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A new approach for fat removal in a discontinuous autotransfusion device–concept and evaluation

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Background Fat present during blood salvage in orthopaedic or cardiac surgery can pose a risk of fat embolism and should be eliminated before transfusion. Based on observations of central fat accumulation at the bottom of Latham bowls, a fat reduction program was developed using two volume displacements, where blood temporarily is removed and respun in the bowl to force the fat through the RBC sediment.

Materials and methods Pooled ABO-matched RBC and FFP were adjusted to a haematocrit of 10%, and human fat tissue added to a concentration of 1·25 vol%. In six experiments, blood was processed with the new-generation cell salvage device CS Elite in a newly developed fat reduction program in bowls of three sizes. Volumetric quantification of fat was performed after centrifugation of blood samples in Pasteur pipettes. From volumes, haematocrits and the concentrations of fat, RBC recovery and fat elimination rates were calculated.

Results Fat removal rates of 93·2 ± 2·8, 97·0 ± 2·1 and 99·6 ± 0·3% were observed with a 70-ml, 125-ml and 225-ml bowl, respectively, and even higher rates when removal rates were calculated one cycle. At the same time, high RBC recovery and plasma elimination rates were maintained, not significantly different to the default program mode.

Conclusion Modifications in process parameters and sequence led to a fat reduction program that significantly improves fat removal with the Cell Saver Elite from 77·4 ± 5·1% in the default mode to an average of 98·6 ± 1·1%, yielding results equivalent to the continuous cell salvage system (C.A.T.S).

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Key words: RBC transfusion, transfusion complications- non-infectious, transfusion practices (surgical).

Introduction

In cell salvage during orthopaedic or cardiac surgery, fat is frequently observed in the wound blood collected in the reservoir or in the product of washed RBC. Although no single clinical case has yet been demonstrated and reported, concerns are that this fat may cause fat embolism after transfusion [1–3]. Generally, fat in blood poses no problem as long as it is emulsified. Fat emulsions are commonly used in parenteral nutrition and certain drug applications like propofol, without embolic complications [4]. Non-emulsified fat on the other hand can easily be prevented from processing by discarding, when observed in wound blood in the reservoir, or from transfusion, when observed in washed RBC in the transfusion bag, respectively. The serious problem is de-emulsifying fat, when with time micro-droplets of fat can fuse to create larger and larger fat particles.

Principally, this not-permanently emulsified fat should be eliminated during cell separation and cell washing.

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Cells and fluids are separated according to their specific densities, and lighter materials are removed by fluid flow, while the denser RBC accumulates in the bowl. Obviously, there exist other factors that in part prevent fat despite its very light density from leaving the separation chamber, both in continuous and discontinuous autotransfusion devices [5]. In experiments with soya bean oil or with fat from human fat tissue, fat removal rates of just 70–80% have been observed for bowl-based separation chambers, significantly lower than with the continuous C.A.T.S system [5, 6]. Further studies showed that fat removal can be increased by filtration, or by modifications in the process parameters and the program [5, 7].

Here, for the first time, an explanation is given and the geometry of the Latham bowl identified as the origin of fat retention. A novel fat reduction program is described and evaluated that allows efficient elimination of fat contamination during cell salvage while preserving key quality metrics such as RBC recovery, Hct and constituent washouts. The rational and principle of the process modifications are described and discussed.

Materials and methods

An experimental study was performed using fresh donor blood supplemented with human fat extracted from fat tissue. The blood/fat mixture was processed with the autotransfusion device Cell Saver Elite (Haemonetics, Braintree, MA, USA) to test a novel fat reduction program (FRP) with bowls of three sizes. Compared to the default mode, this program adds two volume displacements to the process along with an extra wash step. The volume displacements temporarily reduce the spinning volume of the bowl which forces separated fat spinning in the base of the bowl through the packed RBC layer to the outer separation chamber. The wash steps ensure fat now spinning in the outer separation chamber is expelled to the waste bag (see flow chart in Fig. 1).

