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PLASMA COMPOSITION EFFECTS ON MECHANICAL FRAGILITY OF PACKED RED BLOOD CELLS

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Background: Packed Red Blood Cell (pRBC) ability to deform and to withstand mechanical stress without hemolysis is vitally important for post-transfusion cell survival and their participation in microcirculation. These properties may be severely compromised during pRBC storage due to storage lesion effects well documented. Additionally, they may also be affected by intrinsic properties of donors as well as by that of patients receiving the transfusion. pRBC mechanical fragility (MF) has been used previously to assess pRBC propensity to hemolysis, providing a potentially useful test to assess the impact of various factors on pRBC membrane fragility.
Aims: To evaluate the impact of plasma and plasma proteins on pRBC mechanical fragility and to consider its potential impact on improvement of blood transfusion practice.
Methods: pRBC and plasma samples were provided by the University of Michigan Hospital Blood Bank. Essentially fatty acid free plasma was obtained by ultracentrifugation for 1 h at 100,000 g. pRBC stored in AS3 additive solution were diluted with compatible plasma or AS3 supplemented with albumin, polyethylene glycol; subjected to mechanical stress that was applied using a bead mill (oscillation at 50 Hz; durations from 0.25 to 60 min). MF profiles were described in terms of percent hemolysis following stress of specified duration.
Results: Albumin significantly increased pRBC ability to withstand mechanical stress [as compared to that in additive storage solution (AS)] with this 'protection' effect exhibiting strong concentration dependence. While MF of pRBC in AS was not affected by changes in pH (pH 5–7), profiles of cells in AS supplemented by albumin showed pronounced pH dependence, likely due to pH-driven conformational changes of albumin. Lower pH values corresponded with pRBC higher resistance to mechanical stress. Part of the effect of albumin (about 25%) could be attributed to changes in rheological properties, as demonstrated by profiles collected in the presence of equimolar amounts of PEG. The remainder is likely due protein-cell interaction. Paradoxically, compatible plasma did not convey 'protection' from external stress, exhibiting profiles similar to that in AS. Lipemic plasma effected lower 'protection' from induced hemolysis than essentially fatty-acid free plasma. This effect was less dependent on incubation, than on fatty-acid presence during mechanical stress application.

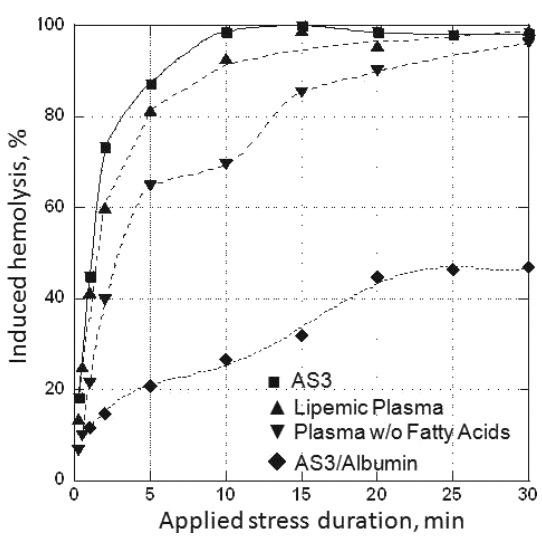

Figure. 1 Mechanical Fragility Profiles of pRBC in AS3 and Plasma

Conclusion: pRBC ability to withstand applied mechanical stress and by extension survive in the blood stream post-transfusion and effectively participate in microcirculation can be significantly affected by blood composition. In particular in the presence of albumin pRBC exhibited much higher resistance to applied mechanical stress. In plasma this effect was partially negated by insoluble fatty acids which composition had been shown previously to be dietary-dependent. It is proposed that the additional presence of cholesterol and triglycerides, also shown to negatively affect Red Cell fragility, accounted for higher MF of pRBC in whole plasma than in solutions containing albumin. While further study of the subject is warranted, this data suggest possible way to improve both the quality of donated blood and transfused pRBC survival and efficacy through monitoring and adjustment (e.g. through medication or dietary changes) of fatty-acid content and composition in donors and patients.

