Improved quiescent endothelial monolayer formation on purified HMW-fibrinogen coatings

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Preliminary data
Abstract

Background: Scaffold coating with fibrinogen results in a non-thrombogenic, endothelialized inner layer of a vascular graft, preventing small-diameter graft failure. However, multiple fibrinogen variants occur in the human circulation and these variants influence endothelial cell characteristics. Objectives: This study aimed to investigate the formation, integrity and morphology of confluent endothelial monolayers on coatings of naturally occurring high molecular weight (HMW) and low molecular weight (LMW) fibrinogen, in order to identify better and more defined fibrinogen coatings for quiescent endothelial monolayer formation. Methods and Results: On HMW-fibrinogen coatings human umbilical vein endothelial cells (HUVEC) spread faster, their confluent monolayers showed higher electrical resistances (24.7 ± 6.1%) and more quiescent F-actin arrangements than those on LMW-fibrinogen coatings. The cell-matrix interactions were responsible for the initial increase in monolayer resistance (0-36h), whereas the cell-cell contacts showed late effects on enhancing monolayer resistances on HMW-fibrinogen (36-72h). The increased monolayer resistance showed to be independent of ERK1/2, RhoA and Rho-kinase signaling. Furthermore, slightly increased macromolecule permeability, but no intercellular gaps were observed in HUVEC on LMW-fibrinogen coatings. Conclusions: The endothelial monolayer on purified HMW-fibrinogen coatings shows a better integrity with a more quiescent phenotype than on LMW-fibrinogen. These findings provide a good starting-point for vascular tissue engineering with HMW-fibrinogen coatings.
Chapter 4A

Introduction

Patients with cardiovascular diseases often require the replacement of diseased or damaged blood vessels. Synthetic conduits of polyethylene terephthalate (Dacron) or expanded polytetrafluoroethylene (Teflon, ePTFE) have been used successfully for large-diameter vessel replacements. Unfortunately, small-diameter synthetic grafts display high failure rates, due to thrombus and plaque formation. Failure of small vascular grafts can be avoided by creating a quiescent endothelial monolayer within the graft. Numerous techniques have been developed with the goal of enhancing endothelial cell coverage and retention on synthetic grafts, e.g. cell settling, hydrostatic delivery, electrostatic attraction and pre-coating/surface modification. However, long-term endothelial cell retention remains problematic. Scaffold pre-coating with the extracellular matrix protein fibrinogen, can allow more precise “engineering” of endothelial cell behavior in the development of vascular tissues.

In blood vessels the endothelial monolayer forms a barrier and regulates extravasation of blood components. Adjacent endothelial cells are in close contact, wherein the adherence and tight junctions form the intercellular sealing. Small gaps between the endothelial cells can lead to extravasation of fluid and macromolecules, for instance during inflammation, while larger gaps may expose the thrombogenic subendothelial matrix. Small G-proteins of the Rho-family play a crucial role in myosin light chain (MLC) phosphorylation. MLC phosphorylation via RhoA, Rho-kinase and MLC-kinase leads to actin-myosin-based cell contraction and results in intercellular gaps. Various compounds were shown to disrupt the barrier function of confluent endothelial monolayers, e.g. vascular endothelial growth factor (VEGF), thrombin and histamine. Intact fibrinogen, fibrinogen fragment Bβ15-42 and the γC-domain added to the luminal side of the endothelial monolayer, reduced the endothelial barrier function via ERK1/2- and/or RhoA-dependent pathways. So far, no barrier dysfunction due to intact fibrinogen or fibrin(ogen) fragments on the basal side of endothelial monolayers has been reported.

Previously, fibrin(ogen) coatings and scaffolds were found to enhance endothelial cell coverage and the formation of shear-stress resistant endothelial monolayers in vascular grafts. Moreover, fibrin(ogen) coatings form a better non-thrombogenic coating than whole ECM or gelatin, and endothelial cells tend to orientate towards the flow direction on fibrin(ogen) coatings. Naturally, fibrin acts as a barrier that prevents blood loss after an injury, and as temporary extracellular matrix it stimulates wound healing. The temporary fibrin matrix is degradable and stimulates matrix remodeling. Moreover, fibrin facilitates cell adhesion and cell-matrix interactions.