In a second set of experiments, the program was validated with regard to other quality parameters using test blood without fat, since fat can interfere with the respective laboratory measurements. In particular, the photometric tests can be affected by fat.

Fat reduction experiments

PRBC and fresh frozen plasma (FFP) (CPDA) were obtained from Research Blood Components, Inc., Boston, MA (NEIRB #04-144 'The Collection of Whole Blood for Research Purposes' end approval date: 4/7/2017) and from Biological Specialty Corporation, Colmar, PA (DHHS Blood Establishment Registration: U.S. License Number 856). Human fat tissue was obtained from All-Cells, Inc., Alameda, CA, after informed consent of the patient, from plastic surgery (liposuction), frozen and stored at −18°C. All donors signed approved inform consent, and no information traceable to the donors was given to the testing laboratory. Liquid fat was extracted by heat at 150°C for 360 min and filtered afterwards to remove tissue debris. Liquid fat was allowed to equilibrate to room temperature before use in experiments. Blood group-matched donations (PRBC and FFP 1:1) were mixed and adjusted with saline solution (0·9% RICCA Chemical Company, Arlington TX), to a haematocrit of 10% to represent typical surgical blood pool concentration. Portions of 45 ml of blood and 5 ml of fat were mixed air-free in two 50-ml syringes connected by a three-way stopcock and added to a blood volume to result in a final concentration of 1·25% fat, as previously described.[5] Thorough mixing was achieved by forcing the blood–fat mixture between the syringes. Portions of test blood were measured in graduated cylinders and processed at room temperature (20°C) with the autotransfusion device Cell Saver Elite. Six experiments were performed with each of three bowls, 225 ml (n = 6), 125 ml (n = 6) and 70 ml (n = 6), in the fat reduction program. To exclude the first loss due to initial surface contact, a prerinse of the system with 50 ml test blood

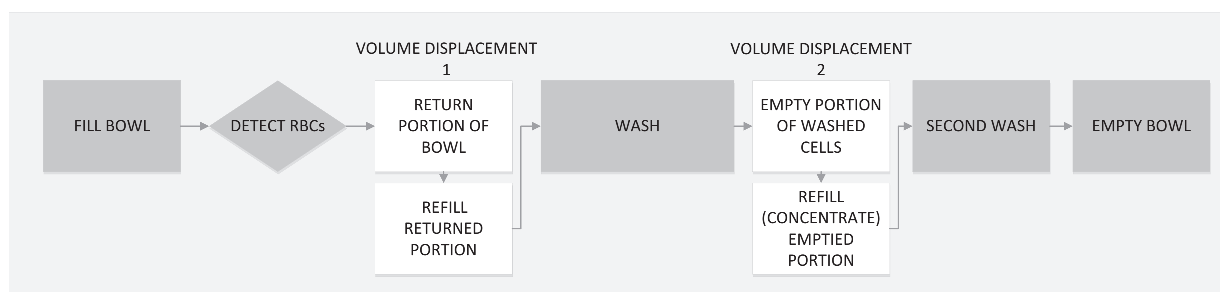

Fig. 1 Flow chart of the fat reduction program. Changes to the default program are shown in white boxes.

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was conducted prior to processing. Fat removal was tested during one process cycle (one bowl filling) including a final emptying. The final emptying adds remaining blood from the system to the product. A defined volume of 1500 ml test blood was placed in an open reservoir (with filter removed) for the 225-ml bowl, 1000 ml for the 125-ml bowl and 500 ml for the 70-ml bowl. After one bowl filling and automatic processing, the amount or blood remaining in the reservoir was measured to determine the processed blood volume. Product volumes were also measured in graduated cylinders. A 20 ml sample was taken from test blood and from the transfusion bag after one bowl had been processed. A second sample was taken from the purge collected in a separate container after inducing a final emptying. Before testing a different bowl, the entire wash set was exchanged to prevent artefacts from fat contaminations in the bowl or tubes. Haematocrit of the blood samples was determined in a haematocrit centrifuge (IEC model MB, Natic, MA).

