The influence of surface micro-structure on endothelialization under supraphysiological wall shear stress

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Interaction between platelets and artificial materials within cardiovascular devices triggers blood coagulation and represents a frequent adverse response to implant deployment. Avoidance of this interaction is obtained through the generation and sustenance under flow of a confluent and stable endothelial monolayer covering the luminal device surface, altogether defined as the process of endothelialization. Supraphysiological wall shear stress (WSS) levels generated within vascular assist devices (VADs) constitute a major challenge toward endothelialization. Here we report the experimental demonstration that stable endothelialization can be achieved at supraphysiological WSS levels by pure means of appropriate surface micro-structuring. Using a custom-designed flow bioreactor we exposed endothelial monolayers to physiological and supraphysiological WSS levels and investigated the resulting integrity of cell-to-cell junctions, the cell density and the cell polarization. At physiological WSS levels, optimal endothelialization was obtained independently from surface topography. However, at higher WSS levels, only monolayers grown on appropriately micro-structured surfaces preserved optimal integrity. Under these flow conditions, endothelial cells polarized by the contact with the micro-structure and, interestingly, oriented themselves in the direction perpendicular to flow. Such endothelial layers withstood WSS levels exceeding of 100% or more the thresholds detected on flat substrates.

1. Introduction

Severe heart failure is the leading cause of mortality in most developed countries, affecting more than 10% of their population above the age of 70 [1]. For these patients, an alternative to heart transplantation is the use of mechanical circulatory support devices (i.e. ventricular assist devices; VADs) with the purpose of improving the quality of life and functional capacity. Although in selected cases with limited comorbidities, one- and two-year survival rates approach the outcomes after heart transplantation [2] a number of unsolved problems remain associated with the implantation of VADs. High flow rates within the device yielding supraphysiological wall shear stress (WSS) levels (e.g. above 4–5 Pa) cause damage of blood cells. Additionally, the contact of blood with artificial surfaces (composed by metallic alloys or polymers) activates blood coagulation [3]. These processes lead to hemolysis and thrombus formation, which may result in pump malfunction and thromboembolic events with potentially fatal consequences [4]. To prevent the activation of blood coagulation, aggressive anticoagulation and platelet inhibition is required which, in turn, increases the risk of bleeding complications [5].

Despite the increased choice of biomaterials for VADs [6], thromboembolic events at the level of the inflow cannular or within the pumping system are still exceedingly frequent [7,8]. New engineering strategies are therefore required to improve the integration of the implant within the body, reducing the incidence of device-related complications. To this end, the long-lasting coverage of luminal VAD surfaces by endothelial cells (ECs) up to the formation of a confluent cell monolayer (altogether defined as the process of endothelialization) is considered as the optimal solution to avoid complications in VAD recipients [8,9]. Here, a stable and confluent endothelium would provide a twofold protection. First, prevent the direct contact between blood and device material and second demote the onset of local inflammatory responses [10,11].
The implementation of surface modifications to known biomaterials represents a promising and cost-effective strategy to modulate cellular processes, which are essential for the development and maintenance of a stable endothelium [12,13]. Engineered surfaces have shown a large potential in modulating critical EC activities [14,15]. Micron-sized gratings contribute to endothelialization under flow by reinforcing EC adhesion to the substrate via a direct modulation of focal adhesion maturation and recruitment of adaptor proteins mediating the interaction with the actin cytoskeleton [16]. Anisotropic topographies can additionally promote the polarization of ECs under flow [14,15] which in turn favors tissue homeostasis [17] and demotes inflammation [18]. Finally, the basal interaction with gratings preserves the integrity of the endothelium upon wounding and under physiological WSS levels [15]. This effect derives from a topography-mediated stabilization of the vascular endothelial cadherin (VEC) based cell-to-cell junction that reinforces the connectivity between neighboring cells in the endothelium [14].

Importantly, all these cellular activities are critically dependent on the local physical conditions, determined both by blood flow and by the geometry of the surrounding vessel walls. Indeed, clinical evidence demonstrates that endothelialization is hampered in regions of disturbed (i.e. increased) flow [19,20]. While topography has been shown to be efficient in promoting endothelialization under physiological WSS values [21], it has yet to be proven whether such benefits can be exploited at supraphysiological WSS levels and, if so, what are the values up to which surface endothelialization of cardiovascular devices can be obtained by pure means of surface structuring.