Many naturally occurring fibrinogen variants are present in human plasma, the most prominent forms are; high molecular weight (HMW), low molecular weight (LMW) and low molecular weight' (LMW') fibrinogen. Unfractionated-fibrinogen is the natural mixture of fibrinogen forms and contains 70% HMW-fibrinogen, representing the intact protein (340 kDa), 24% LMW-fibrinogen, with one partial degraded alpha-chain (305 kDa) and 6% LMW'-fibrinogen, with both alpha-chains partially degraded (270 kDa). After polymerization of soluble fibrinogen into a fibrin network; HMW-fibrin is composed of an open structure with thick fibers and LMW-fibrin of a more dense structure with thinner fibers. Microvascular endothelial cells were shown to increase new capillary formation in HMW-fibrin matrices both in vitro and in vivo, whereas its counterparts on
LMW-fibrin did not. The functional endothelial cell characteristics were improved on HMW-fibrin matrices, as was shown by an increased proliferation and migration, and changes in gene expression of endothelial cells.

In the present study, we investigated the formation of quiescent endothelial monolayers on HMW- and LMW-fibrinogen coatings. Several studies showed superior results using unfractionated-fibrin(ogen) coatings of vascular grafts, compared to collagen or fibronectin coatings and we showed that naturally occurring HMW- and LMW-fibrinogen variants have structural differences and functional effects on endothelial cells. Therefore, we investigated the adhesion, spreading, formation of confluent monolayers, their permeability, intracellular signaling, as well as the arrangements of the cytoskeleton and cell-cell junctions of HUVEC cultured on purified HMW- and LMW-fibrinogen coatings. By using one specific form of fibrinogen, with well-characterized effects on endothelial cells, vascular tissue engineering applications might be further improved.

**Materials and methods**

*Purification of HMW- and LMW-fibrinogen*

Fibrinogen fractions were purified from plasminogen-free fibrinogen (Calbiochem, La Jolla, USA) as described previously by Holm et al. After precipitation the fibrinogen fractions were dissolved in Tris/HCl, dialyzed against phosphate-buffered saline (PBS, Braun, Melsungen, Germany) and treated with gelatin-sepharose beads to remove fibronectin. The purity of the fibrinogen fractions was determined using sodium dodecylsulphate polyacrylamide gel electrophoresis in reducing and non-reducing conditions. Calculations of the fibrinogen concentrations were based on molar extinction coefficients (E1% 280 nm fibrinogen is 15.1).

*Fibrinogen and gelatin coatings*

Fibrinogen coatings were prepared on polystyrene culture plates using 100 µl/cm² with a concentration of 0.2 µM fibrinogen in PBS or with 1% gelatin. Coating was performed 1h at 37°C, followed by removal of the excess solution and washing with PBS. 0.2 µM fibrinogen was found as the lowest concentration that facilitates optimal cell adhesion and proliferation. Additional blocking of the binding sites of culture plates with 1% bovine serum albumin (BSA) did not influence the adhesion and proliferation of endothelial cells.

*Cell culture*

Human umbilical vein endothelial cells (HUVEC) were isolated from umbilical cords, and cultured and characterized as previously described. HUVEC were cultured on 1% gelatin-coated culture plates in Medium 199 supplemented with 100 U/mL penicillin and 100 mg/mL streptomycin (p/s), 2 mM L-glutamine (all Lonza, Verviers, Belgium), 5 U/mL heparin (Leo Pharmaceutical Products, Weesp, The Netherlands), 3.75 µg/mL endothelial cell growth factor (ECGF, crude extract from bovine brain), 10% heat-inactivated human serum (HSi, Sanquin, Amsterdam, The Netherlands) and 10% heat-inactivated newborn calf serum (NBCSi, Lonza), further depicted as complete M199 medium. Confluent monolayers were washed, trypsinized and seeded in 1:3 ratios. HUVEC
were cultured at 37°C in a water-saturated atmosphere of 95% air 5% CO₂ and used until the second passage.