The amount of fat in blood samples before and after processing was measured by volumetric quantification as described elsewhere [5]. In short, each blood sample was injected into two silicone-bottom-sealed Pasteur pipettes. The pipette was weighted before and after the injection for determination of the contained blood volume. Reproducibility of the double determination was tested and proved high reliability (variation <3%). After centrifugation at 2000 g (20°C, 10 min), the fat layer in the tip of the Pasteur pipette was measured in mm, and the content of fat in volume and in percentage was calculated from a standard curve. The detection sensitivity of the method was determined with 0·04vol% or 0·4 µl fat/ml blood.

Fat removal rate was calculated according to the following equation:

$$\text{Fat elimination rate (\%)} = 100 - 100 \times (V_{WRBC} \times \text{Cfat}_{WRBC})/(V_{TB} \times \text{Cfat}_{TB})$$

where V_{WRBC} is the volume of produced washed RBC (ml), V_{TB} the volume of the test blood used therefore, and Cfat the concentration of fat (vol%) in the respective blood [7].

In the fat reduction experiments, two different methods were used to calculate the values of the quality parameters. In general, in the 'total amount approach', all input and all output, including several cycles and the final emptying, are considered. In the 'per cycle approach' that is more feasible for clinical quality controls, only the blood input and the product of one cycle (one bowl filling and automatic processing) are considered [8, 9].

Quality parameter validation experiments

In a second set of six experiments, test blood without fat was prepared to test the plasma washout efficiency of the new program. Instead of fat, the pool was spiked with red blood lysate to a target concentration of 7 g/l of plasma free haemoglobin, corresponding to 20% haemolysis. Heparin was also added to the pool for a target concentration of 2·0 ± 1·0 IU/ml.

For the validation tests, volumes of 4354 ± 25 ml for the 225-ml bowl, 2472 ± 101 ml for the 125-ml bowl and 1283 ± 29 ml for the 70-ml bowl, respectively, were offered to allow for two cycles. The process was finalized by an additional emptying 'procedure complete' step with line purge given to the total product.

The haematocrit was measured using a haematology analyser (Sysmex XE2100 D, Kobe, Japan). Albumin, potassium and plasma free haemoglobin in the supernatant were analysed photometrically with a chemistry analyser (Dimension Xpand, Siemens Healthcare Diagnostics, Eschborn, Germany). Heparin was measured with a coagulation analyser (Stago STA Compact, Diamond Diagnostics, Holliston, USA).

RBC recovery rates were calculated as described elsewhere [8, 10]. The elimination rates of the soluble substances were calculated according to the following equation:

$$\text{Elimination rate (\%)} = 100 - 100 \times ((V_{WRBC} \times (1 - \text{Hct}_{WRBC}/100) \times C_{WRBC})/(V_{TB} \times (1 - \text{Hct}_{TB}/100) \times C_{TB}))$$

where V_{TB} x $(1\text{-Hct}_{TB}/100)$ is the volume of the supernatant in the test blood, while V_{WRBC} x $(1\text{-Hct}_{WRBC}/100)$ represents the volume of the supernatant in the produced washed RBC. C_{WRBC} and C_{TB} are the concentrations of the respective substance in the supernatant of the produced washed RBCs and the therefore used TB [10]. The 'total amount approach' was used exclusively. For comparison, tests were also performed using the default program.

Statistics

Statistical analysis was performed with SigmaStat 3·1 (Jandel Scientific Corp., San Rafael, USA). All values are given as mean and standard deviation (SD). An analysis of variance was performed using a one-way ANOVA with post hoc Bonferroni and a Student's *t*-test. Statistical significance was accepted at a $P < 0{\cdot}05$ after pairwise testing.

