.

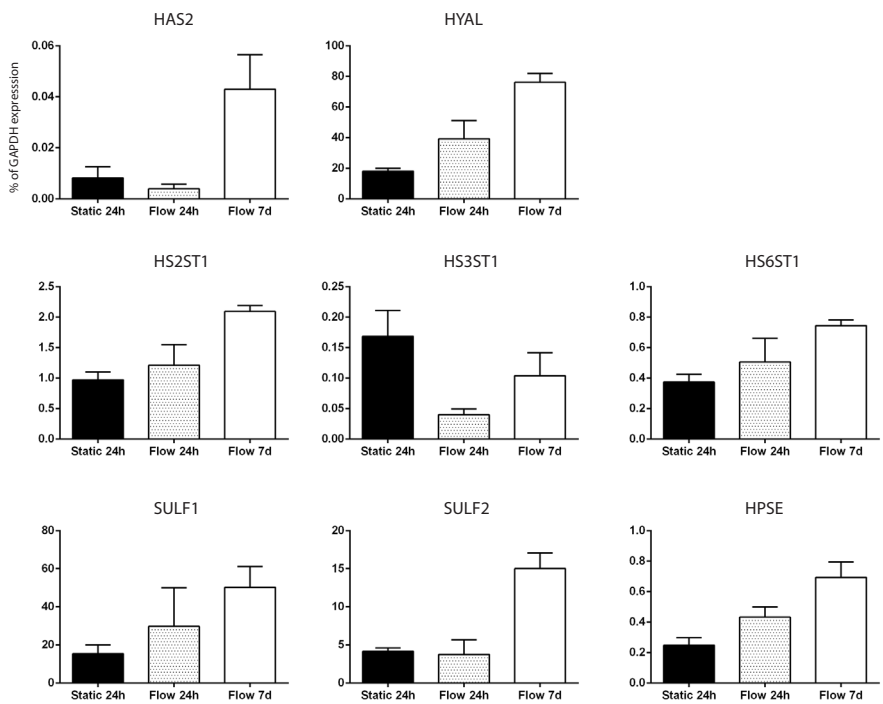

Figure 5: Prolonged shear stress alters gene expression of enzymes involved in HS and HA production and modification. *Gene expressions as percentage of GAPDH expression after static, 1 day flow and 7 days of flow culture for the genes coding for enzymes involved in HA production (HAS2) and breakdown (HYAL) and HS sulfation pattern modification (HS2ST1, HS3ST1, HS6ST1, SULF1, SULF2) and breakdown (HPSE).*

Prolonged Shear stress induces changes in luminal heparan sulfate composition

The HS modifying enzyme gene expression indicates that the chain is compositionally altered upon prolonged shear stress. This was tested further using binding of antibodies against two different HS domains. The expression of specific HS domains was studied after 1 and 7 days of shear stress and was compared to static control conditions. For this purpose, two antibodies against HS domains (JM403, 10E4) were used. Interestingly, total JM403 signal was significantly increased after 1 day of culture under shear stress (total signal intensity of 72290 ± 19400 vs 34928 ± 11937 in static culture, $p<0.05$) (figure 6).

