Sedimentation method for preparation of postoperatively salvaged unwashed shed blood in orthopaedic surgery

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Key points
- Orthopaedic surgery can result in significant blood loss requiring transfusion.
- Salvaged unwashed shed blood can be contaminated with potentially harmful constituents.
- A simple low-cost colloid-based sedimentation method for preparing salvaged unwashed blood is described.
- This approach led to efficient recovery of erythrocytes with reduction in cellular and protein contaminants.
- The safety and efficacy of this approach to improve blood conservation needs to be confirmed.

Background. Salvage and return of unwashed shed blood (USB) after total knee replacement (TKR) is an established blood-saving technique, but some authors question its efficacy and safety and suggest that the shed blood be washed before returning. We evaluated a colloid-based sedimentation method for improving and standardizing the quality of USB collected after TKR without the need for washing.

Methods. Experiments were performed to find the optimal colloid dose and sedimentation time using diluted donated venous blood. USB samples (n = 52) were drawn from the reinfusion bag and mixed with hydroxyethyl starch or gelatine solutions (15–30%, colloid solution volume/total volume × 100). USB red blood cells (RBCs) were allowed to settle by gravity for 30 min, supernatant was evacuated from the syringe, and RBC concentrate was analysed. RBC recovery and other blood cell and chemical removal were calculated according to changes in USB volume and haematocrit. Twenty-five samples from leucodepleted packed RBCs were analysed as a comparator group.

Results. Mean haemoglobin (Hb) of USB was 10.9 g dl⁻¹. After colloid treatment, 90% of RBCs were recovered, and USB Hb was similar to that of leucodepleted packed RBCs (n = 25) (18.9 vs 19.6 g dl⁻¹, respectively; *P = NS). In addition, the procedure reduced USB content of leucocytes (60%), platelets (48%), total protein (76%), cytokines (70–77%), and plasma-free haemoglobin (53%), without major differences between colloids.

Conclusions. Sedimentation of USB with colloid solutions provides a low-cost alternative for improving and standardizing the quality of salvaged USB after TKR.

Keywords: blood, salvage; fluids, colloids; quality, standardization; salvaged blood, unwashed; surgery, orthopaedic

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Unilateral total knee replacement (TKR) can result in substantial blood loss; 30–50% of such patients receive allogeneic blood transfusion. Although allogeneic blood is considered much safer than previously, it is an increasingly scarce and expensive resource, and wrong blood administration episodes are still too frequent (1/15 000–20 000). Concerns about adverse effects of allogeneic transfusion have prompted the review of transfusion practices, with the implementation of restrictive transfusion protocols and the search for transfusion alternatives to decrease or avoid its use in major joint replacement surgery. These strategies include correction of perioperative anaemia, pharmacological measures to reduce blood loss, and different modalities of autologous blood use.

In TKR performed under tourniquet, most blood loss occurs during the postoperative period. Consequently, salvage and return of unwashed filtered shed blood (USB) from postoperative drainage represents an alternative to allogeneic blood in these patients. USB has variable red blood cell (RBC) content and can be contaminated with fat particles, free haemoglobin (Hb), activated coagulation factors, fibrin degradation products, activated white blood cells, or inflammatory mediators, but few relevant side-effects have been witnessed after its reinfusion. However, some authors have questioned the quality and safety of this transfusion product and suggest that shed blood must be washed before return. The different blood-processing devices available differ greatly in their capacity for recovering and concentrating RBCs and for eliminating leucocytes, platelets, fat particles, and plasma chemical contaminants from salvaged blood. In addition, blood-processing devices are expensive and require trained personnel.

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In this study, we evaluated a simple, low-cost procedure for improving and standardizing USB quality that uses a colloid solution in a closed system. This closed-system procedure is based on the ability of colloids to counteract the repulsive electrostatic forces of RBCs, leading to RBC aggregation, rouleaux formation, and accelerated sedimentation, and resulting upward plasma flow.\(^{18-20}\) Thus, the procedure would allow for red cell concentration and plasma, leucocyte, and platelet reduction. In addition, we compared the relative effectiveness of commonly available colloids when used for red cell recovery using this procedure.

