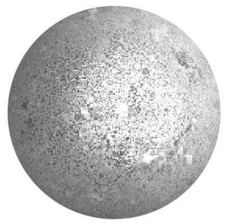
In Situ Removal Of Antibodies, Free Hemoglobin, Cytokines And Bioactive Lipids From pRBCs Using Hemoadsorbent Polymer Beads

Vincent J. Capponi, Thomas D. Golobish, Humayra B. Ali, Matthew J. Gilliland
Robert J. Reynolds, Anthony N. Chiappetta, Syed F. Ali, and Phillip P. Chan
CytoSorbents Corporation, Monmouth Junction, NJ 08852



Goal: To develop an effective, easy to implement, blood purification system based on highly biocompatible, porous polymer beads to improve the quality and safety of blood by broadly reducing contaminants in packed red blood cells (pRBCs) that can cause transfusion reactions, such as bioactive lipids, cytokines, free hemoglobin, and immunoglobulin without the need for antibodies, affinity ligands or other capture agents

Background

An estimated 50 million pRBC transfusions are administered annually each year, with 15 million alone in the US. Despite advances in blood quality, there is still significant room for improvement.

- **Transfusion reactions:** Low but tangible risk of non-hemolytic febrile and allergic transfusion reactions, atypical infections, allo-immunization, and potentially fatal but infrequent reactions including TRALI (transfusion related acute lung injury), anaphylaxis, angioedema, and hemolysis
- **"Old Blood is Bad Blood" Debate:** Significant controversy over whether use of "old" blood is linked to adverse outcome and increased mortality. Two randomized, controlled trials – ABLE and RECESS are ongoing to address this question
- **High risk populations:** Transfusion risk increases in patients receiving multiple pRBCs (e.g. trauma, surgery) and in "primed" susceptible patients (e.g. critical care and high risk surgery)
- **Hidden economic costs:** In addition to the medical burden, these reactions trigger substantial economic costs of treatment, investigation, and follow-up

Blood contaminants cause reactions and potential adverse events. Foreign antigens, antibodies (e.g. anti-HLA, anti-granulocyte, IgA, anti-A, and anti-B), medications, infectious materials (e.g. prions, viruses) and other substances in donated blood can cause serious consequences, including TRALI and anaphylaxis.

During blood storage, pRBC units also accumulate free hemoglobin due to hemolysis, and undergo *in situ* generation of bioactive lipids (e.g. lysophosphatidylcholine – LysoPC), cytokines, and other inflammatory mediators that can trigger transfusion reactions that vary in severity depending on the patient's condition.

Objective

In order to reduce contaminants from pRBCs, we have investigated a novel blood purification technology using an advanced, hemocompatible, porous polymer bead mixture. Each bead is roughly the size of a grain of salt and contains millions of pores and channels that are specifically designed to capture and adsorb different blood contaminants based on pore size and surface adsorption. Some characteristics of this technology include:

- Designed for broad removal of substances <1 kDa to >150 kDa
- Inherent ISO 10993 hemocompatible biomaterials
- Neutrally buoyant beads distribute randomly, require no mixing
- No expensive or leachable antibodies, ligands, or other affinity capture agents are used
- Sterilization is simple (e.g. gamma, steam, others) with long shelf life
- New capabilities can be easily implemented by adding new beads to the mixture
- Theoretically compatible with a wide range of preservatives

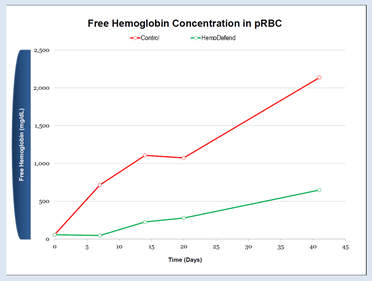
These beads can be used in two configurations. The first is a standard, dock-able, in-line filter that can be used at the point of transfusion. The second involves incubating the beads directly with blood during the entire period of blood storage, called "Beads in a Bag". During bag manufacturing, beads are placed into the bag. A mesh filter integrated into the outflow port prevents beads from escaping during transfusion. When pRBCs are placed into the bag, the neutrally-buoyant beads distribute automatically, and cleansing commences without need for mixing. Blood is administered as usual, with blood going into the patient and beads retained in the bag and discarded.

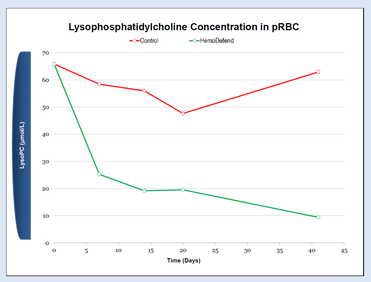
Materials and Methods

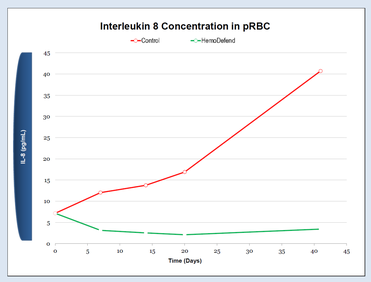
All experiments in this study are from the evaluation of the "Beads in a Bag" concept.

