

Original Article

In Vitro Testing of a Current Cell Salvaging Device for the Removal of Malignant Cells from Contaminated Blood

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ABSTRACT

The efficiency of a current cell washing device for removing tumor cells from bovine blood was examined under laboratory conditions. In the in-vitro laboratory, anticoagulated bovine blood was seeded with known numbers of immunocytochemical stained human malignant epithelial cells (KB) grown in culture. The blood was subjected to cell washing. Blood samples were taken before and after cell washing for identification of the tumor cells. The samples were then analyzed under fluorescence microscopy and pre- and post-cell washing tumor cell counts in 20 microscopic fields were recorded. It was determined that the mean tumor cell removal efficiency was $86\% \pm 13\%$ using the cell salvaging technique. Two-way ANOVA revealed a significant difference between the pre- and post-cell washing samples ($p < 0.001$) with no difference between trials ($p = NS$). The results are discussed in terms of the potential safety of cell salvaged blood for the surgical care patient.

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INTRODUCTION

Blood collected from a patient for retransfusion into that patient at a later time is called "autologous blood." When the guidelines set forth by the American Association of Blood Banks are followed, autologous blood transfusions are considered the safest means of transfusing a patient (1). Intraoperative autotransfusion is the process of collecting shed blood from surgery or a wound, processing it, and reinfusing the blood back into the same patient. In general, blood is collected, filtered, and reinfused as whole blood, or it is collected, processed, and reinfused as washed red blood cells (2). Initially, autotransfusion was reserved for ectopic pregnancies and trauma cases, but today it is used in other emergency procedures and in elective procedures where blood losses in excess of 1,000 ml are anticipated (3). Intraoperative autotransfusion provides clinicians with a safe and effective means to recover and reinfuse blood lost at the surgical field without compromising patient safety (4).

Analysis of intraoperative blood transfusion cost revealed it to be comparable to the use of bank blood, but the cost of bank blood has since risen 30% (5). The autotransfusion machine is easy to operate and an economical method for processing blood lost during surgery. Throughout history, autotransfusion intraoperatively has decreased the strain on hospital blood banks, and decreased the donor exposure of the surgical patient (6). Blood processed by a cell processor has a hematocrit of 55-65% and 2,3 - diphosphoglycerate levels remain normal (5).

Numerous advantages offered by autotransfusion include the prevention of sensitization of the recipient to various antigens in donor erythrocytes, leukocytes, platelets, plasma, and avoidance of transfusion-transmitted diseases, especially viral hepatitis and Acquired Immune Deficiency Syndrome (7). Autologous blood has been classified as the only perfectly compatible blood product (1). In circumstances in which the blood loss exceeds 1,000 ml in the adult, its use has been proven to be cost effective while reducing donor exposure (6). Retransfusion of packed red blood cells freed from cellular debris, heparin, and activated clotting factors significantly reduces blood loss during and after surgery (8). Although autotransfusion is widely recognized as a means of postoperative blood recovery, it use has limitations (4).

Fecal contamination and spillage of malignant cells have been stated as contraindications to intraoperative autotransfusion, without substantial proof of either contention (9). The application of autotransfusion may be limited by the assumption that tumor cells will be retrieved and resuspended with red blood cells and subsequently infused into the patient (10). In previous studies, tumor cells have been shown to pass through the autotransfusion apparatus (9-11).

Advances in cell salvaging equipment have made autotransfusion an effective and efficient method of blood recovery. Despite widespread applications, the presence of a malignant tumor is still regarded as a contraindication to the use of intraop-

erative autotransfusion. In the advent of technological advances in intraoperative autotransfusion equipment, the removal of malignant cells by such equipment has not been well documented. Human malignant epithelial cells (KB) were chosen for this study because of the ability to grow these cells in culture to a clinically relevant number. Because these cells contain abundant cytoplasm, they are more buoyant, and thus theoretically, more susceptible to the principle of centrifugation. The null hypothesis of this study is that there is no difference in the tumor cell count in the product of washed cell salvaged blood containing cultured malignant cells as compared with non-washed cell salvaged blood containing cultured malignant cells.