Here we investigate this important topic, with the help of a custom-designed parallel plate flow bioreactor that is able to reproduce physiological and supraphysiological WSS values (up to 10 Pa) on textured surfaces. The aim of this study is to investigate how surface topography regulates the stability of endothelial monolayers under supraphysiological WSS. We hypothesize that rationally-designed surface textures promote the maintenance of a fully confluent and integral endothelium at WSS values comparable to those experienced in VADs.

2. Materials and methods

2.1. Substrate fabrication

Gratings with depth, line width, and pitch of 1 μm were imprinted on 180 μm thick untreated cyclic olefin copolymer (COC) foils (Ibidi, Germany) using nano-imprint lithography (NIL) as previously reported [14,16]. At the end of the fabrication procedure, the substrates were treated with oxygen plasma (100 W for 300 s), to increase the hydrophilicity of the surface and to promote cell adhesion.

2.2. Antibodies

The following primary antibody was used: goat anti-VEC (Vascular Endothelial Cadherin; #6458) from Santa Cruz Biotechnology Inc. (USA). The secondary antibody was a donkey anti-goat-alexa-488 (A11055).

2.3. Cell culture

Human umbilical vein endothelial cells (HUVEC; Invitrogen, USA) were grown in medium 200PRF supplemented with fetal bovine serum 2% v/v, hydrocortisone 1 mg/ml, human epidermal growth factor 10 ng/ml, basic fibroblast growth factor 3 ng/ml and heparin 10 mg/ml (all reagents from Invitrogen) and were maintained at 37°C and 5% CO2. All reported experiments were performed using cells with less than seven passages in vitro. The substrates were sterilized by overnight treatment with ethanol and rinsed three times with PBS before starting the coating procedure. The substrates were then coated with gelatin according to the protocol by Lampugnani et al. [22]. The substrates were stored at 4°C until the seeding of the cells. To generate a confluent monolayer, cells were seeded on COC substrates at high density (3.5–5 × 10⁴ cell/cm²) and were cultured for three days.

2.4. Flow experiments

A custom-designed parallel plate flow chamber [15] was used to apply a constant shear stress to the monolayers (Fig. 1). The shear stress applied on the cells (τ) can be expressed as function of the channel dimensions (width, w and height, h), medium properties (viscosity, μ) and volumetric flow rate (Q) using the calculation for WSS in a rectangular channel: \( τ = \frac{6Q}{wh^2} \) [21]. While channel dimensions and medium properties were fixed in our experimental setup (w = 20 mm, h = 0.3 mm, \( μ = 8.4 \times 10^{-4} \text{ Pa} \cdot \text{s} \)), the flow rate was controlled using a peristaltic roller pump (Model 66, Harvard Apparatus) to apply WSS up to 10 Pa to the endothelial cell

Fig. 1. Validation of the flow bioreactor at supraphysiological flow levels. a) Scheme of the fluidic channel housing in its center a square patch supporting an endothelial monolayer. The flow velocity (in m/s) along the vertical channel profile is reported as color-coded map with reference to a flow rate of 100 ml/min, yielding a WSS value of around 5 Pa. Black arrows indicate the direction of the flow (positive y). b) Theoretical WSS contour maps at the basal channel surface. The channel area where target WSS values (tolerance of 3%) are obtained is depicted in green. Regions of lower WSS are reported in cyan, while regions of higher WSS are in yellow. A black arrow indicates the direction of flow. An open black square indicates the position in the channel where the substrates are located (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).
monolayer. A compliance element was inserted between the roller pump and the flow chamber to dampen flow pulsation. The flow chamber was connected to the peristaltic pump with Tygon Tubing (E-3603; Saint-Gobain, France) certified for low cytotoxicity.

The CO2 substrate with the monolayer was placed in a CO2 frame on the inferior part of the polycarbonate flow chamber and the upper part was then mounted on top. The assembled flow chamber was filled with medium and incubated at 37 °C and 5% CO2 for 30 min before connecting it to the hydraulic circuit.

The flow bioreactor and the medium reservoir were allocated in an incubated chamber (Life Imaging Services, Switzerland) where the environment was maintained stably at a temperature of 37 °C and CO2 concentration of 5%. The cell monolayer was then exposed to the flow generating the desired shear stress conditions for 16 h.

