disappeared such that by the third transfusion (~150 days post solPEG) normal RBC survival was noted. Moreover, ~80% of mice receiving 16 solPEG exposure (at 1 week intervals) demonstrated essentially normal survival curves with the 1 transfusion. Interestingly, in mice administered soluble mPEG following receipt of 1-3 mPEG-RBC transfusion, no solPEG effect was noted and normal survival of the mPEG-RBC was observed. **Conclusion:** While premature clearance of mPEG-RBC can be seen following a single preexposure to soluble PEG, this is not antibody (anti-PEG) mediated. Indeed, mice pretreated by 16 challenges with solPEG, demonstrated minimal impact on mPEG-RBC survival curves. Furthermore, pre-exposure of naïve mice to mPEG-RBC effectively prevented any effect of solPEG on circulation time of control or mPEG-RBC. These studies demonstrate that pre-exposure to mPEG-RBC effectively blocked/inhibited the pathway by which solPEG was acting. These data continue to support the immunomodulatory effect and potential utility of cellular pegylation.

Disclosure of Conflict of Interest

Wendy Toyofuku, Mark Scott: Nothing to Disclose

SP467

Grafting of Polygylcerols to Red Blood Cell Surface: An Improved Stealth Erythrocyte?

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Background: Although the transfusion of red blood cells (RBCs) are relatively safe, alloimmunization is a concern, with hundreds of minor (non-A/B) blood group antigens existing with varying frequencies in different ethnic groups. Therefore, the immunocamouflage of red blood cell (RBC) surfaces may prove to be the most effective solution in solving the issues of alloimmunization to blood group antigens. Previous research has focused on the attachment of linear and branched poly(ethylene glycol) chains to successfully mask antigenic surface epitopes. The grafting of a hyperbranched polyglycerol (HPG) to red blood cell surfaces and the corresponding physical properties and antigenic masking profiles of the modified cells are presented. **Methods:** HPG containing succinimide groups were grafted onto RBC surfaces via reaction with amine groups present on lysine residues. Efficiency of RBC surface coverage was determined by a number of techniques, including electrophoresis, phase partitioning (using dextran/PEG buffer phases), optical microscopy, and flow cytometry. Toxicity of the HPGs was determined by cell lysis, lipid peroxidation, hemoglobin oxidation, and osmotic fragility. **Results:** HPGs of varying molecular weights (3 kDa to 50 kDa) were grafted to RBCs. The degree of successful grafting was confirmed by a slowing in the electrophoretic mobility of the RBCs and the change in the preferred phase (from dextran to PEG buffer). Efficacy of HPG grafting was found to be a function of molecular weight, concentration, and the number of available succinimide groups. The toxicity of the process was very low, even at high HPG concentrations. **Conclusions:** The use of polyglycerols as grafted polymers is of particular interest due to the dense, highly branched nature of the polymer which may result better protection of surface antigens on the red cell surface. As expected, good blood compatibility was observed, and it is hoped that further functionality will be incorporated onto the blood cell through HPGs by adding functional groups such as biomolecules via the hydroxyl groups available on the outside of the HPGs.

Disclosure of Conflict of Interest

Jayachandran Kizhakkedathu, Mark Scott: Nothing to Disclose
Nicholas Rossi: Canadian Blood Services – Grants or Research Support

SP468

Polymer-Mediated Immunocamouflage of Intact Cells for Transfusion and Transplantation Medicine

