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Autoregulation of thromboinflammation on biomaterial surfaces by a multicomponent therapeutic coating

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ABSTRACT

Activation of the thrombotic and complement systems is the main recognition and effector mechanisms in the multiple adverse biological responses triggered when biomaterials or therapeutic cells come into blood contact. We have created a surface which is auto-protective to human innate immunity by combining three fundamentally different strategies, all developed by us previously, which have been shown to induce substantial, but incomplete hemocompatibility when used separately. In summary, we have conjugated a factor H–binding peptide; and an ADP-degrading enzyme; using a PEG linker on both material and cellular surfaces. When exposed to human whole blood, factor H was specifically recruited to the modified surfaces and inhibited complement attack. In addition, activation of platelets and coagulation was efficiently attenuated, by degrading ADP. Thus, by inhibiting thromboinflammation using a multicomponent approach, we have created a hybrid surface with the potential to greatly reduce incompatibility reactions involving biomaterials and transplantation.

1. Introduction

In modern medicine, artificial materials frequently come into contact with blood and other tissue fluids (e.g., in extracorporeal circulation devices used for hemodialysis, hemofiltration, cardiopulmonary bypass (CPB), extracorporeal membrane oxygenation (ECMO), and plasmapheresis). This contact induces a sequence of events involving protein adsorption; inflammatory reactions, including activation of the complement and coagulation systems; and adhesion of immunocompetent cells to the exposed surfaces. These events result in serious thromboinflammatory incompatibility reactions targeting the implanted materials [1,2]. For example, for hemodialysis patients, the risk of myocardial infarction is 5–10 times higher than for healthy individuals. Chronic whole-body inflammation triggered by hemodialysis likely contributes to arteriosclerosis in uremic patients and is associated with a significantly decreased life expectancy. Also, CPB and ECMO procedures, which have increased over the past decade as a result of vascular bypass surgery and long-lasting infections affecting the lungs (e.g., swine influenza, severe acute respiratory syndrome, SARS), are associated with side effects related to contact between blood and material surfaces that result in cellular and humoral defense reactions known as the systemic inflammatory response syndrome (SIRS). In addition, small implants within the bloodstream react with the cascade systems of the blood; instead of systemic reactions, these interactions produce other deleterious effects: Vascular stents elicit fibrosis, restenosis, and thrombosis at the implantation site, and cardiac aids and pumps can trigger thrombotic reactions, leading to emboli.

Simultaneous activation of innate immunity and the thrombotic cascade also occurs during the transplantation of cells, such as islets of Langerhans [3,4], mesenchymal stem/stromal cells (MSC), and hepatocytes [5]. Graft loss results in part from a thromboinflammatory instant blood-mediated inflammatory reaction (IBMIR). This reaction consists of an innate immune attack triggered by activation of the complement and coagulation systems, followed by rapid binding of activated platelets and infiltration of polymorphonuclear leukocytes (PMNs) [3,4]. The corresponding reactions in whole-organ transplantation are ischemia-reperfusion injury and xenogenic/allogeneic antibody-mediated rejection, the major mediator of cell damage during transplantation [6], which is

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also triggered by complement activation and thrombotic reactions. Thus, it is important to make surfaces of artificial materials and transplanted cells inert against activation of innate immunity and the thrombotic cascade by regulating thromboinflammation.

Our group has studied the regulation of the coagulation/platelet and complement systems on biomaterial surfaces using various approaches. Nilsson *et al.* successfully immobilized an ADP-degrading apyrase on substrate surfaces that inhibits both platelet activation and platelet-dependent activation of the coagulation system [7]. Recently, we also identified peptides with high affinity for various domains of human factor H, an abundant plasma protein that regulates complement activation both in solution and on self-surfaces [8]. One of these peptides (5C6) recruited factor H without interfering with its regulatory function, since it bound to a region of this regulator that does not interact with the C3 convertase [8].

We now describe the creation of a combined surface coating that is autoregulatory against thromboinflammation. This surface modification with 5C6 and apyrase can be applied onto substrates (artificial materials) and cells. An amphiphilic polymer, poly(ethylene glycol)-conjugated phospholipid (PEG-lipid) co-immobilizes 5C6 and apyrase on the cell surface, as outlined in Fig. 1. The PEG-lipid derivatives can be immobilized to cell membranes by hydrophobic interactions with the lipid bilayer membranes without either cytotoxicity or a volume increase [9,10]. So far we have previously studied the effect of surface modification of living cells and islets with PEG-lipid derivatives on graft survival during cell transplantation [9]. The other end of the PEG derivative facing the fluid phase can be functionalized to allow binding of peptides, proteins, or oligonucleotides [10]. The PEG-lipid derivative itself is able to suppress coagulation and the inflammatory reactions of the IBMIR to a certain extent [11]. In this paper, we performed various assays to evaluate 5C6 and apyrase function: detection of complement-activation markers on the modified surfaces, hemolytic assays of the complement alternative pathway (AP), and xenogeneic biocompatibility assays of adherent porcine aortic endothelial cells (PAECs) in contact with human whole blood.