**Adhesion and spreading**

Adhesion and spreading of HUVEC was investigated after seeding 2.0x10⁴ cells/cm² on fibrinogen coatings in M199 supplemented with p/s and 1% human serum albumin (HSA, Sanquin, Amsterdam, The Netherlands). After 1, 2, 4 and 24h the wells were washed and cells were fixated with 2% paraformaldehyde. Pictures were taken using a Qimaging camera on a Zeiss microscope connected to a computer with Optimas image analysis software (Media Cybernetics, Bethesda, USA). Cell counting and cell area quantification were performed using Image J software (NIH, Bethesda, USA).

**Electric Cell substrate Impedance Sensing (ECIS)**

Transendothelial electrical resistance (TEER) of confluent HUVEC monolayers was measured using a technology referred to as ECIS from Applied Biophysics (Troy, USA). The sensing area within a single well of the ECIS array consisted of 10 electrodes in parallel (total 5x10⁻⁴ cm²), and a large counter electrode (0.15 cm²). An alternating electrical field (<1 µA) was applied to the cells, which were electrically connected via the electrolytes of the culture medium on the cells. The ECIS device measured the associated voltage drop across the system and determined the electrical resistance and capacitance of the cell-covered electrodes. Prior to cell seeding, the arrays were coated with gelatin or fibrinogen, as described above. HUVEC (6.0x10⁴ cells/cm²) were seeded in complete M199 medium, and ECIS measurements were performed for at least 72h at 37°C and 5% CO₂. The resistance at 4 kHz serves as a quantitative indicator for the barrier integrity, as determined by the cell-cell and cell-matrix interactions. Using modeling software provided by the manufacturer, the impedance values at 4 kHz were extracted in parameters describing the cell-cell interactions (Rb) and the cell-matrix interactions (alpha). To evaluate the individual spreading and adhesion of the cells the capacitance at 40 kHz was used, as described by Wegener et al. The slope and t½ during the first hour represent the initial cell adhesion and spreading.

Furthermore, ECIS measurements were used to determine the involvement of ERK1/2 on endothelial monolayer integrity, adding 10 µM PD98059 (Cell Signaling Technology, Danvers, USA) immediately after cell seeding. Moreover, these measurements were used to determine the involvement of RhoA and Rho-kinase. RhoA was inhibited with 1 µg/mL C3-transferase (Cell Signaling Technology) and Rho-kinase with 10 µM Y27632 (Tocris Biosciences, Ellisville, USA) or 100 µM H1152 (Calbiochem). RhoA and Rho-kinase inhibitors were added either immediately after cell seeding or after establishment of a confluent monolayer (36h after cell seeding).

**Macromolecule passage**

Transwell filters (Costar, Amsterdam, The Netherlands) were coated with gelatin or 2 µM fibrinogen prior to cell seeding. HUVEC (6.0x10⁴ cells/cm²) were seeded in complete M199 culture medium on coated transwell filters. The exchange of macromolecules through the endothelial monolayer was investigated by the transfer of Horse Radish Peroxidase (HRP)
through the monolayer, as described previously\textsuperscript{26}. After 72h of culture the barrier function of the confluent endothelial monolayer was determined during 15, 30, 45, 60, 90 and 120 minutes. All passage experiments were performed in complete M199 medium.

