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Influence of dynamic flow conditions on adsorbed plasma protein corona and surface-induced thrombus generation on antifouling brushes

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ABSTRACT

The information regarding the nature of protein corona (and its changes) and cell binding on biomaterial surface under dynamic conditions is critical to dissect the mechanism of surface-induced thrombosis. In this manuscript, we investigated the nature of protein corona and blood cell binding in heparinized recalcified human plasma, platelet rich plasma and whole blood on three highly hydrophilic antifouling polymer brushes, (poly(*N*, *N*-dimethylacrylamide) (PDMA), poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC) and poly[*N*-(2-hydroxypropyl) methacrylamide] (PHPMA) using an *in vitro* blood loop model at comparable arterial and venous flow, and static conditions. A fluid dynamics model was used initially to better understand the resulting flow patterns in a vertical channel containing the substrates to arrive at the placement of the substrates within the blood loop. The protein binding on the brush modified substrates was determined using ellipsometry, fluorescence microscopy and the nature of the protein corona was investigated using mass spectrometry based proteomics. The flow elevated fouling on brush coated surface from blood. The extent of plasma protein adsorption and platelet adhesion onto PDMA brush was lower than other surfaces in both static and flow conditions. The profiles of adsorbed protein corona showed strong dependence on the test conditions (static vs. flow), and the chemistry of the polymer brushes. Specially, the PDMA brush under flow conditions was more enriched with coagulation proteins, complement proteins, vitronectin and fibronectin but was less enriched with serum albumin. Apolipoprotein B-100 and complement proteins were the most abundant proteins seen on PMPC and PHPMA surfaces under both flow and static conditions, respectively. Unlike PDMA brush, the flow conditions did not affect the composition of protein corona on PMPC and PHPMA brushes. The nature of the protein corona formed in flow conditions influenced the platelet and red blood cell binding. The dependence of shear stress on platelet adhesion from platelet rich plasma and whole blood highlights the contribution of red blood cells in enhancing platelet adhesion on the surface under high shear condition.

1. Introduction

Synthetic biomaterials used for vascular applications promote surface-induced thrombus generation and inflammation to various extents [1–3]. The host response to biomaterials begins with an immediate and non-specific or specific adsorption of proteins in blood onto the biomaterials surface, followed by platelet adhesion and activation which can lead to surface-initiated thrombus

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generation. The presence or absence of certain proteins on the surface may be related to the thrombogenicity of biomaterials and the direct observation of the reactivity of platelets with an artificial surface is accordingly important for the classification of its hemocompatibility [4–7]. Surface modification can be utilized as a tool to increase the biocompatibility and tailor the biological response to biomaterials. Among the surface modification techniques, the coatings based on hydrophilic polymer brushes, i.e. polymer chains tethered one end on surface at high density, have emerged as a platform technique to modify the surface properties of the materials and minimize complications that arise at the blood-biomaterial interface [8].

At the blood-biomaterial interface, hemodynamic forces of shear stress at the surface play a critical role in blood contacting devices and influence protein adsorption, platelet and leukocyte adhesion other than simply avoiding cell sedimentation [9,10]. The flow gives rise to Vroman effect [11,12], the competitive protein adsorption on a surface and change of the composition and abundance of protein species [13–17]. The complex protein layer on the surface is usually referred to as the "protein corona" confers a new biological identity to the surface and dramatically alters the biological response, including blood coagulation, complement activation and inflammatory reactions [18]. Study on protein corona on nanoparticles under dynamic flow conditions showed difference in the abundance of associated proteins in corona and wider molecular complexity compared to that obtained in static condition [19–21]. The flow also introduces shear stress to increase the extent of platelet activation and aggregation in the fluid phase [10,22]. The collision of platelets with other cells in blood, initial tethering of rolling platelets, subsequent stable adhesion mediated by the adsorbed proteins, and aggregation are all influenced by the shear stress [23]. Therefore when testing the hemocompatibility of a biomaterial surface exposed to blood, one must consider the effect of shear stress on protein and cell behaviour, and therefore the effects of flow.