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THE OVINE PACKED RED BLOOD CELL STORAGE LESION: CHARACTERISATION AND COMPARISON TO THAT OF HUMAN PACKED RED BLOOD CELLS

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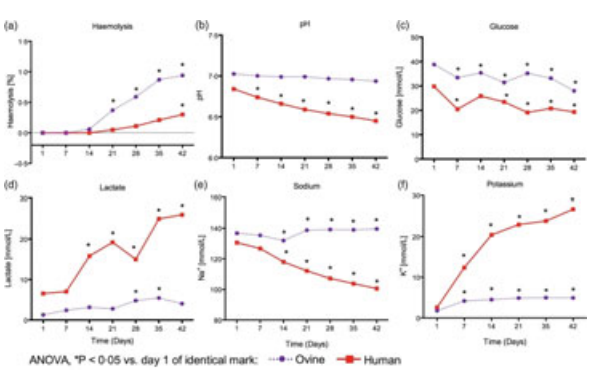
Background: Packed red blood cells (PRBCs) stored under blood-bank conditions undergo morphological and biochemical deterioration, collectively referred as a 'storage lesion', that affect their function and thereby their usability and effectiveness. Indeed, there is growing awareness of the possible association between the transfusion of stored blood and poor clinical outcomes in patients. Ovine transfusion models are of increasing importance in biomedical research because their size, blood volume and the lifespan of their red blood cells are all similar to humans. This enables the collection of a whole unit of blood in standard human blood packs. To validate the ovine transfusion model as one in which the effects of transfusing stored blood can be investigated it is crucial to characterise the storage lesion of ovine PRBCs (ovPRBCs) and to compare this to human PRBCs (huPRBCs).
Aims: We aimed to characterise, throughout 42-day of storage, the haematological and biochemical changes evident in ovPRBCs and compare these to huPRBCs.
Methods: Whole blood units were collected from adult female sheep (n = 5) into blood-packs containing citrate-phosphate-dextrose (CPD) anticoagulant. PRBCs were separated from plasma by centrifugation then leukofiltered and stored for 42 days at 4–6°C in a saline, adenine, glucose and mannitol (SAGM) additive solution. Equivalent huPRBCs (n = 5) were obtained from the Australian Red Cross Blood Service. Samples from ovPRBCs and huPRBCs were collected aseptically at weekly intervals and were tested for haematologic indices, blood-gas values and haemolysis. Haematological parameters, including RBC count, haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular Hb (MCH), mean corpuscular Hb concentration (MCHC) and haematocrit were determined using an automated cell counter. Lactate, glucose, pH, sodium and potassium were evaluated using a blood-gas analyser. The degree of haemolysis in stored samples was determined by measuring supernatant haemoglobin concentration after centrifugation.



Results: Although ovine red blood cells are smaller in size and more frequent in number than human red blood cells, ovPRBC hematology parameters were proportional to the human equivalent and none of the values changed significantly throughout the 42-day storage period (ANOVA, $P < 0.05$ storage duration). Similar to huPRBCs, the rate of haemolysis evident in ovPRBC increased during storage, however it was notably higher from day 21 (0.37% vs 0.05% respectively) and was further increased at day 42 (Fig. A). In contrast with huPRBCs, pH did not decrease in ovPRBC during storage (Fig. B). Similar to huPRBCs, glucose levels decreased and lactate increased over time in ovPRBCs (Fig. C, D). After a slight initial decrease, sodium levels in ovPRBCs increased from day 14 and remained relatively stable throughout storage, this in contrast to huPRBCs in which sodium levels decreased

throughout storage (Fig. E). During storage, potassium levels in ovPRBCs were increased compared to day 1, however this increase was modest compared to that evident in huPRBCs (Fig. F).
Summary: The results of this study allow us to draw parallels between the storage lesions of ovine and human PRBCs and will assist in the design of studies investigating the effects of transfusing stored blood in ovine models.