**Immunofluorescent microscopy**

Confluent HUVEC monolayers were visualized by seeding $6.0 \times 10^4$ cells/cm\textsuperscript{2} on fibrinogen coated 8-well µ-slides (IBIDI, Munich, Germany). After 4, 24 and 48h of culture in complete M199 medium, cells were fixated with 2% cold formaldehyde (Merck, Darmstadt, Germany) for 15 minutes, permeabilized 1 minute with 0.05% Triton X-100 (C2206, Sigma-Aldrich, St Louis, USA) and washed with PBS. F-actin staining was performed during 2h incubation with rhodamine-phalloidine (1:100, Molecular Probes, Eugene, USA) at room temperature. VE-cadherin staining was performed with monoclonal antibody C19 (sc-6458, 1:200, Santa Cruz Biotechnology, Santa Cruz, USA) during overnight incubation at 4°C, followed by secondary antibody rabbit anti goat Alexa 488 (1:100, Dako, Darmstadt, Germany) with incubation at room temperature for 1h. The slides were washed and sealed in Vectashield containing Hoechst (Vector Laboratories, Peterborough, UK). Imaging was performed with a Zeiss Axiovert 200 Marianas \textsuperscript{TM} inverted microscope, controlled with Slidebook 5.0.9 software (Intelligent Imaging Innovations, Denver, USA).

**Statistical analysis**

Statistical analyses were performed using paired Student t-test or One-way ANOVA with Bonferroni post-hoc test. Numbers of replicates and significant P-values are indicated in the text of figures, P\textless{}0.05 was considered significant. All data were obtained in three or four independent experiments with triplicate wells. The results are given as mean ± SEM.

**Results**

**Adhesion and spreading of HUVEC on fibrinogen coatings**

To characterize the adhesion and spreading of endothelial cells on various fibrinogen coatings, HUVEC were seeded on albumin, unfractionated-, HMW- and LMW-fibrinogen coatings. The number of adhered cells was counted, the capacitance and electrical resistance were measured and the cell size was determined after various time intervals. HUVEC did not adhere to BSA-coated wells (Figure 1A, P\textless{}0.05 compared to all fibrinogen variants). No significant differences were found in number of HUVEC adhering to HMW- or LMW-fibrinogen coatings (102 ± 5% vs 89 ± 2%, Figure 1A). This was confirmed using ECIS measurements of the capacitance (as function of cell adhesion and spreading) and resistance (as function of barrier integrity) of endothelial monolayers. No differences in the slope and t½ were found during capacitance measurements at 40 kHz in the first hour (Figure 1B), representing equal cell adhesion to HMW- and LMW-fibrinogen coatings.
Figure 1. Adhesion and spreading of HUVEC on HMW- and LMW-fibrinogen coatings. (A) The HUVEC adhesion to BSA coating (very dark gray line, squares) (P<0.05), unfractionated- (black line, triangles), HMW- (dark gray line, reverted triangles) and LMW-fibrinogen coatings (gray line, diamonds). (B) The capacitance at 40 kHz, representing the cell adhesion and spreading of HUVEC on HMW- and LMW-fibrinogen coatings was measured with ECIS. (C) The initial spreading of HUVEC on HMW- and LMW-fibrinogen coatings after 4h (*P<0.05) and (D) 24h. (E) The resistance at 4 kHz, during the first 4h represents the cell spreading and adhesion, here the resistance of HUVEC on HMW- and LMW-fibrinogen coatings is given. All data are expressed as mean ± SEM of 4 independent experiments with different HUVEC isolations.
However, after 4h the average cell size of HUVEC during the adhesion period was 13.4 ± 2.2% larger on HMW-fibrinogen compared to those on LMW-fibrinogen coatings (Figure 1C, p<0.05). This reflected a difference in spreading rate, as it was transient and disappeared after 24h of culture on fibrinogen coatings (Figure 1D). Resistance measurements by ECIS showed a tendency towards an increased resistance between 1 and 4h (Figure 1E), also suggesting a faster spreading of HUVEC on HMW-fibrinogen. Taken together, the number of endothelial cells adhering to the two fibrinogen coatings was similar, as was the cell size after 24h. However, HUVEC spread faster on HMW-fibrinogen than on LMW-fibrinogen.

