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The mechanism and modulation of complement activation on polymer grafted cells

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ABSTRACT

Cell surface engineering using polymers is a promising approach to address unmet needs and adverse immune reactions in the fields of transfusion, transplantation, and cell-based therapies. Furthermore, cell surface modification may minimize or prevent adverse immune reactions to homologous incompatible cells as the interface between the host immune system and the cell surface is modified. In this report, we investigate the immune system reaction, precisely the complement binding and activation on cell surfaces modified with a functional polymer, hyperbranched polyglycerol (HPG). We used red blood cells (RBCs) as a model system to investigate the mechanism of complement activation on cell surfaces modified with various forms of HPG. Using a battery of in vitro assays including: traditional diagnostic hemolytic assays involving sheep and rabbit erythrocytes, ELISAs and flow cytometry, we show that HPG modified RBCs at certain concentrations and molecular weights activate complement via the alternative pathway. We show that by varying the grafting concentration, molecular weight and the number of cell surface reactive groups of HPG, the complement activity on the cell surface can be modulated. HPGs with molecular weights greater than 28 kDa and grafting concentrations greater than 1.0 mM, as well as a high degree of HPG functionalization with cell surface reactive groups result in the activation of the complement system via the alternative pathway. No complement activation observed when these threshold levels are not exceeded. These insights may have an impact on devising key strategies in developing novel next generation cell-surface engineered therapeutic products for applications in the fields of cell therapy, transfusion and drug delivery.

Statement of Significance

Cell-surface engineering using functional polymers is a fast emerging area of research. Importantly modified cells are used in many experimental therapeutics, transplantation and in transfusion. The success of such therapies depend on the ability of modified products to avoid immune detection and subsequent rejection or removal. Polymer grafting has been shown to modulate immune response, however, there is limited knowledge available. Thus in this manuscript, we investigated the interaction of human complement, part of our innate immune system, by polymer modified cells. Our results provide important evidences on the mechanism of complement activation by the modified cells and also found ways to modulate the innate immune response. These results will have implications in development of next generation cell-based therapies.

1. Introduction

Functional biocompatible polymers have vast applications in biotechnology and medicine to improve therapeutic outcomes that include uses in tissue engineering, regenerative medicine and drug delivery [1–4]. Recent efforts have strategically used cell surface engineered living cells, and therefore, biologically functional cells, to design novel therapeutic systems [5–7]. One approach that takes the advantage of biocompatible polymers is to alter the natural surface chemistry and characteristics of biological cells, and provides the cells with different features and functionalities depending on the polymer graft properties [8–10]. In addition to the

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practical functions of cell-surface engineered products, particular attention is focused on manipulating and controlling the host immune response by creating an environment that physically separates the engineered donor cells from host immune cells and proteins [4,7,11–13]. The success of this emergent scientific field is dependent on the ability of cell surface engineered products to avoid immune detection and subsequent rejection or removal.

Evading immune detection is a fundamental principle in transplantation medicine. For example, in an attempt to develop pancreatic islet cells for transplantation purposes, restricting the contact between the immune system and engineered cells have been of recent interest [12]. In addition to islet cells, chemical modification of other cell types has been explored for different applications. In particular, red blood cells (RBCs) are an attractive model given its abundance, lifespan of ~120 days in humans, and robust function and circulation throughout the vascular system. As a result, modification of RBCs have been investigated for its potential utility in the development of novel cell-based therapeutic products and drug delivery vehicles, thus allowing for direct delivery of therapeutics to the target tissue or organ within the body [1,14–20].

Despite vast applications and experimental success of polymer based cell-surface modification, there are some concerns that remain, including the immunological recognition and subsequent removal of modified cells [7,21,22]; only limited information available in this research area. Protein interaction on polymer modified cell surface may trigger the activation of plasma cascade systems, particularly complement and coagulation [23–25]. Complement is a component of the innate immune system and responds quickly to invading pathogens or foreign substances that enter the blood. The absence of complement regulation on modified cell surface may result in C3b deposition on cell surface that may facilitates further complement fixation [26]. Hence, control of complement deposition by modulating the chemistry and architecture of the cell surface grafted polymers is critical in ensuring the survival of polymer modified cells [8,27,28]. Such information will also aid the design of novel polymer structures specific for next generation cell-surface modification.