2.5. Immunostaining

HUVECs were fixed and permeabilized for 10 min with 3% paraformaldehyde (PFA) and 0.5% Triton-X100 in PBS at room temperature (RT). The cells were then post-fixed with 3% PFA in PBS for 15 min. After washing the samples three times for 5 min with PBS, they were incubated with 5% bovine serum albumin (BSA) in PBS for 1 h at RT. The samples were incubated with TRITC-phalloidin (Sigma, USA) or with goat anti-VEC primary antibody overnight at 4 °C.

Subsequently, the samples were rinsed four times for 1 h with 5% BSA in PBS and then were incubated with anti-goat-alexa-488 secondary antibody for 45 min at RT. The samples were fixed and permeabilized for 10 min with 3% paraformaldehyde (PFA) and 0.5% Triton-X100 in PBS at room temperature (RT). The cells were then post-fixed with 3% PFA in PBS for 2 min in 3% PFA, briefly washed again with PBS, mounted with DAPI-containing Vectashield (Vector Labs Inc., USA) and immediately imaged.

2.6. Image acquisition

The immunostained samples were imaged using an inverted Nikon Ti wide-field microscope (Nikon, Japan) equipped with an Orca R-2 CCD camera (Hamamatsu Photonics, Japan). Fluorescent image stacks of HUVECs immunostained for VEC were acquired with a 40X, 1.3 NA oil immersion objective (Plan Fluor, Nikon, Japan), using a FITC filter.

Confocal images of immunostained HUVECs were collected with a Leica SP2-AOBS (Leica, Germany) microscope using a 63X, 1.4 NA, oil immersion objective (Plan-Apo, Leica, Germany). DAPI emission was excited with the 405 nm wavelength of a solid-state laser and collected in the 410–480 nm optical window. VEC signal was acquired exciting the Alexa-488 emission with the 488 nm wavelength of an Argon laser and collected in the 498–568 nm optical window. F-actin was imaged by exciting the TRITC-phalloidin emission with the 561 nm wavelength of a solid-state laser and collecting the signals in the 732–810 nm optical window.

2.7. Image analysis

Cell density in monolayers was measured using the “Cell Counter” tool of ImageJ (National Institutes of Health, USA). To evaluate the monolayer integrity we defined a connectivity index (CI) based on the distribution VEC fluorescent signal (Supplementary Fig. 1). In confluent and growth-arrested monolayers VEC localizes at the cell-to-cell junctions thus exclusively staining the cell membrane. When the monolayer integrity is compromised, the cell-to-cell junctions are disassembled and VEC diffuses in the cytosol [22]. Practically, the CI was calculated from the average skewness ($R_A$) of VEC signal profile along four directions (i.e. N, W, S, and E). As a result, the value obtained was not sensitive to occasional

![Fig. 2. Monolayer integrity under increasing WSS. a) Connectivity index (CI) measured in monolayers grown on flat substrates under static control conditions (CTRL, white histogram) or exposed to steady state flow yielding a WSS of 1.4, 4, 5, 6 or 8 Pa, respectively (gray histograms). b) Corresponding measure of connectivity index in monolayers grown on gratings under control static conditions or exposed to steady state flow yielding a WSS of 1.4, 4, 6, 8 or 10 Pa, respectively. A horizontal gray bar identifies the threshold value measured in recently confluent endothelial monolayers in static conditions. Error bars correspond to the standard error of the mean. The number of measured fields of view is reported in the upper right corner. Significant differences between the population means are reported (** for $p < 0.01$). Fluorescent images of VEC distribution in the analyzed monolayers grown on flat substrates c) or gratings d) under static conditions or exposed to steady state flow. The corresponding WSS values are reported under each panel. The orientation of the gratings is shown in the lower left corner. The white asterisks highlight the uncovered areas.](image-url)
areas of inconsistent fluorescence distribution due to out of focus signal or background.

\[ CI = \text{average } \left( R_{3d}, R_{z}, R_{z,W}, R_{g}, R_{f,E} \right) \]

CI values close to 1 indicate a monolayer with preserved cell-to-cell junctions (i.e. fluorescent signal remaining distributed in the cell membrane. Supplementary Fig. 1) while values close to zero indicate a diffuse VEC signal in the cytoplasm, typical of cells with disassembled or immature cell-to-cell junctions [22]. Additionally, a functional threshold defining differentiated endothelium was measured comparing long confluent endothelial monolayers (more than three days confluent) to recently confluent ones (one day confluent), as reported in Ref. [22]. The CI is reduced to 0.6 in recently confluent endothelium (Fig. 2). Similarly, a measure for the average cell orientation was obtained from the VEC trace or F-actin signal using the “Directionality” tool of Fiji (National Institutes of Health, USA). The obtained value in degrees was normalized relative to the flow direction. The range of possible alignment was 0 to 90°. Thus a value of 0° indicates perfect alignment.