**Integrity of HUVEC monolayers on HMW-fibrinogen coatings**

In addition to cell matrix interaction and cell spreading, cell-cell interactions are an important determinant of the integrity of the endothelial monolayer. To study the endothelial monolayer integrity, ECIS measurements of endothelial cells on HMW- and LMW-fibrinogen coatings were performed. Starting 4h after cell seeding, the HUVEC monolayer on HMW-fibrinogen coatings displayed a significantly higher resistance than on LMW-fibrinogen (average resistance 24.7 ± 6.1% higher i.e. ~150 Ohm, period 4-72h, P<0.001, Figure 2A). After 72h, the resistance of the HUVEC monolayers on HMW-fibrinogen coatings (868 ± 50 Ohm) was higher than that on LMW-fibrinogen coatings (653 ± 64 Ohm, Figure 2A insert, P<0.05).

From the ECIS impedance measurements the Rb and alpha components were extracted. The cell-cell interaction (Rb) was similar during the first 36h of culture on HMW- and LMW-fibrinogen coatings (2.5 ± 0.2 and 2.2 ± 0.1 Ohm.cm²), however between 36 and 72h after cell seeding, the Rb of HUVEC on HMW-fibrinogen increased to 2.8 ± 0.2 Ohm.cm² at 72h, whereas in HUVEC on LMW-fibrinogen the Rb decreased to 1.8 ± 0.3 Ohm.cm² at 72h (Figure 2B, P<0.001). The cell-matrix interaction (alpha) was lower in HUVEC on LMW-fibrinogen during the first 36h of culture (Figure 2C, P<0.001), after 72h of culture HUVEC on HMW- and LMW-fibrinogen showed similar alpha-values (3.2 ± 0.1 and 3.1 ± 0.1 Ohm⁰.⁵.cm). This can be explained by the extracellular matrix production of endothelial cells after longer culture periods. The resistance, cell-cell (Rb) and cell-matrix (alpha) interactions of HUVEC on unfractionated-fibrinogen tended to be lower as those on HMW-fibrinogen, owing to the presence of ~30% LMW- and LMW'-fibrinogen.

**No alteration in ERK1/2, RhoA and Rho-kinase signaling**

Several investigators reported that soluble fibrinogen can impair endothelial barrier function via ERK1/2 and/or RhoA intracellular signaling. Therefore, we evaluated whether the fibrinogen variants may differentially affect endothelial functioning at the level of ERK1/2 or RhoA/Rho-kinase activation. While HMW- and LMW-fibrinogen induced significantly different basal resistance values (773 ± 3 and 654 ± 5 Ohm, P<0.05) at 72h after seeding, the presence of the ERK1/2 inhibitor PD98059 during 72h did not significantly influence the electrical resistance of HUVEC on HMW- and LMW-fibrinogen coatings (724 ± 26 and 670 ± 64 Ohm, Figure 3A). Moreover, no differences were seen in the degree of ERK1/2 phosphorylation as determined by western blotting of HUVEC-lysates after 48h of culture on HMW- and LMW-fibrinogen coatings (data not shown).
Figure 2. Transendothelial electrical resistance of HUVEC monolayers on HMW- and LMW-fibrinogen coatings. (A) ECIS measurements were used to determine the endothelial monolayer integrity of confluent HUVEC on gelatin (light gray line), unfractionated- (black line), HMW- (dark gray line) and LMW-fibrinogen (gray line) coatings (P<0.001). The insets represent the resistance data after 36h (P<0.05) and 72h (P<0.05) of culture. (B) The Rb represents the cell-cell interaction and shows an effect from 36 to 72h in HUVEC on HMW- and LMW-fibrinogen coatings (P<0.001). (C) Alpha represents the cell-matrix interactions and showed an immediate effect disappearing after 48h of culture on HMW- and LMW-fibrinogen coatings (P<0.001). All data are expressed as mean of 4 independent experiments with different HUVEC isolations.
Subsequently, the roles of RhoA and Rho-kinase on the endothelial barrier integrity were evaluated during ECIS measurement by addition (immediately or 36h after seeding) of RhoA inhibitor (C3) and Rho-kinase inhibitor (Y27632). As expected, the basal resistance of HUVEC on LMW-fibrinogen was reduced (913 ± 79 vs 826 ± 52 Ohm). Inhibition of RhoA, both immediately and 36h after cell seeding, showed no influence on the resistance of HUVEC cultured on the fibrinogen variants. In contrast, addition of the Rho-kinase inhibitor during cell seeding completely inhibited the formation of the barrier by HUVEC on gelatin and the fibrinogen variants (data not shown). However, inhibiting Rho-kinase after establishment of the barrier (36h after seeding) caused a ~30% reduced resistance of HUVEC at 72h, both on gelatin, HMW- and LMW-fibrinogen coatings (Figure 3B, P<0.001). Similar results were obtained when the Rho-kinase inhibitor H1152 was used. Together, these data indicate that the increased monolayer resistance on HMW-fibrinogen coatings is independent of ERK1/2, Rho-A and Rho-kinase activities.
Passage of macromolecules through endothelial monolayers