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Results

Fat reduction experiments

The calculated dilution and fat addition resulted in a test blood of Hct 10·6 ± 0·5% and fat concentration of 1·23 ± 0·8vol%. With the fat reduction program, a mean fat elimination rate of 96·6% was achieved. It was highest (99·6 ± 0·8%) for the 225-ml bowl and lowest (93·2 ± 0·8%) for the 70-ml bowl (Table 1).

Calculation following the 'one cycle approach' (first product without final emptying) resulted in higher values of fat reduction (Table 1). Product Hcts of 48·9 ± 1·8% (70-ml bowl) to 55·1 ± 2·5% (225-ml bowl) were observed. RBC recovery rates were highest for the 125-ml bowl (92·4 ± 7·3%) and lower for the 225-ml bowl (87·0 ± 3·6%) and the 70-ml bowl (72·3 ± 2·9%).

Quality parameters validation experiments

Testing the fat reduction program with test blood without added fat and considering two cycles plus a final emptying resulted in washed RBC with Hcts of 50·7 ± 0·6% (70-ml bowl), 51·4 ± 2·6% (125-ml bowl), and 57·0 ± 2·1% (225-ml bowl) (Table 1). RBC recovery ranged from 90·1 to 94·5%. Plasma elimination according to albumin measurements was highest for the 125-ml bowl (99·6 ± 0·1%) and somewhat lower with the 225-ml bowl (97·3 ± 0·1%) and the 70-ml bowl (97·6 ± 0·1%). Elimination rates of potassium were also high but significantly (P = 0·028 and 0·037) lower than for albumin with the 125 ml and the 225-ml bowl (Table 1). Plasma free haemoglobin (PFH) was eliminated at rates above 90% for all bowls with a wide variation. Heparin was eliminated at a rate of exceeding 99% for all bowls. Results with the default program are shown in Table 2 for comparison.

Table 2 Performance of the default program

Quality parameter	Bowl size 225 ml	125 ml	70 ml
Haematocrit (%)	55·7 ± 0·5	49·8 ± 1·0	51·4 ± 0·5
RBC recovery rate (%)	94·5 ± 0·9	91·4 ± 2·0	90·7 ± 2·1
Albumin elimination rate (%)	99·0 ± 0·1	98·7 ± 0·1	95·5 ± 0·4
Potassium elimination (%)	96·4 ± 0·3	97·2 ± 0·1	96·6 ± 0·6
Processing time per cycle (min)	6·5 ± 0·1	7·5 ± 0·5	8·0 ± 0·1

Values calculated following the 'total amount approach', that is considering two cycles plus final emptying.

Time for one cycle increased by 87%, when switching from default mode to the fat reduction program (FRP).

Discussion

The fat reduction program described and evaluated here originated from the observation that during centrifugation of fat-contaminated blood in the Latham bowl, fat accumulates at the base of the separation chamber (see F in Fig. 2b). While in the upper part of the bowl, the formed fat layer is forced by the growing sediment of RBC and by fluid flow towards the outlet located there (F1 in Fig. 2a), at the bottom of the bowl, the buoyancy of the light fat is a force directed towards the inlet (F2 in Fig. 2a). In addition, while the pump-driven fluid flow pushes incoming plasma and saline through the RBC sediment, fat does not easily pass the tightly packed RBC. Thus, at the bottom of the bowl, fat accumulates at the RBC interface (F3 in Fig. 2b). The presented fat removal program is based on two 'volume displacements', where a certain volume of blood is temporarily withdrawn from the separation chamber and then returned to let this trapped fat rise through the collecting red cells (Fig. 1). In

