Biomaterials 35 (2014) 8479–8486

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

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

Francesco Robotti [a], Davide Franco [a, b], Livia Bänninger [a], Jair Wyler [a], Christoph T. Starck [b], Volkmar Falk [b], Dimos Poulikakos [a], Aldo Ferrari [a, *]

[a] Laboratory of Thermodynamics in Emerging Technologies, Department of Mechanical and Process Engineering, ETH Zurich, Sonneggstrasse 3, 8092 Zurich, Switzerland
[b] Clinic of Cardiac and Vascular Surgery, University Hospital Zurich, Raemistrasse 100, 8091 Zurich, Switzerland

ARTICLE INFO

Article history:
Received 16 May 2014
Accepted 22 June 2014
Available online 10 July 2014

Keywords:
Endothelialization
Endothelial cells
Topography
Adhesion
Vascular endothelial cadherin
Wall shear stress

ABSTRACT

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].

* Corresponding author. ETH Zurich, Laboratory of Thermodynamics in Emerging Technologies, Sonneggstrasse 3, ML J 27.1, CH-8092 Zurich, Switzerland. Tel.: +41 44 632 25 88; fax: +41 44 632 11 76.
E-mail address: aferrari@ethz.ch (A. Ferrari).

http://dx.doi.org/10.1016/j.biomaterials.2014.06.046
0142-9612/

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 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 nanoimprint 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% CO_2. 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 ($3.5–5 \times 10^4$ cell/cm^2) 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: $\tau = 6Q\mu/wh^2$ [21]. While channel dimensions and medium properties were fixed in our experimental setup ($w = 20$ mm, $h = 0.3$ mm, $\mu = 8.4 \times 10^{-4}$ Pa × 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

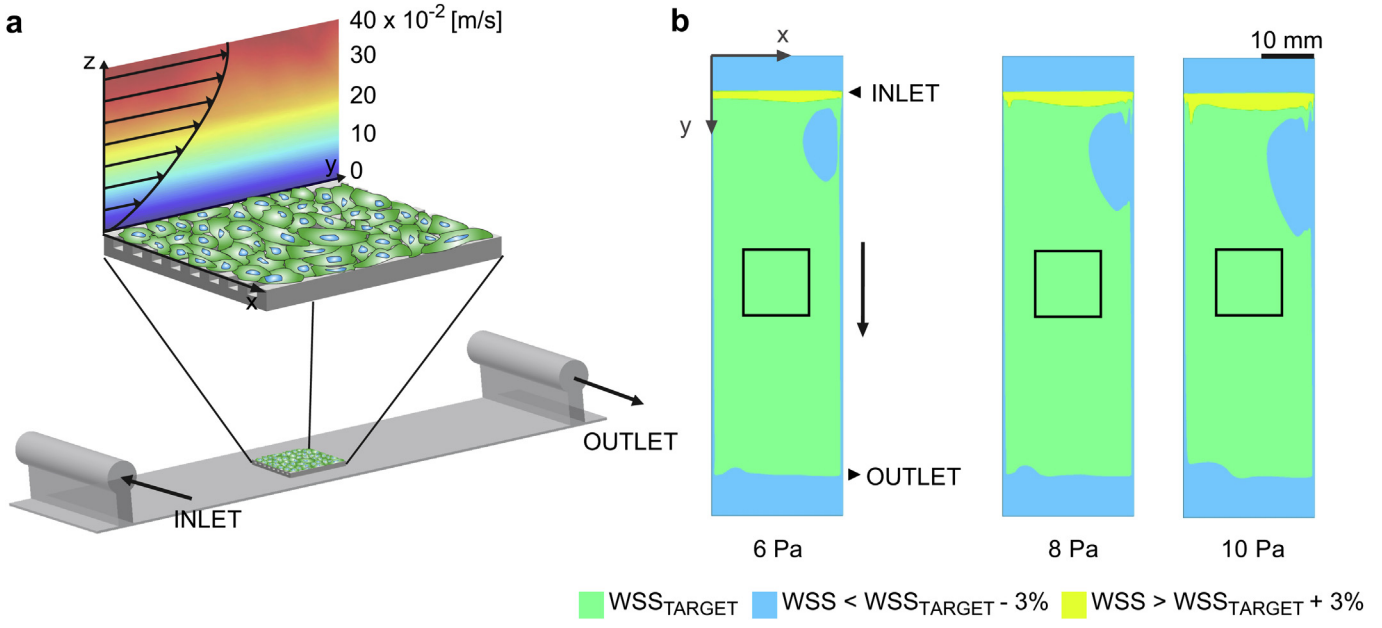

Fig. 1. Validation of the flow bioreactor at supraphysiological flow levels. **a)** Scheme of the fluidic channel housing in its center a square patch supporting an endothelial monolayer. The flow velocity (in m/s) along the vertical channel profile is reported as color-coded map with reference to a 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.).