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The effect of topography of polymer surfaces on platelet adhesion

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ABSTRACT

In this study, the effect of surface topography on fibrinogen and platelet adsorption was investigated. High aspect ratio surface features, in the submicron to nanometer range, were constructed on the poly-(lactic-*co*-glycolic-acid) (PLGA) films. The topographic surfaces were fabricated by solvent-mediated polymer casting on a master template. Fibrinogen adsorption and platelets adhesion on these topographic surfaces were quantified by enzyme linked immunosorbent assay (ELISA) and lactate dehydrogenase (LDH) assay respectively, while the activation of platelets was quantified by flow cytometric analysis using fluorescein isothiocyanate (FITC) tagging. The lowest fibrinogen adsorption amount and platelet activity was observed on surfaces with specific topographical features in the submicron range with a significant reduction in adhesion when compared to the pristine PLGA films. The topographical parameters found to induce low levels of fibrinogen adsorption and platelet response were high aspect ratio structures (>3:1) with reduced interspacing (<200 nm) or high density. The results signify that topographical manipulation of thrombogenic surfaces of biodegradable polymers is a feasible approach for reducing their thrombogenicity.

1. Introduction

Biomaterials are widely used in all fields of medicine and surgery for a range of medical applications from long-term implants to short term dressings. Despite the wide use of biomaterials in the clinical environment, the biocompatibility of these materials is still far from ideal and a variety of foreign body reactions may be triggered when the medical device comes into direct contact with tissue. Typically, blood is the first body fluid that comes into contact with a foreign material. Usually, rapid adsorption of plasma proteins occurs on the foreign surface which leads to platelet adhesion which triggers the coagulation of blood leading to thrombus formation [1,2]. The minimization of protein adsorption and platelet adhesion is therefore critical to improve the blood compatibility of biomaterials.

Strategies adopted to increase the hemocompatibility of biomedical materials include surface modification such as inorganic coatings (carbon based) on artificial heart valves [3], bioactive coatings with fragments of biomacromolecules such as in the immobilization of heparin [4], passivation of surfaces in the formation of a covalently linked coating layer by employing brushes of long-chained hydrophilic molecules like poly-ethylene oxide (PEO) [5], chemical composition modifications of polymer surfaces [6], and chemically patterned surfaces achieved via spatial organization and immobilization of biological molecules with controllable positioning and size [7]. However, one of the limitations in using coatings is its delamination under shear stress in blood flow, thereby causing complications downstream. In addition, the cost and complexity of these coatings could further complicate the regulatory approval process. Most recently, cellular interactions on topographic surfaces with physical attributes have received tremendous attention [8,9] and numerous studies on the effect of surface topography in several aspects of cell biology have been published [10–13]; particularly in areas of orientation [13], proliferation rate [14] and gene expression [15], cell function regulation [7], cell adhesion [16], morphology [17,18], tissue engineering [19] and cytoskeletal arrangement [20]. In contrast, the effect of substrate topography on platelet adhesion has not received as much attention, because most efforts have been focused on chemical modifications [21–23]. In our previous study [24], platelet adhesion on poly(lactic-*co*-glycolic-acid) multi-walled carbon nanotube PLGA-MWCNT composites with different surface topographies has shown a significant reduction level of platelet adhesion on a vertically aligned PLGA-MWCNT composite and was ascribed to the sharp topographic features presented by the MWCNT. Recently, other researchers have also employed colloidal-derived topographies [25], polymer demixing [26], dual-scale structures with nano and micron dimensions [27] as model

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Table 1
Summarized table showing an overview on the topographical dimensions and aspect ratio for all substrates.

Substrate	Interspacing (*I*) nm	Width (*W*) nm	Height (*H*) nm	Aspect ratio
P(1,1,**2**)*	120 ± 27	90 ± 28	**200 ± 68**	~2
P(1,1,**8**)	130 ± 29	90 ± 26	**800 ± 131**	~8
P(1,**5**,8)	140 ± 27	**500 ± 5**	840 ± 161	~2
P(1,**2.5**,8)	140 ± 32	**250 ± 6**	840 ± 159	~4
P(**8**,10,0.4)	**840 ± 12**	1000 ± 60	40 ± 4	~0.04
P(**14**,6,0.4)	**1400 ± 15**	600 ± 55	40 ± 6	~0.07
P(9,30,150)	900 ± 35	3000 ± 12	15 ± 3.7 μm	~5
C(~,1.5,190)	≠	150 ± 47	19 ± 5.8 μm	~130

Substrate notation: P refers to the textured PLGA film and C refers to vertically aligned carbon nanotubes on a silicon substrate; (1,1,2) → refers to the dimensional aspect (interspacing, width, height) of the pillars and is expressed in (×100 nm) unit.
Numbers in bold refer to the area of investigation between substrates; i.e. P(1,1,**2**) refer to height. ≠ Not measurable due to the high density of carbon nanotubes.

substrates to investigate for the impact of surface topography on platelet response. In another study, Milner et al., [28] showed that submicron polyurethane features with low aspect ratio positively influenced platelet adhesion when compared to that of pristine surfaces.