Table 1 Performance of the fat reduction program

	'Total Amount Approach' With Procedure Complete			'One Cycle Approach' Without Procedure Complete		
	Bowl size			Bowl size		
Quality parameter	225 ml (n = 6)	125 ml (n = 6)	70 ml (n = 6)	225 ml (n = 6)	125 ml (n = 6)	70 ml (n = 6)
Fat elimination rate (%)	99·6 ± 0·3[a]	97·0 ± 2·1[a]	93·2 ± 2·8[a]	99·6 ± 0·3[a]	99·1 ± 0·6[a]	97·0 ± 2·6[a]
Haematocrit (%)	57·0 ± 2·1	51·4 ± 2·6	50·7 ± 0·6	55·1 ± 2·5[a]	52·3 ± 1·9[a]	48·9 ± 1·8[a]
RBC recovery rate (%)	94·5 ± 0·9	92·0 ± 1·8	90·1 ± 0·7	87·0 ± 3·6[a]	92·4 ± 7·3[a]	72·3 ± 2·9[a]
Albumin elimination rate (%)	97·3 ± 0·1	99·6 ± 0·1	97·6 ± 0·1			
Potassium elimination rate (%)	87·4 ± 7·9	90·4 ± 7·8	96·1 ± 1·3			
Processing time (min)	12·6 ± 0·4	13·6 ± 0·3	14·9 ± 1·3			

Values calculated following the 'total amount approach', that is considering two cycles plus final emptying by 'procedure complete and the 'one cycle approach', that is considering one cycle without procedure complete.
[a]From fat reduction experiments, that is with fat-contaminated test blood.

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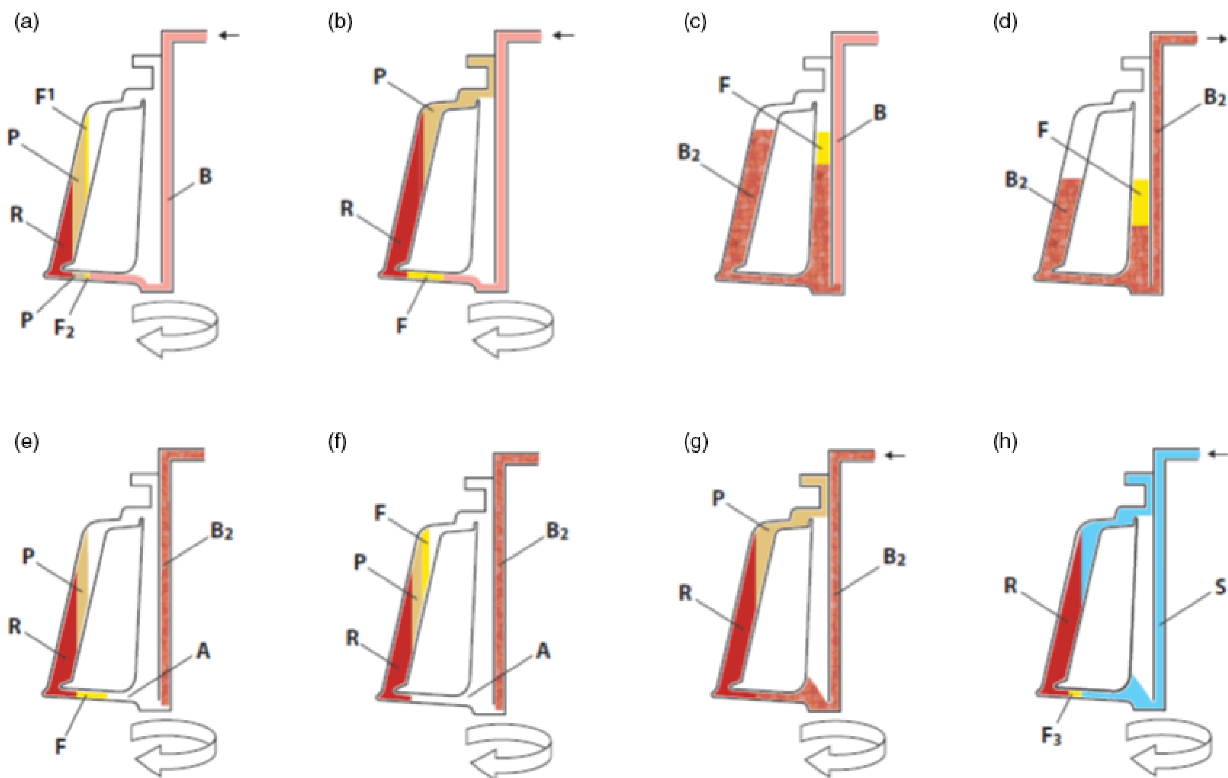