2.8. Numerical validation of the flow chamber

The fluid dynamics of the chamber was validated by a computational fluid dynamics (CFD) simulation in Ansys CFX (Ansys, USA). The volume was drawn with a CAD software (Autodesk Inventor, USA) and consequently imported in CFX, with very high fidelity (10^{-8} m with a critical geometrical feature in the order of 10^{-5} m). Water was chosen as fluid, assuming the following properties: Molar mass \( \mu = 0.0180 \) kg/kmol, density \( \rho = 997.048 \) kg/m³, viscosity \( \mu = 8.4 \times 10^{-3} \) Pa·s. Finally, the geometry was meshed with approximately 1.8 \times 10^6 hexahedral elements. A second fluid dynamics simulation was performed in COMSOL Multiphysics (COMSOL Inc., USA), in order to evaluate the local velocity profile in the region of fully developed laminar flow, where the sample is allocated. In this case, the Fluid Dynamics Module and in particular the stationary laminar flow subection were used, meshing a slice of the channel (2 mm × 20 mm) with approximately 1.1 \times 10^6 tetrahedral elements.

2.9. Statistical analysis

All statistical comparisons were performed using a non-parametric Mann–Whitney (\( \alpha = 0.05 \)) or a Kolmogorov–Smirnov test. All quantitative measurements reported are expressed as average \( \pm \) the standard error of the mean. The total number of measured events in the analysis is always indicated in the presented graphs.

3. Results

3.1. Validation of the fluidic bioreactor

We developed a fluidic bioreactor based on a parallel plate design (Fig. 1). The bioreactor featured a longitudinal fluidic channel with length 60 mm, width 20 mm and a constant height of 300 μm. The center of the fluidic channel (Fig. 1a) housed a square 1 cm² substrate supporting a fully differentiated and growth-arrested endothelial monolayer. Substrates were composed of a transparent and thermoplastic polymer that could be structured via standard hot embossing. Similar thermoplastic polymers are used for the fabrication of various parts of cardiovascular devices [24]. Specifically, control monolayers were grown on flat substrates, while the effect of topography was tested on anisotropic micro-gratings. Importantly, the open design of the fluidic bioreactor allowed placing the substrates with arbitrary orientation with respect to the direction of flow (Fig. 1a).

This set up enabled conditioning primary human endothelial cell monolayers (HUVECs) for 16 h under physiological flow conditions [21], yielding WSS values which ranged from venous (0.35–1.4 Pa) to arterial levels (up to 5 Pa; [21]). To validate this configuration in supraphysiological conditions we simulated steady state flow patterns within the fluidic channel (Fig. 1b). The results show that under flow conditions yielding target WSS values up to 10 Pa the central housing of the channel (i.e. where the cells are allocated) featured a homogenous and fully developed shear distribution (Fig. 1b). In all, the fluidic bioreactor is suitable for testing the effect of supraphysiological flows on human endothelial cells.

Our tests aimed at investigating the phenotype of endothelial monolayers under flow conditions yielding increasing WSS levels. For this, the integrity of cell-to-cell junctions, the cell density, and the cell polarization were evaluated in endothelia grown on flat substrates or gratings, before and after exposure to flow.

3.2. Integrity of endothelial monolayers under increasing WSS

The definition of endothelial monolayer integrity goes beyond the coverage of a target substrate and is manifested by the maturation of cell-to-cell junctions connecting neighboring cells [25]. In physiological conditions, in vivo, a network of well-defined cell junctions enables the monolayer activities in regulating the transport phenomena between blood and surrounding tissue, and is essential to maintain homeostasis [26]. On the other hand, an incomplete endothelial differentiation affects cell connectivity thus resulting in poor or incomplete endothelial function [27].

To quantitatively discriminate fully differentiated, growth-arrested endothelium from immature, non-differentiated ones, we introduced a connectivity index (CI; Supplementary Fig. 1), based on the cellular distribution of the pivotal protein vascular endothelial cadherin (VEC; [22]). CI values measured in control, long confluent HUVEC monolayers grown following the protocol described in Ref. [22] were set to 1 indicating optimal cell connectivity. Consistently, CI values measured in recently confluent endothelia were reduced to 0.6 (Fig. 2). This value was set as threshold to discriminate monolayers featuring full integrity to monolayers providing surface coverage but compromised integrity.