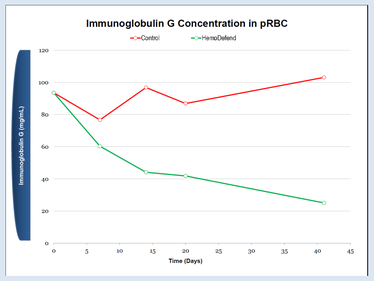
Experimental protocol: Two liters of non-leukoreduced pRBCs (AB+) were obtained in standard blood storage bags (Hemerica Inc., RI) containing CPD anti-coagulant at 4°C, within 7 days of collection. pRBCs were pooled and divided into 450 mL volumes amongst four new PVC blood storage bags containing either no beads (control), or 30 mL of a proprietary, highly porous, biocompatible, styrene-divinylbenzene copolymer bead prototype, and incubated at 2-8°C for 41 days, reflecting the useful lifespan of pRBCs. In the case of hemoglobin, the bags were gently rocked on top of a platform rocker (WorldWide Medical Products Inc., NJ), representing a bad case hemolysis scenario. In all other cases, the blood was stored in a stationary position, unmixed, during the incubation period. On Days 0, 7, 14, 20 and 41, 5 mL blood samples were removed from each bag, spun down, and supernatant removed and frozen at –20°C. Supernatants were later analyzed using an Assay Kit for LysoPC (Azwell, Japan), an ELISA Kit for immunoglobulin G (Zeptometrix, NY), an ELISA Kit for free hemoglobin (Bethyl Laboratories, TX) and a Human Cytokine Magnetic Bead Panel Assay for cytokines (Millipore, MA).

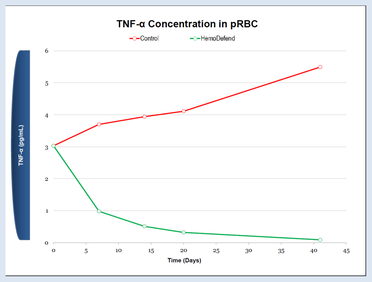
Contaminant	Arm	Starting Value (Day 0)	Ending Value (Day 41)	% change from Day 0	% change vs control (Day 41)
Immunoglobulin G (mg/mL)	Control	93.5	103.1	10%	---
	Treatment	93.5	25.1	-73%	-83%
Free Hemoglobin (mg/dL)	Control	57.0	2,135.0	3,746%	---
	Treatment	57.0	648.0	1,137%	-2,609%
Lyso-PC (µmol/L)	Control	65.9	62.9	-5%	---
	Treatment	65.9	9.4	-86%	-81%
TNF-α (pg/mL)	Control	3.0	5.5	81%	---
	Treatment	3.0	0.1	-97%	-178%
IL-7 (pg/mL)	Control	0.9	2.0	222%	---
	Treatment	0.9	0.00	-100%	-322%
IL-8 (pg/mL)	Control	7.2	40.70	468%	---
	Treatment	7.2	3.44	-52%	-520%

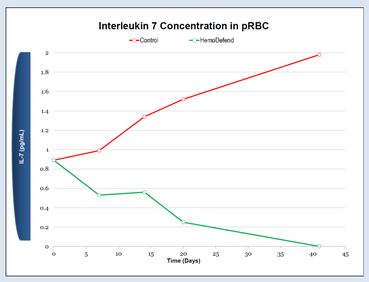












Results

The data for these experiments can be found in the center lower panels (Fig 1). Starting concentrations represent naturally occurring levels found in these pRBCs (Table 1). Other cytokines concentrations were below the assay sensitivity including IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, p70, IL-13, IFN-γ, and GM-CSF. Values at Day 41, percent (%) change compared to Day 0, and percent (%) change versus control at Day 41 are listed in Table 1. The data demonstrate a rise in concentration of each of these substances over time in the control. In the treatment arm, with the exception of hemoglobin, the concentrations declined. An example of neutrally-buoyant beads is demonstrated in Fig. 2. Fig. 3 demonstrates a visual representation of free hemoglobin by these porous polymer

Fig. 2: Neutrally-buoyant beads (right) versus normally buoyant beads (left) after incubation for 1 hour in pRBCs

Fig. 3: Removal of free hemoglobin from pRBCs using an early generation of porous polymer beads (left) versus no bead control (right) following rocking incubation for 14 days at 4°C. Plasma fractions are shown following centrifugation.

Conclusions

We have demonstrated the ability to substantially reduce free hemoglobin, immunoglobulin G, lysophosphatidylcholine, and cytokines from pRBCs compared to control using novel, hemocompatible, porous polymer beads in a "Beads in a Bag" concept that takes advantage of time during blood storage. This prototype technology potentially represents an easy to implement and effective way to increase the quality and safety of blood by "washing" blood without the time, cost, and logistical difficulties of actually washing it. It does not require any additional equipment or capital expenditures to use, and importantly, is invisible to the end user and does not require any changes in the practice of medicine. Other than this technology and blood washing, there are few if any alternative non-leachable technologies capable of cost-effectively removing a broad range of contaminants from pRBCs.

Future Work

One of the limitations of this study was the use of non-leukoreduced blood. We plan to repeat these experiments in the near future using leukoreduced blood. We also plan to examine whether this system can specifically remove anti-HLA IgG, and larger immunoglobulins such as IgA dimers (~300 kDa), and anti-A and anti-B IgM pentamers (~970 kDa). We will investigate whether prolonged treatment with these hemocompatible porous polymer beads results in any changes in pRBCs, in terms of storage lesions, post-transfusion stability and oxygen carrying capability. Ultimately, studies may be conducted to demonstrate reduced reactions or adverse events in animals or humans. Interestingly, this technology may also have benefit in the purification of platelets, though monitoring of platelet activation will be a major consideration.