The ECIS resistance measurements give a reflection of the barrier function of endothelial monolayers. To determine the endothelial monolayer permeability for macromolecules directly, we investigated the passage of HRP, which has a comparable Stokes radius as albumin, through HUVEC monolayers on filters coated with gelatin, HMW- or LMW-fibrinogen. As shown in Figure 4, the HRP-passage after 72h of culture is slightly higher through HUVEC monolayers on fibrinogen-coated filters, than through HUVEC monolayers on gelatin. Although not significant, HUVEC on LMW-fibrinogen coatings displayed a slightly higher permeability than those on HMW-fibrinogen coatings.

F-actin cytoskeleton is altered, cell-cell junctions not

To investigate the barrier function and cell-cell junctions of endothelial monolayers, the F-actin cytoskeleton and VE-cadherin in the adherence junctions were stained in HUVEC cultured 24h on HMW- or LMW-fibrinogen coatings. No differences in HUVEC arrangement and cell-cell junctions on HMW- and LMW-fibrinogen were visible by phase contrast microscopy (data not shown). The F-actin arrangement showed a thin and regular peripheral ring in HUVEC cultured on HMW-fibrinogen. However, HUVEC cultured on LMW-fibrinogen displayed a thick and irregular F-actin peripheral ring and stress fibers (Figure 5A,B). In contrast, the VE-cadherin staining pattern showed no gaps and the intensity of the adherence junctions was similar after 24h of culture in HUVEC on HMW- and LMW-fibrinogen coatings (Figure 5C). Taken together, the confluent HUVEC monolayers on LMW-fibrinogen reflected aspects of an activated or stressed phenotype, whereas HUVEC cultured on HMW-fibrinogen were more quiescent.

Figure 4. Passage of macromolecules through HUVEC monolayers on fibrinogen-coated filters. 72h after HUVEC seeding on gelatin, unfractionated-, HMW- and LMW-fibrinogen coated filters, the percentage of HRP-passage through the confluent HUVEC monolayer during a 2h period was determined. All data are expressed as mean ± SEM of 3 independent experiments with different HUVEC isolations.
Figure 5. Immunohistochemical staining of HUVEC in confluent monolayers on HMW- and LMW-fibrinogen coatings. (A) Filamentous-actin in HUVEC cultured on HMW- and LMW-fibrinogen coatings was visualized using F-actin staining and fluorescent microscopy, 63x magnification. (B) represents an enlargement of the box indicated in Figure 5A. (C) VE-cadherin in the adherence junctions was visualized with antibody staining and fluorescent microscopy. The light intensity in all pictures was set at similar levels. Pictures were taken after 24h of culture in complete medium, the scale bars represent 10 µm.
Discussion

In the present study, we provided evidence that purified HMW-fibrinogen coatings provide a better support for endothelial cells to form a tight, quiescent monolayer than LMW-fibrinogen coatings. Specifically, HUVEC spread faster, their monolayers show higher electrical resistances and more quiescent F-actin arrangements on HMW-fibrinogen coatings, whereas the adhesion, permeability for macromolecules and VE-cadherin distribution of HUVEC were similar on HMW- and LMW-fibrinogen coatings. Taken together, the endothelial monolayer on purified HMW-fibrinogen coatings shows a better integrity with a more quiescent phenotype.