Hyperbranched polyglycerol (HPG) has been an excellent choice for cell-surface modification for a number cell-based therapies [8,9,18,19,28]. HPGs are hydrophilic, compact and highly biocompatible [29,30], and the abundance of hydroxyl groups located on the periphery allows for easy chemical modification to achieve better presentation of ligands on the surface. There is limited information currently available on the interactions between HPG modified cell-surface and the innate immune system. Thus to further our current understanding of the interactions involved at the interface of modified cell surface and the host immune system, we investigated the mechanism of complement activation by HPG modified RBCs as a model system. In an effort to optimize the cell-surface modification and modulate the complement activation, the influence of various polymer graft properties is investigated. We anticipate that this information would aid the design of next generation cell-surface modification strategies to address the challenges imposed by the host immune system, thereby significantly improving current cell based therapeutics.

2. Materials and methods

2.1. Synthesis and functionalization of hyperbranched polyglycerols (HPG)

HPGs of different molecular weights (8.7–98.5 kDa) were synthesized and characterized according to the established literature protocols [8,9,18]. Succinimidyl succinate (SS) groups functionalized HPGs were synthesized according to our established laboratory procedures [9,18,28]. For a typical reaction for 7 SS group functionalized HPG (42 kDa, 7 SS groups per molecule), 0.5 g (0.012 mmol) of lyophilized HPG was dissolved in 3 mL of pyridine prior to being dried under vacuum at 90 °C overnight. One drop of 5 mg/mL of dimethylaminopyridine dissolved in pyridine was added to the mixture as a catalyst. To this mixture, 8.4 mg of succinic anhydride dissolved in 0.5 mL of pyridine was added. The mixture was stirred overnight at room temperature (RT) (22 °C) under argon and was precipitated in cold acetone (40 mL), and the polymer was dried over argon. The resulting polymer was dissolved in dimethylformamide and 9.4 mg of N-hydroxysuccinimide and N, N′-diisopropyl carbodiimide (10.5 mg) were added to activate the carboxyl groups. The solution was stirred overnight under argon at RT. Cold acetone precipitation was performed to purify the succinimidyl succinate (SS) functionalized HPG (SS-HPG). The SS group modified HPG was characterized by proton NMR to determine the degree of functionalization. A similar protocol was used to develop other SS functionalized HPG.

2.2. Membrane grafting of HPG to red blood cell surface

Ethics approval for collecting human blood from donors was obtained from the University of British Columbia's clinical research ethics board (UBC CREB: H07-02198). Whole blood was collected into citrated vacutainer tubes from consented volunteer donors and centrifuged at 1000*g* for 10 min. Using a Pasteur pipette, the plasma, as well as the platelet and white blood cell rich Buffy coat was removed. The RBCs were then washed three times with 0.9% saline, and re-suspended in PBS (pH 7.0). Cell concentrations were determined using ADVIA 120 Hematology System (Siemens, Germany) to adjust the RBCs to a final concentration of 2.25×10^{12} cells/L.

To covalently attach SS-HPG to RBC surfaces, a typical reaction involves aliquoting the RBC suspensions into 1 mL samples and centrifuged at 1000×*g* for 3 min. Various volumes of the supernatant (0 μL, 50 μL, 100 μL, 150 μL, etc.) was removed, and the same volume was replaced with a 10 mM stock solution of the SS-HPG prepared in PBS (pH 8.0) containing 150 mM NaCl and 50 mM K_2HPO_4. The samples were gently mixed prior to placing onto an orbital shaker for 1 h at RT to allow for covalent attachment of HPG to RBC membranes. The modified RBCs were washed twice with PBS buffer and once with 0.9% saline, and then re-suspended in 0.9% saline to the initial 1 mL volume. Different molecular weights (8.9–98 kDa) of SS-HPG, HPGs with different number of SS groups per molecule, and different concentrations (0.5–2.5 mM) were used for RBC grafting by following a similar protocol. The HPG grafted RBCs were characterized by measuring their partitioning in aqueous two phase systems.

2.3. Pooling of serum

ABO matching blood types from 8 healthy human donors were collected into glass serum tubes, and left at room temperature for 30 min to allow for the blood to clot. Samples were centrifuged at 1500*g* for 15 min and the serum was collected using a Pasteur pipette. Serum from different donors are pooled and pipetted into 1 mL aliquots, which were frozen and stored at −80 °C. The frozen serum was thawed on ice as needed.

2.4. Lysis of HPG modified RBCs in human serum

The HPG modified RBCs were incubated in 20% pooled ABO matched serum for 1 h at 37 °C to investigate for any lysis as a result of exposure to human serum. Lysis was measured using Drabkin's method [31]. The amount of modified RBCs lysed was quantified by treating 3.9 μL of the cell suspension with 296 μL