Fig. 2 Scheme of fat removal by volume displacements. R = packed RBC, P = plasma, F = fat, S = saline, A = air, B = incoming RBC, B2 = washed RBC.

detail, blood is filled into the bowl as usual, until the RBC sediment is detected by the optics sensor and the bowl stops (Fig. 2c), which leads to resuspension of RBC (but not of fat) in supernatant. At this time, 25 mL of resuspended blood, a volume equivalent to the capacity of the input line, is pumped back to the reservoir (Fig. 2d) and the bowl is respun. Under centrifugation, air replaces removed blood in the centre of the bowl (A in Fig. 2e) , which assists in pushing the fat through the temporarily loosely packed RBC layer towards the upper part of the bowl. In contrast to the filled bowl in the default mode, the fat layer now forms a white band clearly visible at the air interface (F in Fig. 2f, and Fig. 3). This fat band is subsequently pushed out of the bowl through pump-driven flow by refilling the bowl with the temporarily removed blood and with the wash solution (S in Fig. 2h) during the normal wash phase. To remove any remaining fat (F3 in Fig. 2h), the second volume split is performed, after this wash phase, by emptying two-third of the product (washed RBC) into the product bag. Upon centrifugation of the remaining content (Fig. 1), a white fat band is visible (Fig. 2f), and the fat is discarded to the waste bag by refilling with the product previously removed ('concentrate') and by a final 100ml wash (Fig. 2g and 2h).

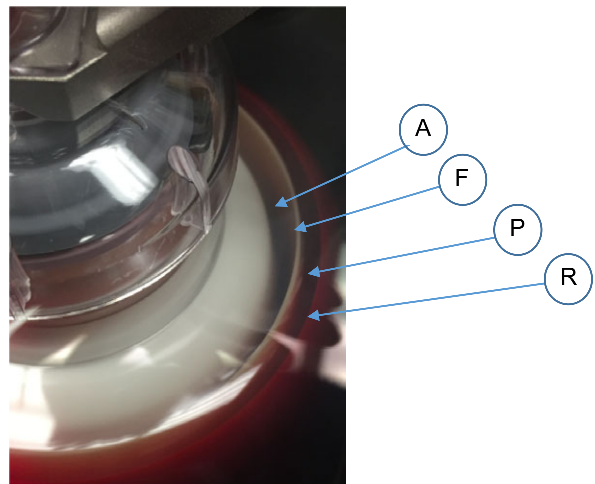

Fig. 3 View into the spinning bowl after the first volume displacement. A white band of fat separated from RBC sediment and plasma interlayer is visible. A= air, F= fat, P= plasma, R= packed RBC.

The evaluation of this novel program showed high fat elimination rates (see Table 1).