**Methods**

**Blood samples and colloids**

**Diluted venous blood samples**

Venous blood (300 ml) was drawn from five healthy volunteers into bags containing citrate–phosphate–dextrose as an anticoagulant (Kawasumi Laboratories, Tokyo, Japan). Each blood donation was split into two bags and diluted with iso-group out-date fresh frozen plasma to obtain 10 batches of diluted venous blood with a final haematocrit (Hct) of \(\sim 25-30\%\). All diluted venous blood batches were used for the different experiments within 24 h.

**USB samples**

After approval by the Ethics Committee of the University Hospital ‘Virgen de la Victoria’ (Málaga, Spain), 52 consecutive patients undergoing TKR who gave informed consent entered the study. In all subjects, the collection blood canister (ConstaVac CBC II\(^{\circledR}\); Stryker, Kalamazoo, MI, USA) was connected at the end of surgery to two drainage catheters through a y-connector, and USB was collected without an anticoagulant at a negative pressure of 25 mm Hg. The blood first passed through a 260 \(\mu\)m filter before entering the container. The canister is connected to the return bag to which the USB is transferred, discarding the last 60–80 ml to minimize fat particles and other debris being transfused to the patient, and allowing for several returns if needed. If at least 400 ml of blood was collected within 6 h after surgery, the USB was returned to the patient. During return, the blood passed through an additional 40 \(\mu\)m screen filter (SQ40SJKL\(^{\circledR}\), Pall Biomedical, Portsmouth, UK) intercalated in the given set. Samples of USB (20 ml) were obtained directly from the reinfusion bag and immediately used for the different experiments.

**Leucodepleted packed red cell samples**

Samples of stored leucodepleted packed RBCs were drawn into 4 ml EDTA-K2 tubes (BD Vacutainer K2E\(^{\circledR}\); Becton Dickinson, Plymouth, UK) from 25 U transfused at our haematology/ oncology day hospital unit, as part of our internal quality control programme. After measurement of haematometric parameters, plasma samples were obtained by means of centrifugation (3000 rpm for 10 min), and aliquot samples were stored at \(-70\)°C until assayed.

**Colloid solutions**

Low molecular weight (130 kDa) hydroxyethyl starch 6% solution (HES-130; Voluven\(^{\circledR}\), Fresenius Kabi, Louviers, France), intermediate molecular weight (200 kDa) hydroxyethyl starch 6% solution (HES-200; Hemospheres\(^{\circledR}\), BBraun, Melsungen, Germany), high molecular weight (450 kDa) hydroxyethyl starch 6% solution (HES-450; Hes Grifols\(^{\circledR}\), Grifols, Barcelona, Spain), and succinylated gelatine 4% solution (gelatine; Gelafundina\(^{\circledR}\), BBraun) were used in these experiments.

**Experimental procedures**

**Experiments with diluted venous blood**

A modification of the procedure of Rubinstein and colleagues\(^{21}\) for RBC depletion of placental/umbilical cord blood was used. Diluted venous blood samples were collected from blood bags into 20 ml syringes. Subsequently, one of the four different colloids was added to the diluted venous blood to attain a final concentration of 15%, 30%, or 45% (colloid solution:volume total volume, v v\(^{-1}\)). After obtaining an aliquot of the mix (Fig. 1a), the syringe was set upright and left for 20 or 30 min at room temperature to allow for RBC sedimentation by gravity (Fig. 1a). Thereafter, the supernatant was evacuated from the syringe (Fig. 1c), and the remaining syringe content was shaken and harvested for analysis. After measurement of haematimetric parameters, plasma samples were obtained by means of centrifugation (3000 rpm for 10 min), and aliquot samples were stored at \(-70\)°C until assayed.

