

# Removal of Free Hemoglobin, Potassium, Cytokines, Bioactive Lipids and Antibodies from pRBCs with Hemocompatible Porous Polymer Beads



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**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 potassium, free hemoglobin, cytokines, bioactive lipids, and immunoglobulins without the need for antibodies, affinity ligands or other capture agents

## Background

Packed red blood cell (pRBC) units contain reactive donor antibodies (e.g. anti-HLA, anti-granulocyte, anti-A, and anti-B), free hemoglobin, high extracellular potassium and biologically active inflammatory mediators that can lead to transfusion reactions and adverse effects.

- **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.
- **RBC storage lesion:** During storage RBCs release potassium and free hemoglobin due to cold storage and hemolysis, that can lead to adverse effects such as hyperkalemia and hyperbilirubinemia. Stored pRBCs also undergo *in situ* generation of bioactive lipids and other inflammatory mediators that can trigger transfusion reactions that vary in severity depending on the patient's condition.
- **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, documentation, additional blood testing and follow-up.

## Study Objective

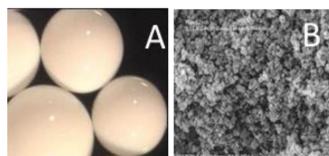
- In this study we evaluated the effectiveness of a small, in-line filter, called HemoDefend, containing hemocompatible, porous polymer beads to remove a broad range of contaminants present in aged RBCs including K<sup>+</sup>, immunoglobulins, free hemoglobin, cytokines, bioactive lipids and other inflammatory mediators.
- Each bead is roughly the size of a grain of salt and contains millions of pores and channels that capture and adsorb different blood contaminants based on pore size and surface adsorption. The beads are used in a standard, dock-able, in-line filter that can be used at the point of transfusion.

## Technology Overview

Some characteristics of this technology include:

- Designed for broad removal of substances <1 kDa to >150 kDa.
- Inherent ISO 10993 hemocompatible biomaterials.
- 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 at room temperature.
- Compatible with a wide range of RBC storage solutions.

A) 500 micron bead; B) interior of polymer bead at 10K magnification.

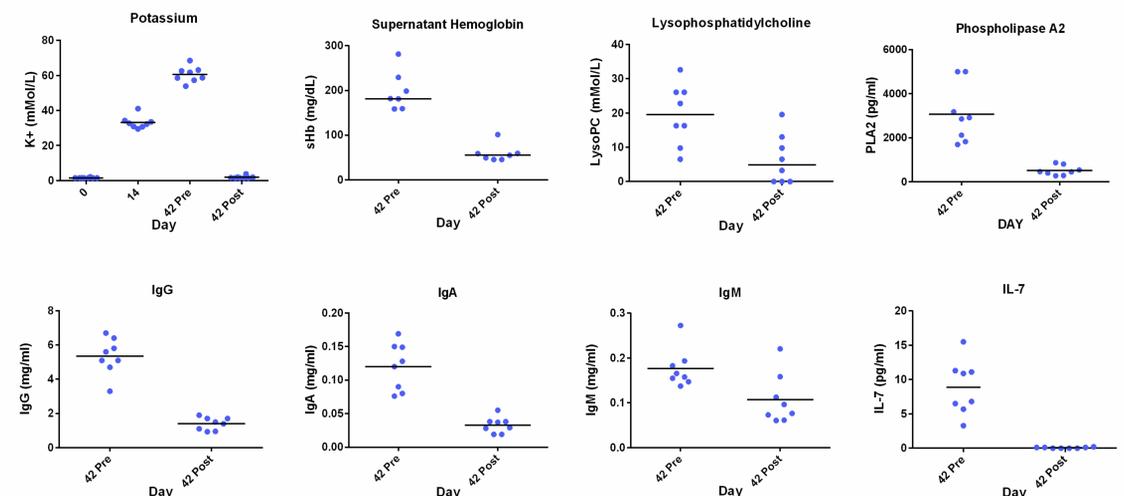
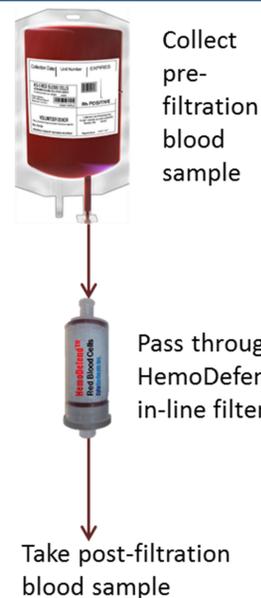