Following our previous results [24,29,30], here we aim to fabricate a variety of topographic features on PLGA films to investigate the role of geometrical parameters, (i.e. size, aspect ratio and density), in influencing platelet adhesion to ultimately determine an optimum topography with much reduced thrombogenicity. We also envision that the study of platelet adhesion on specifically defined geometrical features with homogeneous chemical composition may help to better understand the complex platelet behavior on surfaces.

The topography of interest in this study is high aspect ratio protruding features in the submicron to nanometer range. Methods of fabricating micron to nanometer scale patterns in polymers include hot embossing [31] and nanoimprint lithography [32] respectively, where a master template is used to transfer the features onto the polymer by mechanical deformation at precise conditions of heat and pressure. Technologies such as polymer casting [33] and capillary force lithography [34] allow for the fabrication of high aspect ratio features in polymers and are therefore the techniques adopted in this work. Additionally, we investigate the platelet response on topographical surfaces with similar features on different materials; namely the vertically aligned multi-walled nanotubes (MWNTs). The material used as control was the highly ordered pyrolytic graphite (HOPG) as it is well recognized as being thromboresistant in blood contacting implants [35] and coatings [36]. The adsorption of fibrinogen and subsequent platelet adhesion from platelet-rich plasma (PRP) on the topographic surfaces were quantified and analyzed. The morphology of platelets after exposure to the topographically modified substrates was also studied to determine the degree of activation inflicted in relation to the specific topographic features tested.

2. Materials and methods

2.1. Materials

2.1.1. Substrates

80/20 poly(lactic-*co*-glycolic-acid) 5.01 (PLGA) was obtained from PURAC Biochem. Vertically aligned multi-walled nanotubes (MWNTs) were purchased from

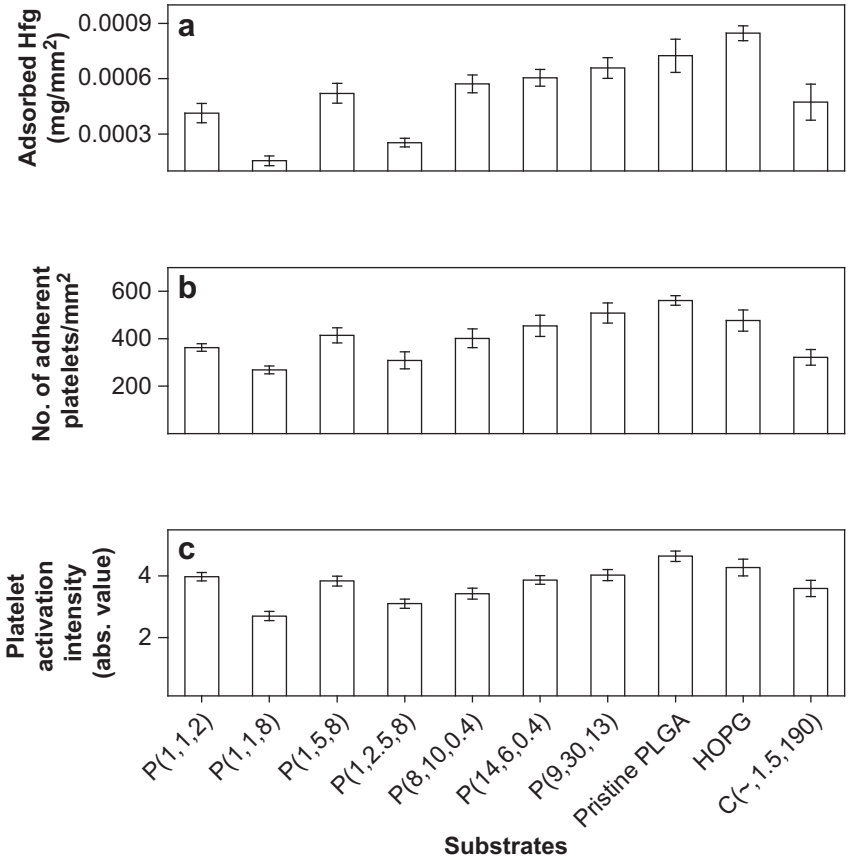

Fig. 1. Quantification graphs showing the a) amount of fibrinogen adsorbed on substrates determined by ELISA, b) number of adherent platelets determined by LDH assay and c) PAC -1 expression on platelet activation derived from flow cytometric analysis. Data were run in triplicate ($n = 3$) and expressed as mean ± standard deviation of 3 repeated independent experiments.