**Experiments with USB**

USB samples were collected from the reinfusion bags into 20 ml syringes. Subsequently, HES-200, HES-450, or gelatine was added to the USB to attain a final concentration of 15–30% (v v\(^{-1}\)). After obtaining an aliquot of the mix, the syringe was set upright and left for 30 min at room temperature to allow for RBC sedimentation, the supernatant was evacuated from the syringe, and the remaining syringe content (colloid-processed USB) was handled as above.

**Analytical procedures**

**Haematological parameters**

Red cell count (RBC), Hct, Hb, total and differential leucocyte counts, and platelet counts were determined in colloid-processed and -unprocessed samples using a Pentra 120 Retic cell counter (ABX, Montpellier, France). Phenotypic identification of lymphocyte subpopulations in processed and unprocessed samples was performed by flow cytometry using monoclonal antibodies as described elsewhere\(^{22}\) (see details in Supplementary material 1).

**Biochemical parameters**

Concentrations of total proteins, glutamate-oxalacetate aminotransferase (GOT), lactate dehydrogenase (LDH), and creatine kinase (CK) were measured using the Dimensions RxL Max analyser (Siemens Healthcare Diagnostics, Deerfield,
IL, USA). Measurements of haptoglobin levels were performed using a BN-II nephelometer (Siemens Healthcare Diagnostics). Plasma-free haemoglobin (PFHB) was measured using a HemoCue Plasma/Low Hb photometer (HemoCue, Angelholm, Sweden). Plasma concentrations of pro-inflammatory cytokines [interleukin-6 (IL-6), interleukin-8 (IL-8), and tumour necrosis factor-alpha (TNF-α)] were assessed using a solid-phase enzyme-labelled chemiluminescent immunometric assay (Immulite I, Diagnostics Products Corporation, Los Angeles, CA, USA). All these parameters were assessed in plasma from processed and unprocessed samples.

**Recovery and removal calculations**

The RBC mass recovery was calculated using the following equation:

\[
\text{RBC mass recovery (\%)} = \frac{V_{pb} \times \text{Hct}_{pb}}{V_{ub} \times \text{Hct}_{ub}} \times 100
\]  

where \( V_{pb} \) is the processed blood volume, \( \text{Hct}_{pb} \) the processed blood haematocrit, \( V_{ub} \) the unprocessed blood volume, and \( \text{Hct}_{ub} \) the unprocessed blood haematocrit.

The removal of leucocyte or platelet mass was calculated using the following equation:

\[
\text{Leucocyte or platelet removal (\%)} = \left(1 - \frac{V_{pb} \times \text{BC}_{pb}}{V_{ub} \times \text{BC}_{ub}}\right) \times 100
\]  

where \( V_{pb} \) is the processed blood volume, \( \text{BC}_{pb} \) the leucocyte or platelet counts in processed blood, \( V_{ub} \) the unprocessed blood volume, and \( \text{BC}_{ub} \) the leucocyte or platelet counts in unprocessed blood.

The per cent removal of plasma volume was calculated using the following equation:

\[
\text{Plasma removal (\%)} = \left[1 - \frac{V_{pb} \times (1 - \text{Hct}_{pb})}{V_{ub} \times (1 - \text{Hct}_{ub})}\right] \times 100
\]  

where \( V_{pb} \) is the processed blood volume, \( \text{Hct}_{pb} \) the processed blood haematocrit, \( V_{ub} \) the unprocessed blood volume, and \( \text{Hct}_{ub} \) the unprocessed blood haematocrit.

The per cent removal of chemical contaminants (CC; e.g. PFHB, cytokines, etc.) was calculated using the following equation:

\[
\text{CC removal (\%)} = \left(1 - \frac{P_{pb} \times \text{CC}_{pb}}{P_{ub} \times \text{CC}_{ub}}\right) \times 100
\]  

where \( P_{pb} \) is the processed blood plasma volume, \( \text{CC}_{pb} \) the CC in processed blood, \( P_{ub} \) the unprocessed blood plasma volume, and \( \text{CC}_{ub} \) the CC in unprocessed blood.