Other approaches have been proposed and developed to increase fat removal. The stickiness of human fat at room

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temperature (in contrast to oil used in some studies) can facilitate separation in a special bag system [11, 12] or in blood filters [6, 13]. But these methods, which are additional to the autotransfusion systems, are time-consuming and increase RBC loss and haemolysis. Alternatively, modifications in process parameters have been used based on the observation that fat removal is altered with variation in wash volume or flow, as well as with different program modes [5, 7]. Turbulences in the bowl induced by these changes have been discussed to underlie the resulting fat washout. In contrast, the program modifications used here are based on an understanding of fat retention in Latham bowls to foster fat mobilization out of the bowl. A 'concentration' step, that is the refill of product into the bowl before washing, as in the 'second volume split', has been evaluated in previous studies [7]. But in the present study, the major principle is the prior removal of part of the blood from the bowl (prior to the 'concentration' step) to use air to overcome the resistance of the RBC sediment to passage of fat and its subsequent accumulation in the bottom of the Latham bowl. The result is visible in the formation of a clear fat band (Fig. 3).

It is essential that any modifications in the established process variables do not impair the high quality standard of cell salvage. Namely, an improvement in fat elimination rate should not be at the expense of RBC recovery and plasma elimination, representing the major quality parameters for autotransfusion devices [8, 10]. Our evaluation demonstrates that high performance is maintained with the described fat reduction program. Plasma elimination was reflected by measurement of albumin and in addition by measurement of potassium and heparin (Table 1). Albumin is confirmed to best represent plasma elimination, while PFH and potassium show markedly lower values and wider variation. Calculation of their washout is obscured by the fact that their concentration decreases during the wash phase but concomitantly increases with some haemolysis occurring during the process [8, 14]. Heparin results overestimate plasma elimination due to additional removal by adsorption to cells and artificial surfaces [14]. Plasma elimination was slightly decreased for the 225-ml bowl, but increased for the smaller bowls (compare Tables. 1 and 2) with the additional washing and concentrate steps of the FRP. These modifications lead to a higher product Hct, but also increase processing time. However, in clinical practice, this delay does not create a disadvantage, since production of one unit of autologous PRBC still is far faster than ordering an allogeneic unit from the blood bank.

RBC recovery is not impaired by switching program mode from default to FRP and comparable to the literature [15]. This second main quality parameter was evaluated for FRP both with (data not shown) and without added fat. The conditions for its determination differed. The result shown on the left side in Table 1 originated from measurements of Hct with a cell counter and from consideration of two process cycles including final emptying, those on the right side in Table 1 from measurements of Hct with a Hct centrifuge (to avoid distortions by the contained fat) and from consideration of only one cycle without final emptying.

In this study, these quality parameters were calculated from the comparison of total input and total output, including an additional emptying. A different approach usually is used in clinical quality controls, where washed RBC is transfused as soon as prepared and final emptying of the wash system is restricted to the end of surgical bleeding. There, compared input and output are related to one cycle of a preparation [7, 10]. This principle was additionally used in the fat reduction study for determination of RBC recovery and fat removal (see Table 1). This approach resulted in lower values for RBC recovery rates and higher values for the fat elimination rates compared to the 'total amount' approach. Thus, the high fat washout is confirmed. The larger bowls fulfil the intended quality criteria also with this calculation approach, with a RBC recovery exceeding 80% [8]. The 70-ml bowl did not reach this level, with an average RBC recovery of 72·3%. This is due to the fact that in the small bowl a larger proportion of the product volume is retained in the line and the bowl after each completed cycle with the 70-ml bowl. This volume is only returned to the patient after the additional final emptying step at the end of the procedure. Additionally, the discrepancies in the values of product Hct and RBC recovery in Table 1 are partially due to the used test blood without (left) or with (right) added fat.

The 'one cycle approach' however allows comparison of the results of this study with other investigations using the same experimental set-up and evaluation method [5, 7]. Figure 4 shows the fat elimination rates of different autotransfusion devices, namely Cell Saver 5plus (Haemonetics), XTRA (LivaNova) and C.A.T.S (Fresenius), and fat removal programs. With 99·6% (for the 225-ml bowl), the performance of the tested fat reduction program is markedly and significantly ($P < 0{\cdot}001$) higher than the predecessor model in the default mode (77·9 ± 9·7%), and not significantly different to the high performance of the fat removal program Pfat (99·6 ± 0·07%, $P = 0{\cdot}974$) and C.A.T.S (99·7 ± 0·2%, $P = 0{\cdot}378$). This confirmed that a high fat elimination is not an exclusive feature of the continuous system; a lower elimination rate is not a general and necessary disadvantage of discontinuous systems.