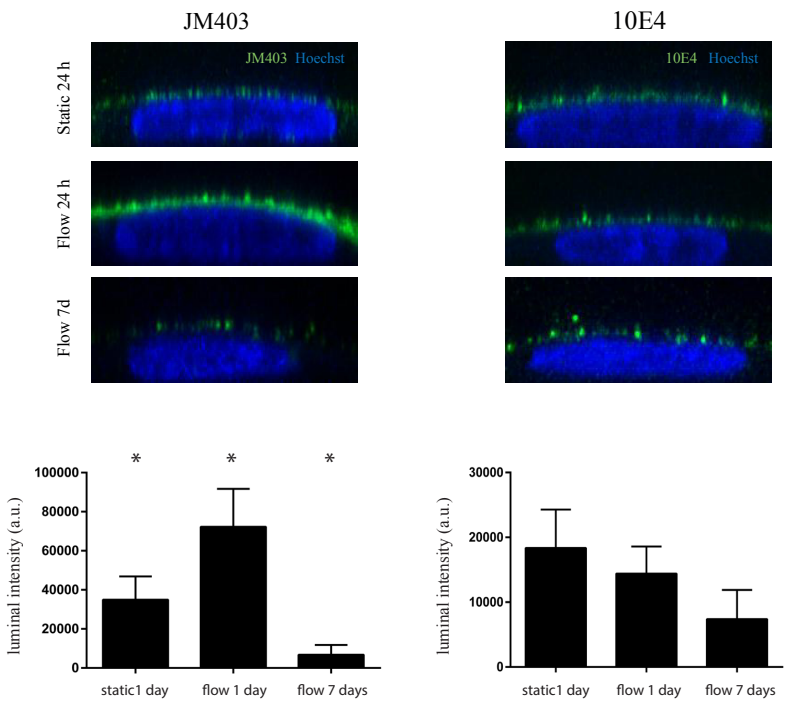

Figure 6: Prolonged shear stress alters the expression of heparan sulfate domains. *A) Representative examples of HS staining on top of the endothelial cells with JM403 (left) and 10E4 (right) after static, 1 day flow and 7 days of flow culture. B) Quantification of staining with specific HS-domain antibodies JM403 (left) and 10E4 (right). For quantification, isotype control staining is subtracted from the stainings shown in figure 6A.*

However, after 7 days of flow the total luminal staining signal was markedly reduced for JM403 compared to both static culture and 1 day shear stress (total signal intensity of 6786 ± 5022, $p<0.05$) (**figure 6**). In contrast, total 10E4 signal was not significantly changed, although a trend towards a reduction could be observed during 7 days of flow. Both antibody expression levels thereby suggest that exposure to shear stress results in the modification of the luminal heparan sulfate composition in HUVECs bearing less N-substituted residues

Laminar shear stress induces an anti-inflammatory glycocalyx

Both JM403 and L-selectin have been previously shown to require the presence of N-unsubstituted glucosamine residues to bind to HS [27,31]. Consequently, we predicted that the JM403 epitope could be a pro-inflammatory HS domain. In our study, this would imply that the increase in JM403 after 24 hours and the decrease after 7 days flow, reflect a pro- and anti-inflammatory phenotype, respectively. To study this hypothesis, the capacity of the JM403 epitope to mediate monocyte recruiting and adhesion was tested. For this purpose, static cultured HUVECS were stimulated with TNF-α to enable inflammatory cell adhesion. Consequently, these cells were perfused with THP-1 monocytes. To determine the role of the JM403 epitope in adhesion, the assay was performed after blocking the epitope with JM403 antibodies or an appropriate isotype control. Blocking the JM403 domain on the endothelial cell surface with JM403 antibody specifically reduced adhesion of the mononuclear THP-1 cells (71.7% of control adhesion, $p<0.01$) (**figure 7**).

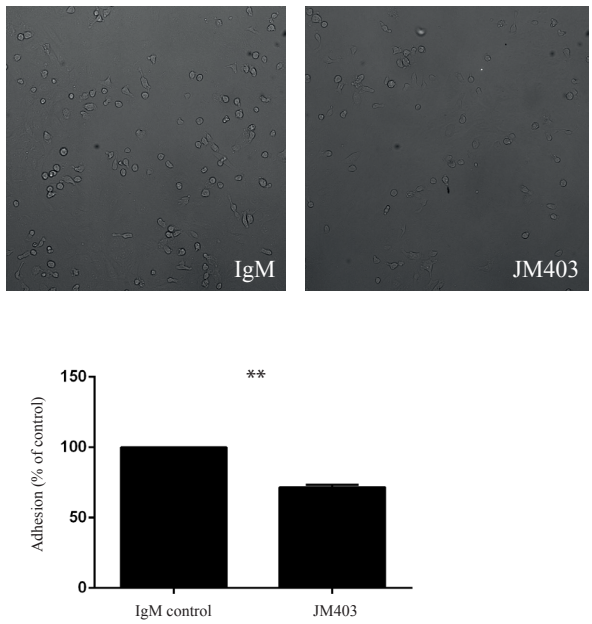

***Figure 7*: Monocyte adhesion after blocking the JM403 epitope in cultured HUVECs.** *A) Examples of monocytes adhered to the endothelial layer after blocking with JM403 (right) and a control IgM antibody (left). B) Quantification of A. Number of adhered monocytes where corrected after blocking for JM403 were corrected for the number adhered in the control situation.*

Discussion:

Changing the endothelial environment in vitro through exposing the cells to a prolonged uniform laminar shear stress not only induces a more homogeneously distributed and thicker glycocalyx but also changes heparan sulfate composition of surface expressed HSPG leading to a non-inflammatory, non-adhering phenotype.