**Statistics**

Data are reported as mean (SD) of \( n \) determinations. Statistical differences were determined by the non-parametric Wilcoxon’s rank test for data from diluted venous blood.
experiments, and Kruskal–Wallis H test (with Mann–Whitney U-test for post hoc analysis) for data from experiments with USB and for comparison between leucodepleted packed RBC and colloid-processed USB parameters. Statistical tests were performed using the SPSS 15.0 package (SPSS Inc., Chicago, IL, USA), licensed to the University of Málaga, Spain. All P-values reported are two-sided and are considered statistically significant at < 0.05.

Results

Incubation of diluted venous blood with colloids (dose- and time-finding experiments)

Haematological and biochemical characteristics of the 10 diluted volunteer donor venous blood batches are shown in Tables 1 and 2. Incubation of samples from five of the diluted venous blood batches for 20 or 30 min at room temperature with each of the different colloid solutions to a final concentration of 15%, 30%, or 45% (v v⁻¹) led to RBC sedimentation to a variable extent. After the evacuation of supernatant, the remaining syringe content was analysed and the percentages of RBC recovery and blood cell and chemical removal were calculated. Compared with control samples incubated without colloid, there was a concentration- and time-dependent increase in the percentages of leucocyte, platelet, and plasma removal. Overall efficacy of HES-200, HES-450, and gelatine was similar and higher than that of HES-130. However, there were no colloid-dependent differences in the percentages of RBC recovery (full data sets from 20 and 30 min incubation experiments are provided in Supplementary material 2).

On the basis of these results, an incubation time of 30 min and a colloid concentration of 30% were selected for subsequent experiments on removal of lymphocyte subsets, except for HES-450 which was tested at 15% (HES-450-1) and 30% (HES-450-2) concentrations. The remaining five diluted venous blood batches were used in these experiments, and one control sample was run with each of the

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Haematological and biochemical characteristics of diluted venous blood and after operation salvaged USB. Values are expressed as mean (SD) of n determinations. *P&lt;0.05, USB vs. diluted venous blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diluted venous blood (n=5)</td>
<td>Unwashed shed blood (n=40)</td>
</tr>
<tr>
<td>Haemoglobin (g dl⁻¹)</td>
<td>10.5 (0.4)</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>31 (2)</td>
</tr>
<tr>
<td>Total leucocytes (x 10⁶ μl⁻¹)</td>
<td>5.2 (0.8)</td>
</tr>
<tr>
<td>Platelets (x 10⁹ μl⁻¹)</td>
<td>124 (45)</td>
</tr>
<tr>
<td>Plasma-free haemoglobin (g dl⁻¹)</td>
<td>0.09 (0.03)</td>
</tr>
<tr>
<td>Total protein (g dl⁻¹)</td>
<td>4.7 (0.3)</td>
</tr>
<tr>
<td>Interleukin-6 (pg ml⁻¹)</td>
<td>3.1 (1.4)</td>
</tr>
<tr>
<td>Interleukin-8 (pg ml⁻¹)</td>
<td>10.2 (4.3)</td>
</tr>
<tr>
<td>Tumour necrosis factor-α (pg ml⁻¹)</td>
<td>6.5 (2.8)</td>
</tr>
<tr>
<td>Glutamate-oxalacetate transaminase (U l⁻¹)</td>
<td>20 (10)</td>
</tr>
<tr>
<td>Creatine kinase (U l⁻¹)</td>
<td>64 (10)</td>
</tr>
<tr>
<td>Lactate dehydrogenase (U l⁻¹)</td>
<td>153 (13)</td>
</tr>
<tr>
<td>Haptoglobin (mg dl⁻¹)</td>
<td>109 (5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Haematological characteristics, including lymphocyte subsets, of diluted venous blood and after operation salvaged USB. Values are expressed as mean (SD) of n determinations. *P&lt;0.05, USB vs. diluted venous blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diluted venous blood (n=5)</td>
<td>Unwashed shed blood (n=12)</td>
</tr>
<tr>
<td>Haemoglobin (g dl⁻¹)</td>
<td>8.8 (0.1)</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>26 (1)</td>
</tr>
<tr>
<td>Total leucocytes (x 10⁵ μl⁻¹)</td>
<td>3.8 (0.3)</td>
</tr>
<tr>
<td>Neutrophils (x 10⁵ μl⁻¹)</td>
<td>2383 (167)</td>
</tr>
<tr>
<td>Lymphocytes (cells μl⁻¹)</td>
<td>947 (68)</td>
</tr>
<tr>
<td>T cells (cells μl⁻¹)</td>
<td>624 (27)</td>
</tr>
<tr>
<td>Helper T cells (cells μl⁻¹)</td>
<td>398 (19)</td>
</tr>
<tr>
<td>Cytotoxic T cells (cells μl⁻¹)</td>
<td>197 (11)</td>
</tr>
<tr>
<td>B cells (cells μl⁻¹)</td>
<td>55 (7)</td>
</tr>
<tr>
<td>Natural killer cells (cells μl⁻¹)</td>
<td>168 (33)</td>
</tr>
<tr>
<td>Monocytes (x 10³ μl⁻¹)</td>
<td>252 (23)</td>
</tr>
</tbody>
</table>
triplicate experiments with colloid. The incubation of diluted venous blood with HES-200, HES-450-1, HES-450-2, and gelatine resulted in a variable (65–95%) but significant reduction in the total content of the different leucocyte populations and lymphocyte subsets with respect to control. Incubation with HES-130 was not as effective and this colloid solution was not used further (full data sets are shown in Supplementary material 3).