Distinct from previously studied surfaces at flow conditions (mostly hydrophobic, e.g. silicone, glass and hydrophobic SAM layer) [13–16], the highly hydrophilic polymer brush layers do have functional groups with tightly bounded water. The protein adsorption on hydrophilic surfaces requires different set of driving forces [3,24]. The information regarding changes in the protein corona from complex media like blood plasma or whole blood with flow on highly hydrophilic surfaces or brushes has not been investigated previously. Protein corona gives polymer brush coated biomaterials a new biological identity, which may be substantially different from their pristine state. It acts as a critical parameter which defines the biological fate of the biomaterials [25,26]. We anticipate that the flow will alter the protein corona composition and determine the biological response to the hydrophilic surfaces. The types of proteins and their abundance in the corona encode information that edicts surface hemocompatibility. We have chosen three different types of antifouling polymer coating with distinct chemistry, poly(*N*, *N*-dimethylacrylamide) (PDMA), poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC) and poly[*N*-(2-hydroxypropyl) mehacrylamide] (PHPMA) in the present study to illustrate the concept. These polymer brush coatings present three major polymer groups, including polyarylamide, zwitterionic polymer and hydroxyl containing functional polymer, which are intensively investigated for generating "non-fouling" polymer brushes [27]. They are reported to be protein and cell adhesion resistant, and thus could be a good model to study the changes in protein and cell binding in dynamic flow conditions [28–33]. In addition, the types of adsorbed proteins which were previously indicated as contributing factors to the thrombogenicity of surfaces (e.g. platelet adhesion) such as fibrinogen, von Willebrand factor are also low on antifouling brushes compared to hydrophobic surfaces. Thus we anticipate that other potential contributing factors could be identified using these surfaces by analysing the nature of the adsorbed protein corona. Moreover, protein binding shows preference on the functionality of the polymer coating [34–37], which could be also altered by the flow conditions.

In addition to the surface chemistry and flow, the type of biological fluids used can also contribute to the observed thrombogenicity of surfaces *in vitro*. Although previous studies of using single protein solutions and platelet rich plasma (PRP) have provided some beneficial information, the contribution of erythrocytes and leucocytes as well as other blood cells were not taken into account. The "non-physiological" nature of evaluation with certain fluids does not stand up to scrutiny [3]. It is known that leucocytes interact with the adsorbed protein layer and trigger reactions which can further compound platelet activation and adhesion [1,2,38]. Erythrocytes could bring the lateral diffusion of platelets to the surface and increase the probability of platelet colliding with the surface [39,40]. The liberation of adenosine diphosphate (ADP) and other factors from red blood cells could also enhance the platelet aggregation and adhesion on surface [40]. Therefore studies using both whole blood and PRP in flow conditions are needed to be examined. In addition, the enzymatic activity in the blood or plasma is an important factor. Most of the studies published on biomaterial surface-blood interaction in the literature used sodium citrate or EDTA anticoagulated plasma, PRP or whole blood, however, in these cases the enzymatic activity (coagulation & complement) was minimized by the chelation of calcium and magnesium. The reactive nature of blood should be restored (closer to *in vivo* conditions) for better understanding of surface interaction of biomaterials with blood.

Thus in the present work, we investigated the protein and blood cell binding on well-defined antifouling hydrophilic brushes with distinct chemistry in flow (arterial and venous) and static conditions using a blood loop. Our aim is to determine whether protein corona composition and cell binding on the surface will be altered under dynamic flow in comparison to static condition. We also want to establish possible correlation of protein corona and surface-induced thrombus generation with respect to polymer chemistry and the test conditions (i.e. static vs. dynamic incubation). For this purpose, initially we used a simplified computational fluid dynamics model based on COMSOL Multiphysics 5.2a to better understand the resulting flow patterns in a vertical channel containing flat substrates. The adsorbed protein corona from the heparinized recalcified blood was investigated by using ellipsometry, fluorescence microscopy and by mass spectrometry based proteomics analyses. The platelet adhesion from whole blood and PRP was analyzed by scanning electron microscopy (SEM) and lactate dehydrogenase (LDH) assay. The data presented here will help to understand the role of protein corona and its changes with flow, and chemistry of the surfaces on surface-induced thrombus initiation in experimental conditions that closely mimic the *in vivo* conditions.

2. Materials and methods

2.1. Materials

N,*N*-Dimethylacrylamide (99%), 2-methacryloyloxyethyl phosphorylcholine (97%), copper(I) chloride (99.9%), copper(II) chloride (99.9%), tris[2-(dimethylamino)ethyl]amine (Me_6TREN) (97%), 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA) (97%), methyl-2-chloropropionate (97%), were purchased from Sigma-Aldrich (Oakville, ON). *N*-(2-Hydroxypropyl) methacrylamide (97%) was purchased from Polysciences. *N*,*N*-Dimethylacrylamide