## Materials and Methods

Whole blood (500 mL) units were collected from healthy volunteers into citrate-phosphate-dextrose (CPD) sets (Fenwal), leukocyte-reduced (LR), component processed within 8 hours of collection into ADSOL<sup>®</sup> solution (AS-1), and the RBCs held at 1-6°C for 42 days. Bags were sampled on days 0, 14, and 42 prior to and following passage through the in-line bead filter at a flow rate of 8.6 ±1.0 ml/min. For the free hemoglobin experiments, LR RBCs (6 units) stored into AS-1 were obtained from a regional blood donor center (Blood Centers of America) at expiration (42±2 days) and passed through a 30 ml porous polymer device, equilibrated to minimize osmotic changes, at a flow rate of 3.5 ml/minute. Supernatants were analyzed using assay kits for free hemoglobin (Arbor Assay), LysoPC (Azwell), human IgG, IgA and IgM (AssayPro), Arginase I (Hycult), phospholipase A2 (Bethyl Labs) and IL-7 (Life Tech).

## Results

- Filtration of 42-day AS-1 units through the porous bead device reduced K<sup>+</sup> levels from 60.6 mM pre-filter to 1.9mM post-filter.
- In-line filtration removed 73% and 72%, respectively of immunoglobulins IgG and IgA and 39% of the much larger 900 kDa immunoglobulin IgM. Filtration reduced IL-7, phospholipase A2 and lysophosphatidyl choline by 99, 81 and 64%, respectively. RBC counts, pH, ATP and morphology were maintained post-filtration.
- Filtration demonstrated a 70.4% reduced the amount of free hemoglobin in 42-day AS-1 units from a total of 198.6 mg per unit pre-filtration to 59.3 mg per unit post-filter.



**Fig. 1.** Levels of potassium, supernatant (free) hemoglobin, lysophosphatidylcholine, phospholipase A2, immunoglobulins G (IgG), A (IgA), M (IgM), Samples were collected on day 42 pre- and post-filtration. Samples for potassium levels were also collected on days 0 and 14.

Table 1.	Pre-filtration	Post-filtration	% Reduction	P value
Potassium (mM)	60.6 ±4.4	1.9 ±0.8	96.9	<0.0001
Free Hb (mg/unit)	198.6 ±48	59.3 ±21.2	70.1	<0.01
IL-7 (pg/ml)	8.9 ± 3.9	0.1± .07	98.9	<0.0001
IgG (mg/ml)	5.34 ± 1.0	1.4 ±.4	73.8	<0.0001
IgA (mg/ml)	0.12 ±0.035	0.033 ±0.011	72.7	<0.0001
Phospholipase (pg/ml)	3075 ±1301	516.6 ±219.7	83.2	<0.001
LysoPC (µmol/L)	18.3 ±9.0	6.5 ±7.2	64.4	<0.05
RBC (x10 <sup>6</sup> /µl)	6.78 ±0.42	6.61 ±0.46	2.4	0.476
ATP (µmol/gHb)	3.14 ±0.45	2.89 ±0.48	7.95	0.302
RBC morphology	83.8 ±14.1	89.8 ±5.6	-7.2	0.279

## Conclusions

- The HemoDefend in-line filter dramatically reduced extracellular K<sup>+</sup> concentration and significantly reduced levels of free hemoglobin, immunoglobulins, cytokines and bioactive lipids with minimal alteration in metabolic markers.
- This filter may represent an effective method to remove deleterious constituents of stored pRBC and avoid adverse clinical effects.
- The technology does not require any additional equipment or capital expenditures allowing it to be easily incorporated in transfusion practice, providing 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.
- Ultimately, our goal is to demonstrate reduced reactions or adverse events in animals and humans.

## Acknowledgements

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