**Incubation of USB samples with colloids**

Haematological and biochemical characteristics of the 52 USB samples obtained from the reinfusion bags are shown in Tables 1 and 2. USB showed similar haematimetric values (Tables 1 and 2), but higher concentrations of PFHB, cytokines, and enzymes, and lower concentrations of haptoglobin and platelets, when compared with diluted venous blood (Table 1).

Incubation of USB samples with HES-200 (30%), HES-450-1 (15%), HES-450-2 (30%), or gelatine (30%) for 30 min at room temperature yielded similar results to those obtained with diluted venous blood, except for lower leucocyte and platelet removal. As depicted in Figures 2 and 3, incubation with colloid led to a substantial reduction in the content of leucocyte (60%), platelets (48%), plasma (77%), total protein (76%), PFHB (58 (SD 23)%), IL-6 (74%), IL-8 (77%), TNF-α (70%), and enzymes (65–74%), and to a 90% recovery of RBCs, without major differences between the results obtained with the different colloids. Similarly, the incubation of USB with each of the different colloids resulted in a variable but significant reduction in the total content of the different leucocyte populations and lymphocyte subsets, without major differences between the results obtained with the different colloids, except for a smaller reduction in some lymphocyte subsets when HES-450-1 was used (full data are shown in Supplementary material 4).

**Comparative analysis of colloid-processed shed blood and leucodepleted packed red cells**

After being concentrated by incubation with colloids and plasma removal, the haematological and biochemical characteristics of 40 processed shed blood samples were compared with those of 25 leucodepleted packed RBC samples obtained from standard units with a mean storage time of 14 (10) days (min: 3 days–max: 36 days). As shown in Table 3, there were no differences in Hb concentration or Hct level between colloid-processed USB and leucodepleted packed RBCs, although colloid-processed USB had higher concentrations of leucocytes, platelets, proteins, enzymes, haptoglobin, and PFHB.