Our ECIS data revealed that the endothelial monolayers on HMW-fibrinogen have a higher resistance and therefore a better barrier integrity, than their counterparts on LMW-fibrinogen over the full period of 72 hrs. The integrity of HUVEC on LMW-fibrinogen was sufficient to prevent gap formation and macromolecule hyperpermeability. Unfractionated-fibrinogen has been known to provide a better support than collagen and fibronectin for endothelialization and long-term performance of vascular grafts. Our data adds in showing that these coatings can be further optimized by using one specific form of fibrinogen. Since all commercially available unfractionated-fibrinogen batches consist of ~30% LMW- and LMW'-fibrinogen, the presence of these might influence the endothelial barrier integrity negatively. It has been shown that soluble fibrinogen disturbs barrier integrity by inducing the formation of contractile stress fiber formation, similar to barrier-disrupting vaso-active agents such as thrombin. In the present study, we showed that coating of fibrinogen molecules on the apical-side of endothelial monolayers did not induce barrier dysfunction, neither with HMW- nor with LMW-fibrinogen.

The integrity of endothelial monolayers is determined both by the cell-cell, and the cell-matrix interactions. The increased resistance from 0 to 36h of HUVEC on HMW-fibrinogen was mainly caused by better cell-matrix interactions, as became evidenced by the alpha-values. At later time-points (36 to 72h) the increased resistance of HUVEC on HMW-fibrinogen was mainly caused by better cell-cell interactions, as became evident by the Rb-values. Previously, it has been shown that the adhesive interactions of cells activate various intracellular signaling pathways that direct cell fate, viability and proliferation. Integrins often provide this physical interaction of endothelial cells with the fibrinogen matrix. In addition, it has been shown that HUVEC synthesize new extracellular matrix proteins during culture. In our study, HMW-fibrinogen caused better spreading, barrier integrity and cell-cell contacts on of HUVEC, this suggests an altered intracellular signaling. Previously we showed that the surface expression of integrins did not change in endothelial cells upon culture on HMW- and LMW-fibrin coatings. The differences in cell-matrix interactions became less pronounced in time, probably due to replacement of fibrinogen after matrix synthesis of the endothelial cells. Herewith, we showed that both the cell-matrix as the cell-cell interactions determine the barrier integrity of HUVEC on HMW- and LMW-fibrinogen coatings.

The more quiescent F-actin arrangement in HUVEC on HMW-fibrinogen coatings further confirmed our findings. As stated in the introduction, actin and myosin are important in the contractile machinery in endothelial cells. Previously, it has been shown that soluble fibrinogen γC-domains increase endothelial monolayer permeability via RhoA and stress fiber formation. Fibrin peptide Bβ15-42 was shown to interact with endothelial cells and protected against barrier
dysfunction via intracellular signaling with RhoA and Rho-kinase activities. Moreover, soluble fibrinogen was shown to induce hyperpermeability via ERK1/2 and stress fiber formation. In the present study, the actin arrangement was altered upon culture on HMW- and LMW-fibrinogen, but this was not mediated by alterations in RhoA, Rho-kinase and ERK1/2 intracellular signaling. Herewith, the most apparent signaling pathways were studied on their involvement in endothelial barrier integrity on HMW- and LMW-fibrinogen coatings. Alternative explanations might involve the activity of the other Rho-GTPases; Rac1 and Cdc42 that regulate the endothelial barrier integrity. Also focal adhesion kinase might play a role.

In conclusion, we provided evidence that specifically on HMW-fibrinogen more quiescent endothelial monolayers are formed with a better barrier integrity, when compared to LMW-fibrinogen. Our data argue for a preferential position of purified HMW-fibrinogen as coating in vascular tissue engineering. Taken together, these findings provide a good starting-point for vascular tissue engineering with HMW-fibrinogen coatings.

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Reference List