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Background: Prevention of immunological rejection of allogeneic cells is of crucial importance in transfusion medicine. To this end, the immunocamouflage of donor cells via covalent grafting of methoxypolyethylene glycol (mPEG; PEGylation) has emerged as a promising technology. While previous studies on red blood cells (RBC) demonstrated decreased antigenicity and immunogenicity of the modified human and murine RBC in vitro and in vivo, the underlying biophysical mechanisms are poorly understood. **Methods:** To elucidate the biophysical mechanisms of biological protection, an aliphatic amine polystyrene latex model was used to quantify the effects of polymer size and density on electrophoretic mobility and protein adsorption. Specific protein adsorption was further investigated using isobaric tags for relative and absolute quantification labeling followed by mass spectrometry analysis (iTRAQ/MS). **Results:** PEGylation of latex particles with succinimidyl carbonate-mPEG (SCmPEG) resulted in a molecular weight (MW) and concentration dependent camouflage of surface charge. At a grafting concentration of 0.2 mM SCmPEG, electrophoretic mobility decreased by 16.118.8 and 73.26.2% for the 2 and 20 kDa polymers relative to control beads. Increasing the SCmPEG grafting concentration to 2 mM further enhanced surface charge camouflage for the 2, but not 20, kDa polymer as mobility decreased by 54.423.5 and 85.44.8%, respectively. Interestingly, human plasma adsorption to the latex surface, while significantly decreased by all polymer sizes, was most effectively attenuated by the low molecular weight mPEGs. While bare latex adsorbed 159.911.0 protein/cm latex, 0.5 mM grafting concentrations of 2, 5 and 20 kDa SCmPEG resulted in adsorption of 24.36.2, 54.010.1 and 70.416.6 ng protein/cm latex (respectively). Only at higher grafting concentrations (≥2 mM) did the three polymer sizes demonstrate equivalent adsorption characteristics. Analysis of the bound plasma proteins by iTRAQ/MS further illuminated the efficacy of surface PEGylation. Of note, the abundance of adsorbed complement and coagulation proteins was decreased in an mPEG-dose dependent manner on the modified latex. **Conclusions:** The immunocamouflage of cell surfaces by polymer grafting involves biophysical mechanisms including charge camouflage and the prevention of surface-macromolecule interactions. These effects in turn are influenced by polymer size and density. By understanding these relationships we can improve the immunomodulation of donor cells.

Disclosure of Conflict of Interest

Yevgeniya Le, Mark Scott: Nothing to Disclose

SP469

The Development of Synthetic Peptidolipids, Glycolipids and Other Lipid-linked Structures to Create Designer Red Cells

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Background: Red cells used in immunohematology assays are restricted by their natural phenotype and membrane characteristics. More recently a technology has been developed which allows for the attachment of a large variety of specific carbohydrates, peptides and other structures to the surface of cells – known as FSL cell surface coating (KODE™ technology). FSL constructs are water dispersible and consist of three components; a F functional head group, a S spacer and a L diacyl lipid tail. This technology allows users to introduce novel antigen profiles or characteristics at the membrane surface of cells. **Methods:** FSL constructs were synthesised bearing carbohydrates, peptides, and fluorophores. One part (0.05 ml) of FSL construct 1 mg/ml and one part of packed red cells were combined, mixed and incubated at 37°C for 2 hours, washed and then analysed for the presence of constructs by direct-indirect agglutination and fluorescence serological methods. **Results:** In all cases red cells were shown to be able to be precisely labelled with serologically detectable FSL construct. Red cells were modified to carry ABO, H, Lewis, acquired-B, the animal antigen Galili, hyaluronic acid, fluorophores and blood group peptides (Miltenberger). **Conclusion:** FSL cell surface coating technology allows for the creation of red cells expressing controlled levels of normal and/or novel antigens or structures. Such cells can be used for quality control, specialised antibody screening and identification panels and have potential for novel future applications.

Disclosure of Conflict of Interest

Nicolai Bovin: Nothing to Disclose
Stephen Henry: KODE Biotech Limited – Stocks or Bonds,
Travel Support or Honorarium, including proposed educational program

Red Blood Cell: Molecular Genetics & Testing

SP470

Prevalence of Yt^a Antigen in Selected Blood Donors in a Regional Rare Donor Program

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Background: The Cartwright (Yt) blood group system comprises two antithetical antigens, Yt^a and Yt^b, that represent a His353Asn amino acid substitution in acetyl cholinesterase on red cells. Yt^a is a high-prevalence