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MEASURING DEFORMATION OF RED BLOOD CELLS (RBCS) OVER STORAGE USING A LUMIFUGE® ANALYSER

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Background: RBCs in vivo change shape and become more rigid as they age, which affects their ability to deform and circulate through narrow capillaries to deliver oxygen to tissues. Storage of RBCs in vitro using standard blood banking procedures results in accelerated changes to RBC shape and membrane rigidity, reducing their ability to deform. RBC deformation may therefore be a relevant quality control criterion for stored RBCs. Using a LUMiFuge® analyser, the settling or sedimentation of RBCs can be determined at various centrifugal speeds as a function of time. RBC biophysical properties include size, shape and density change over storage and these can influence the settling velocities of RBCs.
Aim: In this study we investigated the use of the LUMiFuge® technology to measure changes in deformation of RBCs during storage.
Methods: Leukocyte-depleted RBCs (n = 6) were processed and stored according to standard Blood Service procedures in SAGM additive solution. Samples were removed asceptically from stored RBCs at day 1, 21 and 42 of storage. Standard routine parameters including; full blood examination, haemolysis and pH were tested on units at each sample time. RBC samples were analysed using a LUMiFuge® (LUM, Germany) and SEPView® software according to methods described [1]. Solids concentrations and viscosities for RBCs and supernatants were used to calculate settling velocities. RBC morphology was observed using light and fluorescent microscopy after fixation and staining with fluorescein-labelled phalloidin to detect actin. Images were captured on a CCD camera attached to a fluorescence microscope and associated imaging software. RBCs were counted and the percentage of RBCs with discoid morphology was calculated. Loss of membrane via the generation of microparticles (MPs) positively staining for Glycophorin A (GPA+) and annexin V binding was quantified from RBC supernatants using flow cytometry as described [2]. Statistical analysis used paired *t*-tests, repeated measures ANOVA and Pearson correlations. Significance was defined as $P < 0.05$.
Results: All RBC units complied with Council of Europe recommended guidelines for RBC concentrates. The percentage of RBCs with normal discoid morphology decreased significantly over storage ($P < 0.006$), which corresponded to significant increases in GPA+ ($P < 0.003$) and annexin V binding MP ($P < 0.001$) accumulation in the supernatant. LUMiFuge® analysis demonstrated that settling velocities of RBCs decreased significantly during storage ($P < 0.004$). A good correlation between the settling velocities of stored RBCs and loss of discoid morphology was also evident ($r = 0.6491$). The mean cell haemoglobin concentration (MCHC) decreased in RBCs over storage and correlated with decreasing settling velocities ($r = 0.527$).
Summary/Conclusion: Changes to RBC deformation characterised by loss of RBC membrane normal discoid shape, showed good correlation with decreased settling velocities measured for RBCs during storage. These results suggest that stored RBCs settle more slowly with increasing storage time and changes in RBC deformation can be monitored using a LUMiFuge® analyser.
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THE EFFECT OF STORAGE ON RED BLOOD CELL DEFORMABILITY: A MICROFLUIDIC APPROACH

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Background: Throughout their life span, red blood cells (RBCs) undergo extensive deformation and, at the same time, resist fragmentation when travelling through the microcapillaries. Deformability, the combined result of properties of the membrane, the surface area-to-volume ratio and the hemoglobin content, is a critical determinant of capillary blood flow. During blood bank storage and under pathophysiological conditions, RBCs become more rigid, which can result in the blockage of the microvascular system. Various experimental techniques provide information on the rheological properties of RBCs, but their clinical significance is controversial. Therefore, we developed a microfluidic approach for evaluating RBC deformability in a physiologically meaningful and clinically significant manner.
Aim: To study RBC behaviour in a microcirculation-mimicking network and measure the effect of storage time on deformability.
Methods: RBC deformation and relaxation were measured in microfluidic PDMS channels with dimensions similar to (micro)capillaries.
Results: Our approach yielded a high-throughput determination of changes in deformation capacity with statistically significant data, while providing morphologically information at the single cell level. These data strongly suggest that deformation and relaxation capacity kinetics of transfused red blood cells in the patients' circulation decrease with storage time.
Conclusions: Microfluidic analysis of RBC behaviour in a capillary-like network enables visual assessment, which is highly instructive in understanding the morphological dynamics and behaviour of RBCs. Unlike other techniques, our method also enables a high-throughput determination of changes in deformation capacity providing biologically and statistically significant data.