Long confluent HUVEC monolayers grown on flat substrates (Fig. 2a) and exposed to flow yielded physiological WSS values (up to 4 Pa) preserved optimal integrity. At a WSS value of 5 Pa the CI was reduced to 0.8, while it dropped dramatically at higher WSS values (0.4 and 0.25 at 6 and 8 Pa, respectively) indicating that in these conditions endothelia grown on flat substrates could not preserve their integrity. Indeed, areas of poorly connected or completely disconnected cells appeared (Fig. 2c).

Similarly, long confluent HUVEC monolayers grown on gratings in control, static conditions displayed optimal connectivity (Fig. 2b). CI values close to 1 were preserved under flow conditions yielding WSS values up to 6 Pa. A moderate CI reduction was detected at 8 Pa, while connectivity dropped below threshold at 10 Pa (Fig. 2b and d).

These data demonstrate that endothelial monolayers grown on gratings oriented parallel to the flow, could preserve their integrity under supraphysiological flow conditions. In all, the simple implementation of surface structure enabled stable endothelialization of artificial substrates at WSS values which were 60% higher than on identical, flat substrates.

3.3. Density of endothelial monolayers under increasing WSS

The cell density within an endothelial monolayer is a relevant parameter determining the confluency and differentiation state of an endothelium and is inversely proportional to the cell spreading (i.e. the average cell area; [28]). Long confluent HUVEC monolayers are typically characterized by high density (600 cells/mm² or higher) while lower density (2–300 cells/mm²) is typical of recently confluent monolayers [22,28]. Values below 200 cells/mm² indicate an incomplete coverage of the substrate.

Initial monolayers grown on flat substrates or gratings were highly confluent and growth-arrested (Fig. 3). High cell density was maintained in endothelium grown on flat substrates when exposed to flow yielding WSS values up to 6 Pa. A significant density decrease was recorded at 8 Pa. These data demonstrate that under increasing WSS on flat substrates the monolayer integrity is compromised at
The alignment of endothelial cells is shown by the distribution of actin filaments and by the orientation of cell-to-cell junctions [14]. We therefore visualized filamentous actin and VEC in endothelial monolayers grown on flat substrates or gratings under increasing WSS values (Fig. 4). Control endothelial cells grown on flat substrates in static conditions did not show a preferential orientation (Fig. 4a). Exposure to flow conditions yielding physiological WSS values induced a significant alignment toward the direction of flow, which increased up to WSS values of 5 Pa (Fig. 4c). At higher WSS values this coordinated cell orientation was lost (Fig. 4a and c and Supplementary Fig. 2).

Control endothelial cells grown on gratings in static condition aligned to the direction dictated by the topography (Fig. 4b). Cell orientation was further reinforced by exposure to co-aligned flow yielding physiological WSS values (Fig. 4b and d). Interestingly, at higher WSS values a significant fraction of cells displayed a polarization perpendicular to the direction of flow and of the underlying topography (Supplementary Fig. 2). Finally, at WSS levels inducing the loss of monolayer integrity (i.e. 10 Pa), cell polarization along the direction of flow was reestablished (Fig. 4b).

3.5. The effect of endothelial monolayers pre-polarization

Based on the observation that under high WSS values (Fig. 4), ECs on gratings tend to re-align perpendicular to the flow, we wondered whether a pre-polarization of the endothelial monolayer could prove beneficial. Gratings were therefore oriented perpendicular to the direction of flow and the overlying endothelia was subsequently exposed to high, supraphysiological WSS values (10 Pa; Fig. 5).

In this configuration, cells maintained the original polarization dictated by the direction of the gratings (Fig. 5d). Importantly, the measured CI was close to 1, indicating that the monolayer integrity was fully preserved. This effect scaled with the cell density that was significantly reduced. Consistently, ECs in the monolayer were more spread, with an average cell area which was 1.5 times larger than on the static control (Fig. 5c).

Altogether these results demonstrate that a pre-polarized endothelium by means of surface topography can withstand WSS values exceeding by 100% or more the threshold values of flat surfaces.