The retransfusion of wound blood contaminated with lipids can constitute a serious risk in different ways.

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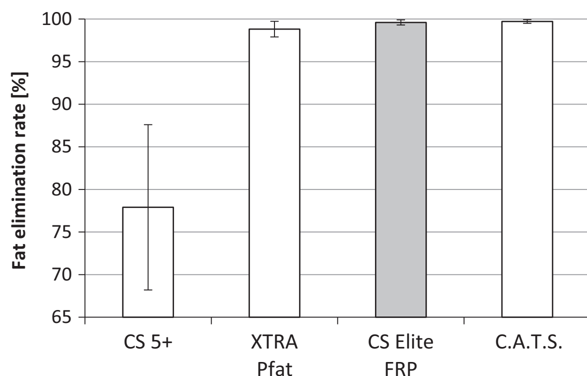

Fig. 4 Fat removal with Cell Saver Elite (225-ml bowl) and the fat reduction program (FRP) (grey column) compared to other autotransfusion devices and programs (data from Seyfried *et al.* 2015 [5] and Seyfried *et al.* 2016 [7]).

Circulating lipids can lead to disturbances in the coagulation system and to cerebral and pulmonary embolism. Membrane lipids derived from cellular debris are known to be procoagulant and can also cause immunological reactions [16–18]. Contamination of wound blood with fat particles frequently occurs in cardiac or orthopaedic surgery, where fat layers and circulating particles have been observed in wound blood [5, 19]. These particles are linked to cerebral dysfunction caused by 'small capillary and arteriolar dilations' (SCADs), which have been detected in human and animal brains after cardiopulmonary bypass surgery [1, 13]. Adverse pulmonary effects were reported by Eyjolfsson *et al.*[20] where the retransfusion of lipid contaminated blood led to an increase in pulmonary vascular resistance.

While a discussion is going on about different extend of fat removal with various autotransfusion devices, other authors recommend the retransfusion of unwashed blood in orthopaedic surgery [21, 22] with no fat reduction by centrifugation and washing. The retransfusion of unwashed wound blood, however, is associated with undesirable effects, and filters are less efficient than autotransfusion devices in removing fat [5, 23]. In contrast, Djaiani *et al.* report on reduced cognitive deficit after the processing of pericardial suction blood with an autotransfusion device [24]. An experimental study by Kinacid *et al.* has also shown a reduction in cerebral lipid microembolism, when scavenged blood was processed with an autotransfusion device [13]. However, in a randomized, double-blind study of Rubens *et al.*, no advantage of processing shed blood during cardiopulmonary bypass prior to transfusion was observed with regard to postoperative neurocognitive function [25]. More studies obviously are needed to clarify the risks associated with fat in wound blood and to prove the benefit of its removal before retransfusion. Meanwhile, all attempts should be undertaken to prevent lipids and fat from entering the circulation during intra- and postoperative autologous transfusion. The fat reduction program described and evaluated here is a further step to ensure the safety and high quality of autologous transfusion.

In addition, this article shows and discusses the difference of quality parameter results when determined in experiments to evaluate a cell salvage device or program (total amount approach), or in periodically, clinical quality controls (one cycle approach) that meanwhile in several countries are state of the art.

Acknowledgements

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Conflict of interests

RJM is director of the testing laboratory contracted by Haemonetics for this project. SK is in current employment at Haemonetics Corporation. EH and TFS have repeatedly cooperated with Fresenius, Haemonetics and LivaNova in studies. All authors declare that they have no conflict of interests relevant to the manuscript being submitted to Vox Sanguinis.

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