Abstract

Repeated transfusion of red blood cells (RBCs) is the only treatment modality currently available for certain blood related genetic disorders such as thalassemia and sickle cell anemia. Due to chronic transfusion of RBCs in these patients, clinical problems surrounding alloimmunization develops in approximately 30% of patients. The pathology arises from adverse immune reactions to minor antigens that are either not routinely typed for, or cannot be readily matched. Hence, the development of donor RBCs that reduces the risk of alloimmunization would be highly beneficial.

An innovative approach to address this problem involves the use of polymers to mask the immunogenic blood group antigens on RBC membranes. Given potential applications of polymer grafted RBCs, non-toxic and non-immunogenic materials are desired. In this research, we have investigated the covalent attachment of hyperbranched polyglyecerols (HPG), a highly biocompatible polymer, to red blood cell surfaces. The aim is not only to shield immunogenic blood group antigens, but also to prevent the degradation of biomaterial modified cells by the immune system, particularly by the proteolytic convertases of the complement system.

We investigated the mechanism of complement activation on HPG modified cells, and the influence of various polymer properties, including: grafting concentration, molecular weight, and degree of HPG functionalization in an effort to optimize the grafting process on cells. Traditional assays using antibody sensitized sheep erythrocytes and rabbit erythrocytes were used to assess the overall complement activation. Complement activation products C4a, C3a, Bb, and SC5b – 9 were quantified by ELISAs to determine the specific pathway of complement activation by HPG modified RBCs. Flow cytometry was also performed to demonstrate the effectiveness of antigen protection by the different graft properties.

HPGs with a molecular weight greater than 28 KDa at grafting concentrations greater than 1.0 mM, as well as a high degree of HPG functionalization result in the activation of complement via the alternative pathway. No activation was observed when these threshold levels were not exceeded. These insights may have an impact on devising key strategies in developing novel therapeutics, especially in the fields of both transfusion and transplantation medicine.