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COMPARISON OF MICROPARTICLE PRODUCTION IN PACKED RED BLOOD CELLS STORED UNDER ANAEROBIC AND CONVENTIONAL COLD STORAGE CONDITIONS

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Background: The potential negative clinical effects of the transfusion of older packed red blood cells (PRBCs) is widely disputed in the literature. Findings include biochemical and metabolic changes: decreased pH, depletion of 2,3,DPG, SNO-Hb, ATP, increased lactate, decreased use of glucose in the pentose phosphate shunt in oxygenated red blood cells (RBCs) stored >15 days. Biomechanical/structural changes include increased hemolysis, morphological changes, decreased membrane surface volume, decreased deformability, increased percentage of free cholesterol and 8-isoprostane in PRBC supernatant, alterations in surface membrane proteins, RBC surface blebbing, microparticle shedding. Oxidative damage to RBCs results in increased hemolysis, hemoglobin oxidation, denaturation, and lipid peroxidation.
Aims: In the current study, we evaluated the ability of storing PRBCs in an anaerobic environment to decrease the formation of microparticles as compared to conventional PRBC storage.
Methods: Fifteen units of whole blood were collected at the Mayo Clinic Rochester Blood Donor Centerfrom normal healthy volunteers utilizing a Pall Corporation Leukotrap® WB 500 ml collection bag set containing 70 ml CP2D. Within 1 h the whole blood was passed through an in-line leucoreduction filter and centrifuged at 4563× g for 5 min. Plasma was expressed. The unit was divided into two equal units in 600 ml storage bags. OFAS3 additive[1] was added to one unit (test), AS3 was added to the other (control). The set of test units were depleted of oxygen pre-storage using Ar gas exchange (in Ar-filled 600 ml bag, 10 min agitation in a platelet mixer, for six exchanges) and stored under Ar atmosphere[2]. At weeks 0, 1, 2, 3, and 6, samples were removed using a sterile connecting device from the PRBC units. Red cell microparticles (RMPs) were isolated from each sample by transferring 5 ml of stored RBCs into 15 ml conical tubes. Samples were diluted with 10 ml isotonic saline (4°C). Samples were mixed for 30 s, then centrifuged at 250× g for 10 min. Supernatants were transferred to 15 ml conical tubes for RMP tagging. Two fluorescent markers were utilized: Glycophorin A with phycoerythrin (PE) and Annexin V with fluorescein isothiocyanate (FITC). After a 20-min incubation in the dark, analysis was performed using a FACS Canto Flow Cytometer. RMP data was captured as the number of events counted per 1 min and normalized by total hemoglobin concentration of blood units.
Results: A large variation was observed in MP production rates among blood donors throughout the entire storage period. Generally, the anaerobic arm exhibited lower values compared to the conventional control arm during 2–6 weeks of storage (minimum of 80% of units in anaerobic arm had lower MP). A statistical significance was achieved at 3 weeks of storage ($P < 0.003$) for GPA (control '25.1 ± 10.9'; test '15.5 ± 5.4' MPs events/min/g Hb) and Annexin MPs (control '95.0 ± 31.8'; test' '47.0 ± 14.3').
References: 1. Transfusion 2008; **49**:458.
2. Vox Sanguinis 2007; **93**:184.