**Discussion**

The use of devices that recover and transfuse postoperative shed blood to the patient in TKR surgery has been shown to decrease the frequency of exposure to allogeneic blood. However, there are concerns regarding the quality and safety of USB, and some authors recommend the use of blood-processing devices, which increases blood management costs and requires trained personnel. We evaluated here an alternative, simple, low-cost procedure for improving and standardizing USB quality that uses a colloid solution for rapid RBC sedimentation in a closed system. This procedure allows for RBC concentration, reduction of leucocytes and platelets, and elimination of most plasma and chemical contaminants from USB, yielding a product with haematimetric characteristics similar to those of standard packed RBC. In addition, we compared the

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**Fig 2** Recovery of RBCs and removal of leucocytes (WBC), platelets, plasma, total protein, and PFHB after processing postoperative USB by the addition of different colloid solutions to a final concentration of 15% (HES-450-1) or 30% (HES-200, HES-450-2, and gelatine) (colloid solution volume/total volume × 100) and incubation for 30 min, according to the procedure described in Figure 1. Each colloid concentration was tested in 10 separate experiments.
relative effectiveness of commonly available colloids when used for RBC recovery using this technique.

In normal plasma, RBCs do not aggregate because the external surface of the membrane is negatively charged due to the carboxyl groups in sialic acid, which cause the cells to repel each other.\textsuperscript{18} Different macromolecules of colloid solutions [including gelatine, dextran, and hydroxyethyl starch (HES), but not albumin] can adhere to the membrane surface to allow interactions between adjacent RBCs. This adhesion, which is believed to be favoured by van der Waals forces, hydrogen bonds, or electrostatic attractions, counteracts the negatively charged repulsive forces of RBCs leading to RBC aggregation, rouleaux formation, and accelerated sedimentation, with consequent upward plasma flow.\textsuperscript{18, 20, 24}

Modified fluid gelatine (Gelafusin\textsuperscript{®}, Gefundine\textsuperscript{®}, BBraun) is a solution of electrostatically charged polypeptides with a wide range of molecular masses (mean molecular weight 30 kDa). It increases RBC aggregation by reducing electrostatic repulsive force after adsorption onto the RBC membrane, an effect that has also been observed with other anionic polymers, such as polyglutamic acid (50 kDa).\textsuperscript{25} At physiological pH (7.4), gelatine is non-helical due to electrostatic repulsion of negative charges, and bind to the RBC surface through van der Waals forces, hydrogen bonding, or both.\textsuperscript{25} In addition, gelatine interacts specifically with plasma fibronectin, thus enhancing the formation of bridges between RBCs.\textsuperscript{26} As a result, RBC sedimentation rate can increase up to 100-fold when one volume of blood is mixed with two volumes of Gelafusin\textsuperscript{®}.\textsuperscript{18}

![Figure 3](https://academic.oup.com/bja/article-abstract/105/4/457/239104)

**Figure 3** Removal of IL-6, IL-8, TNF-a, GOT, CK, and LDH after processing postoperative USB by the addition of different colloid solutions to a final concentration of 15% (HES-450-1) or 30% (HES-200, HES-450-2, gelatine) (colloid solution volume/total volume × 100) and incubation for 30 min, according to the procedure described in Figure 1. Each colloid concentration was tested in 10 separate experiments.

### Table 3: Haematological and biochemical characteristics of banked allogeneic blood (leucodepleted packed RBCs), and postoperative USB after being processed with one of the four colloids for 30 min at room temperature (colloid-processed USB). Values are expressed as mean (SD) of n determinations. *P<0.01, colloid-processed UBS vs leucodepleted packed RBCs