4. Discussion

When exposed to physiological WSS, the cells within a confluent endothelium rearrange to align themselves to the direction of flow, thus establishing a characteristic polarization that is observable both in vivo and in vitro [17]. This process requires a remodeling of the cytoskeleton and of the inter-cellular adhesions within the monolayer [21,31]. To this end, early upon flow stimulation, the cell-to-cell junctions become more dynamic and overall weaker, allowing cells to reshape. This process is modulated through the control of VEC localization at the cell membrane [28]. Physiological WSS values favor VEC withdrawal from the membrane into the cytoplasm, thus improving the dynamic instability of the cell-to-cell junction. The interaction with surface topography counteracts this process therefore increasing the connectivity between ECs [15,21].

Based on the data presented here, the response of a fully differentiated endothelium to increasing WSS values can be distinguished into three regimes. Under low WSS (i.e. up to 4 Pa) ECs respond by gradually re-orienting along the direction of flow (Fig. 4). This polarization can be achieved without compromising the monolayer integrity and does not require cell proliferation (Figs. 2 and 3). Additionally, the interaction with substrate
topography is not necessary to withstand the mechanical stimulation induced by WSS. At high WSS values (i.e. up to 8 Pa) cells, surprisingly, re-orient along the direction perpendicular to the flow, and the presence of an underlying surface structure becomes necessary to achieve a complete polarization without compromising the monolayer integrity (Figs. 2 and 4). Under these conditions, the interplay between flow stimulation and interaction with topography may preserve a delicate equilibrium at which ECs coordinately reshape while maintaining a sufficient junctional strength (Fig. 4). At higher and supraphysiological WSS values (i.e. 8 Pa and higher) the reorientation of ECs becomes unsustainable and inevitably leads to a loss of monolayer integrity and substrate coverage. Therefore, a correct pre-polarization of the endothelium is necessary to withstand the flow-generated stress. In this case cell reorientation is not required and the endothelium maintains the original polarization perpendicular to flow (Fig. 5).

Several hypothesis have proposed that endothelial cells orient themselves in response to hemodynamic forces by minimizing their exposure to stress [33]. However, a number of findings indicate that flow-induced cell alignment cannot be solely regulated by force minimization. Different cell types select opposite orientations under identical WSS conditions and a cell polarization perpendicular to the direction of flow is typical of vascular smooth muscle cells (SMC) [32]. Similar to ECs, the alignment of SMC depends on the flow dynamics and relies on the active remodeling of the cell cytoskeleton and the cell-to-cell junctions [32,33]. Additionally, the here-presented results reveal that ECs orient either parallel or perpendicular to flow depending on the imposed WSS levels. This observation finds a physiological reference in valvular ECs, a unique population of cells performing endothelial function at the valve leaflet, which align parallel to the underlying collagen fibers but perpendicular to flow, both in vivo and in vitro [34]. Together, these observations imply that endothelial response to flow is an active cell process. Therefore, different endothelialization strategies may be enforced in regions of the vasculature experiencing different WSS levels and thus performing specialized functions.

5. Conclusions

The set of data presented in this work demonstrates the possibility of achieving a stable endothelialization of artificial substrates under supraphysiological flow conditions by pure means of surface structuring. The requirements of such strategy are strictly dependent on the WSS range, necessitating both the contact with a specific surface topography and the correct pre-polarization of the endothelium. In all, the implementation of specific textures on the luminal surface of cardiovascular devices can be envisioned as a viable strategy to improve their biointegration and avoid adverse

Fig. 4. Cell polarization under increasing WSS. a) Average cell alignment measured in monolayers grown on flat substrates under static control conditions (CTRL, white histogram) or exposed to steady state flow yielding a WSS of 1.4, 4, 5, 6 or 8 Pa, respectively (gray histograms). b) Corresponding measure in monolayers grown on gratings under control static conditions or exposed to steady state flow yielding a WSS of 1.4, 4, 6, 8 or 10 Pa, respectively. The vertical dashed line depicts the threshold WSS value for endothelialization. Error bars correspond to the standard error of the mean. The total number of measured cells is reported in the upper right corner. Significant differences between the population means are reported (* for p < 0.05 and ** for p < 0.01). Distribution of filamentous actin (red, left column) and VEC (inverted fluorescent signal, right column) in endothelial monolayers on c) control flat substrates or d) gratings under control static conditions (upper row) or high WSS values (lower row). The orientation of the gratings is shown in the lower left corner. A white arrow indicates the direction of flow (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).
reactions generated by the contact between blood and device material.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.biomaterials.2014.06.046.

References