2. Materials and methods

2.1. Preparation of factor H and peptides (5C6 and Sc)

2.1.1. Biotinylation of factor H

Human factor H was prepared from human serum [12]. Biotinylation of factor H was performed using biotinamidohexanoic acid *N*-hydroxysuccinimide ester (Sigma–Aldrich, Inc., St. Louis, USA). A solution of factor H (300 μg/mL in PBS) was mixed with biotinamidohexanoic acid *N*-hydroxysuccinimide ester (1.76 mM in DMSO) for 30 min at room temperature (RT), followed by dialysis against PBS at 4 °C overnight.

2.1.2. Synthesis of factor H-binding peptide or control peptide

Analogs of the factor H-binding peptide 5C6 (ASSSRCTYSHWCSH) were prepared using solid phase peptide synthesis (SPPS) and cyclized via oxidation of its cysteine residues as described previously [8]. For surface attachment, the sequence was expended at the C-terminus by a short spacer group ($[PEG_3]_2$) followed by either

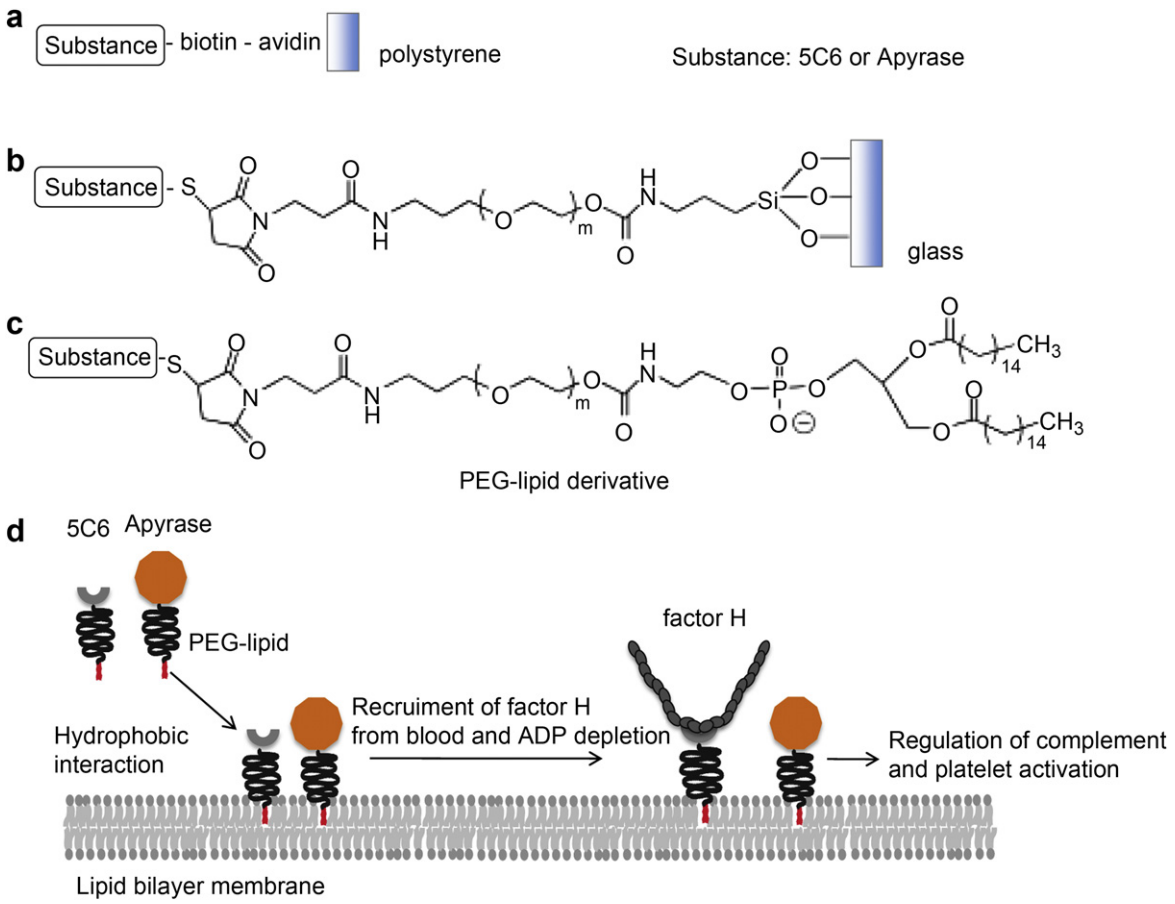

Fig. 1. Surface modification of substrate and cell surfaces with 5C6 and apyrase. (a) Immobilization of biotinylated peptide onto polystyrene surfaces via avidin. (b) Immobilization of thiolated peptide and apyrase onto glass surfaces via maleimide-conjugated PEG. Thiolated factor H-binding peptide 5C6 and apyrase are conjugated to the end of PEG chains via a thiol-maleimide reaction. (c) Chemical structure of 5C6-conjugated PEG-lipid for surface modification of cells (erythrocytes, endothelial cells). (d) Schematic representation of a cell surface modified with 5C6 and apyrase, which are co-immobilized on cell surfaces by incorporation into the lipid bilayer membrane. Factor H is recruited to the surface by 5C6 from human blood to impair complement activation, and apyrase degrades ADP to suppress platelet and coagulation activation.