<table>
<thead>
<tr>
<th></th>
<th>Leucodepleted packed RBCs (n=25)</th>
<th>Colloid-processed UBS (n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g dl\textsuperscript{-1})</td>
<td>19.6 (1.5)</td>
<td>18.9 (2.8)</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>59 (4)</td>
<td>57 (8)</td>
</tr>
<tr>
<td>Leucocytes (×10\textsuperscript{3} µl\textsuperscript{-1})</td>
<td>0.04 (0.04)</td>
<td>2.7 (1.3)*</td>
</tr>
<tr>
<td>Platelets (×10\textsuperscript{3} µl\textsuperscript{-1})</td>
<td>2 (1)</td>
<td>51 (4)*</td>
</tr>
<tr>
<td>Plasma-free haemoglobin (g dl\textsuperscript{-1})</td>
<td>0.25 (0.14)</td>
<td>0.87 (0.49)*</td>
</tr>
<tr>
<td>Total protein (g dl\textsuperscript{-1})</td>
<td>0.9 (0.5)</td>
<td>4.3 (0.9)*</td>
</tr>
<tr>
<td>Glutamate-oxalacetate transaminase (U l\textsuperscript{-1})</td>
<td>19 (5)</td>
<td>110 (50)*</td>
</tr>
<tr>
<td>Creatine kinase (U l\textsuperscript{-1})</td>
<td>64 (27)</td>
<td>1279 (1218)*</td>
</tr>
<tr>
<td>Lactate dehydrogenase (U l\textsuperscript{-1})</td>
<td>164 (83)</td>
<td>1101 (501)*</td>
</tr>
<tr>
<td>Haptoglobin (mg dl\textsuperscript{-1})</td>
<td>6 (7)</td>
<td>55 (36)*</td>
</tr>
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albumin is a more symmetrical protein and does not seem to contribute to RBC sedimentation, and was therefore not evaluated in this study.\textsuperscript{18} Dextran is a glucose polymer of quaternary structure. Because of its high dielectric constant and positively charged property, higher molecular weight (110 kDa) dextran induces RBC aggregation and rouleaux formation in a similar way to gelatine, and has been used for RBC reduction in umbilical cord blood before cryopreservation.\textsuperscript{19} However, lower molecular weight dextran (60 kDa) has very little effect on RBC aggregation.\textsuperscript{18} HES is a polar glucose-based artificial colloid with low antigenicity and an established safety profile as a plasma expander. HES is also used clinically to increase RBC sedimentation during leukapheresis.\textsuperscript{27} Additionally, an increased RBC sedimentation rate has been observed in blood samples drawn from patients receiving HES-200.\textsuperscript{20} Again, the insertion of the polar macromolecules of HES between adjacent RBCs increases RBC aggregation by decreasing electrostatic repulsive forces. Therefore, this effect may depend on both molecular weight and concentration of the colloid.\textsuperscript{20-25}

In a survey of the extent of use of colloids by anaesthesiologists in Spain, HES solutions were the colloids most often used (73%), followed by gelatine (28%), whereas the use of dextrans as therapeutic plasma expanders has been discontinued in Spain due to the risk of anaphylaxis caused by circulating dextran-reactive antibodies of the immunoglobulin G class found in most adults.\textsuperscript{28} Consequently, we evaluated the effects on RBC sedimentation of gelatine and three commercially available HES solutions (6% in normal saline), selected on the basis of their molecular weight. Normal saline alters the shape of RBCs and increases their mechanical fragility, but has only minor effects on RBC sedimentation.\textsuperscript{18} Therefore, a control group of USB treated with normal saline was not deemed necessary, and data from colloid-processed USB were compared with those obtained with USB without the addition of colloids.

In order to find the appropriate colloid type, colloid concentration, and incubation time, a series of experiments were performed using venous blood which was previously diluted to obtain haematimetric values similar to those of postoperative USB. With the exception of HES-130, the highest rates of RBC recovery (94%) and leucocyte (88%), platelet (87%), and plasma (80%) removal at the lowest colloid concentration were observed after 30 min incubation with HES-200 (30%), HES-450 (15% and 30%), and gelatine (30%). Interestingly, these results inversely correlated with those of RBC depletion (89% and 96%), WBC recovery (78% and 84%), and mononuclear cell recovery (81% and 85%) obtained after processing of human cord blood with HES-450 and gelatine solutions, respectively.\textsuperscript{29}

Consequently, these later colloid types and concentrations were selected for subsequent experiments with USB samples. As with diluted venous blood, incubation of USB with the selected colloid led to a reduction in the content of leucocytes, platelets, and plasma, and to a high recovery of RBC (90%), without differences between colloids.