While previous studies already have suggested a role for shear stress in expression and function of an endothelial glycocalyx [21,25,32,33], we focused in this study on the compositional and functional changes in the luminal endothelial glycocalyx upon exposure to long-term shear stress. To quantify the luminal stained endothelial glycocalyx we reconstructed this layer to a 3-dimensional image to properly distinct luminal from abluminal staining. In contrast to the observed effects of long-term shear stress exposure, we confirmed with the observed increase in IL-8 levels after 24 hours of shear stress previous data that short-term exposure to high laminar shear stress evokes a profound inflammatory response in endothelial cells instead of inducing a quiescent phenotype [29]. Furthermore, the subsequent decrease in IL-8 after 7 days reveals that prolonged exposure to such shear stress induces the quiescent phenotype, as exemplified by induction of the well-known shear-responsive genes, such as KLF2 and eNOS. Together, with rearranging of VE-cadherin and eNOS proteins into cell-cell contacts and the appearance of typical short stranded f-actin filaments at the abluminal endothelial domain.

After subjecting the cells to shear stress we observed an increase in WGA staining intensity and thickness after 7 days. WGA binds N-acetyl-D-glucosamine which enables us to estimate changes in the endothelial glycocalyx dimensions, since this carbohydrate is present in most of the glycocalyx components (except for chondroitin and dermatan sulfate). Interestingly we also observed a more homogeneous distribution of the WGA staining on top of the endothelial layer. Previously a decrease of WGA staining was observed upon stimulating endothelial cells with ROS [34]. Furthermore, WGA staining on endothelial cells in rat glomeruli was decreased in a model of spontaneous kidney failure in aged rats [35].

Since WGA stains most of the subcomponents of the endothelial glycocalyx, the biochemical composition of the glycocalyx cannot be studied directly using this lectin. Since the specific composition could be crucial for endothelial cell function we used the following HS-domain specific antibodies 10E4 and JM403. While 10E4 mainly recognizes mixed HS domains that contains both N-acetylated and N-sulfated disaccharide units [26], JM403 typically needs the relatively rare N-unsubstituted glucosamine residues within the HS chain to be able to bind [27]. The presence of this N-unsubstituted domain within the HS sequence mostly likely depends on the activity and efficiency of the NDST enzymes. These enzymes modify the HS sequence during the modification process by substituting the acetyl group from glucosamine residues with sulfate, via an N-unsubstituted intermediate. However, sometimes this modification is partial, which then results in N-unsubstituted

domains in mature HS [36-38]. Since under normal, healthy, circumstances this domain occurs relatively rare, it has been proposed that due to pathological processes this domain contributes to selective protein, and or receptor binding [39].

To the best of our knowledge, we are the first to study the specifically luminal expressed JM403 epitope in HS, since most studies only investigated JM403 binding within the ECM. Interestingly, JM403 staining was significantly increased after 24 hours. After 7 days of shear culture, hardly any JM403 staining was observed. The observed increase after 1 day coincided with a pro-inflammatory phenotype, since IL-8 expression was 100–fold increased. This might imply a relation between the JM403 epitope within HS and a pro-inflammatory endothelial phenotype. The observation that this epitope for JM403 binding, a domain with N-unsubstituted glucosamine residues, is involved in THP-1 adhesion further supports this hypothesis. Since it has been shown that endothelial HS that are able to bind L-selectin are enriched for N-unsubstituted glucosamine residues [31], the observed reduction in monocyte adhesion might be L-selectin dependent. In addition to the quiescent endothelial phenotype, this indicates that prolonged shear stress induces an anti-inflammatory HS modification. In contrast, a short period of shear stress leads to a pro-inflammatory phenotype of the cell (high Il-8 gene expression) and glycocalyx (high JM403 staining).

Although the changes in EG are clearly observed after 7 days of shear stress culture, we cannot be sure that shear stress is the single responsible stimulus. Prolonged culture of endothelial cells under static conditions has been shown previously to also induce endothelial glycocalyx growth [40]. However, when endothelial cells were cultured under static conditions for 7 days, they slowly continued to proliferate and eventually formed several cell layers without any organization. This made it practically impossible to compare the glycocalyx on top of 7 days cultured cells in static conditions with the monolayer in flow conditions (data not shown).

Altogether we demonstrated that prolonged fluid shear stress alters the composition and structure of the endothelial glycocalyx. Shear stress induces the production of a thicker and more homogeneously distributed layer which. Furthermore, the altered HS composition results in the decrease of the epitope for JM403 binding, which is indicative for a more anti-inflammatory endothelial glycocalyx upon prolonged shear stress stimulation.

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