The reduction of RBC mass in the processed samples is probably due to loss of intact RBCs during the evacuation of the supernatant from the syringe, and a certain degree of haemolysis induced by the use of citrate as an anticoagulant in diluted venous blood or the presence of inflammatory mediators in USB.\textsuperscript{32-34} However, a substantial portion of haemolysis products were eliminated from USB during the procedure, as reflected by removal of PFHB and enzymes.

The RBC recovery rate found in this study is similar to or higher than that described for several blood-processing devices (50–90% of the initial RBC mass), although these devices produced a more efficient removal of PFHB (65–92%) and platelets (68–99%).\textsuperscript{32-34} Nevertheless, high concentrations of PFHB were still observed in colloid-processed USB, and this most probably accounts for its reduced haptoglobin levels. However, it has been previously reported that if postoperative USB is reinfused up to 15% of the total blood volume or 1000 ml, there seems to be enough haptoglobin (an acute-phase reactant) in the circulation to bind PFHB, avoiding possible renal damage.\textsuperscript{10}

The ability of the colloid-induced RBC sedimentation procedure to remove leucocytes from USB (60%) is similar to that of most blood-processing devices (30–80%).\textsuperscript{32-34} In addition, total leucocyte content in a 250 ml colloid-processed USB unit will be $0.7 \times 10^9$ cells, which is similar to that of a standard buffy-coat removed RBC unit in additive solution ($1.2 \times 10^9$ cell unit$^{-1}$).\textsuperscript{35} To reduce further the likelihood of possible leucocyte-mediated adverse events after USB return, it might be useful to remove these blood cells using a leucocyte filter which also allows for a complete removal of fat particles.\textsuperscript{9} However, recent studies failed to show any differences in inflammatory reaction after TKR surgery between patients receiving USB, with or without leucocytes, washed shed blood, or no transfusion.\textsuperscript{11, 12, 36, 37}

One of the concerns regarding the use of USB is its content of biologically active peptides (e.g. activated coagulation factors, fibrin degradation products, and inflammatory mediators). Several studies have shown that USB return did not modify the humoral or cellular response that follows TKR surgery,\textsuperscript{11, 12, 14} but resulted in a transient yet clinically uneventful activation of coagulation and fibrinolysis.\textsuperscript{38} As we found that colloid-induced RBC sedimentation removes 75% of plasma and proteins (e.g. total protein, CK, or IL-6); from USB, it is conceivable that it also reduces other biologically active peptides (e.g. activated coagulation factors). Even though its capacity for removal of chemical contaminants is lower than that of blood-processing devices (90–95%),\textsuperscript{32-34} the procedure evaluated here can reduce the bulk of biologically active peptides, thus increasing USB quality and safety.

There is also some concern regarding the use of HES, especially those with high molecular weight, high molar substitution, and high C2/C6 ratio, as they might cause haemostatic derangements.\textsuperscript{39} However, if 300 ml of colloid solution is added to 700 ml of USB with an Hct of 30%, and 75% of the USB total supernatant (plasma + colloid, 790 ml) is removed after incubation for 30 min, $<100$ ml of colloid solution will
be transfused to the patient (~1 ml kg⁻¹), a colloid volume that should have a negligible effect on coagulation or fibrinolysis.⁴⁰–⁴²

No differences were observed in Hb concentration or Hct between colloid-processed USB and standard leucodepleted packed RBCs, and its leucocyte content was similar to that of a buffy-coat removed RBC unit in additive solution.⁴³ Thus, the procedure will allow for a standardization of this transfusion product, which may be important for its inclusion in a quality management programme.

In conclusion, processing of USB with commercially available colloid solutions allows for the concentration of RBCs, the elimination of most plasma and proteins, and the reduction of leucocytes and platelets. Therefore, it might provide a low-cost alternative for improving and standardizing the quality of USB before autotransfusion. However, more clinical research is needed to ascertain the impact of this procedure on patient outcome.

**Supplementary material**

Supplementary material is available at *British Journal of Anaesthesia* online.

**Conflict of interest**

None declared.

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