MATERIALS AND METHODS

Human malignant epithelial cells (KB) were cultured in a medium^a containing 5% fetal bovine serum at 37°C. After the KB tumor cell line was cultured for a minimum of 48 hours, twenty flasks were labeled with 4',6-Diamidino-2-phenylindole (DAPI) diluted to a concentration of 20 mg/ml in sterile DMSO. The flasks were then incubated for 15 minutes at 37°C to allow for the absorption of the DAPI into the nucleus of the KB cells. After incubation, each flask was washed in a serum free medium and trypsinized for ten minutes at 37°C to remove the cultured cells from the flask. The cells were spun at 1000 g for 10 minutes and resuspended with 10 ml of a phosphate buffered solution (PBS+).

The cells were then combined with 100 ml of bovine blood and mixed thoroughly. Citrate-phosphate-dextrose was added to bovine blood to ensure anticoagulation. The mixture was processed through a high speed pediatric cell processor^b system at a fill rate of 200 ml/min and washed at a rate of 200 ml/min with one liter of 0.9% NaCl. The pediatric cell processor was chosen because of its smaller bowl volume which was used to achieve a clinically relevant amount of cultured KB cells in 100 mls of bovine blood. The system contained a non-filtered cardiotomy and no additional filters in line. Samples were drawn pre- and post-cell salvaging. The 3 ml samples were then washed in PBS+, centrifuged, and resuspended each time to a volume of 3 mls.

A 20 ul aliquot of each specimen was then placed on a 35 mm dish that was treated with 1 mg/ml of polylysine and PBS+ to achieve a monolayered adhesion. The 35 mm dishes were then spun at 1000 g for ten minutes, and fixed in 25% formalin and covered in glycerol for viewing. The dishes were then viewed by a registered cytotechnologist to count the number of malignant cells. Twenty random microscopic fields were chosen, and the DAPI labeled cells were counted and recorded from each

a Roswell Park Memorial Institute, Number 1640

b Basic Small Volume High Speed Cell Saver, List No. 241. Haemonetics Corporation, Braintree, MA 02184

field. Each trail represents a matched pair. The cells in a single specimen were counted. That specimen was then processed with the cell salvager, and the cells in this sample were counted.

The post-cell salvaged samples were compared to pre-cell salvaged samples in which a clinically relevant amount of cultured tumor cells were seeded into 100 ml of bovine blood and subjected to the staining assay described above. The pre- and post-filtration samples were compared using a computer statistical software package^c and graphed on a graphical software package^d. A p value <0.05 was considered significant. Paired t-tests were done to evaluate differences between samples and two-way analysis of variance done to evaluate differences between trials.

RESULTS

The efficiency of the cell salvaging system for the removal of tumor cells from whole blood is shown in Table 1. Cancer cells were detected in all but one of the post-washing samples. The recovery of cancer cells was low at each constant concentration, but the overall recovery rates were reproducible. The recovery for each trial is seen in Table 2. The mean removal efficiency of the cell salvaging device was 86% ± 13%.

The difference between the pre- and post-tumor cell counts was analyzed using a paired t-test and was found to be significantly different with a p<0.001, as shown in Figure 1. Two-way analysis of variance revealed no statistical difference between trials (p=0.905).

Figure 2 (A and A') are photomicrographs taken from pre-processing samples. Figure 2 (B and B') are photomicrographs taken of a microscopic field in a post-processed sample. The ultraviolet photomicrographs show the DAPI labeled cells which appear white in Figures 2(A) and 2(B). Figures 2(A') and 2(B') were taken under phase contrast microscopy to show the same pre- and post-processed KB cells in relationship to the red blood cells.

DISCUSSION

In the present investigation, the cell processing unit proved to be an effective means of removing most cancer cells from the bovine blood under constant cancer cell concentrations. The present study indicates that 86% ± 13% of cultured tumor cells were removed by the cell processing system. The standard deviation of 13% was attributed to cellular clumping. This removal efficiency was greater than that reported by Dale, et

al, who, in an in vitro study in 1973, reported a 24% or less removal efficiency of radiolabeled tumor cells by a cell processing unit (10). In this study, there was no discussion of the washing techniques or fill rates utilized during cell salvaging. A more recent in-vitro study using a gravity dependent autotransfusion

Table 1: Efficiency of cell salvaging for the removal of KB cultural tumor cells

	N	Mean	Median	STDev	Min	Max
Pre	10	47	47	±11	29	61
Post	10	7	4	±6	0	18

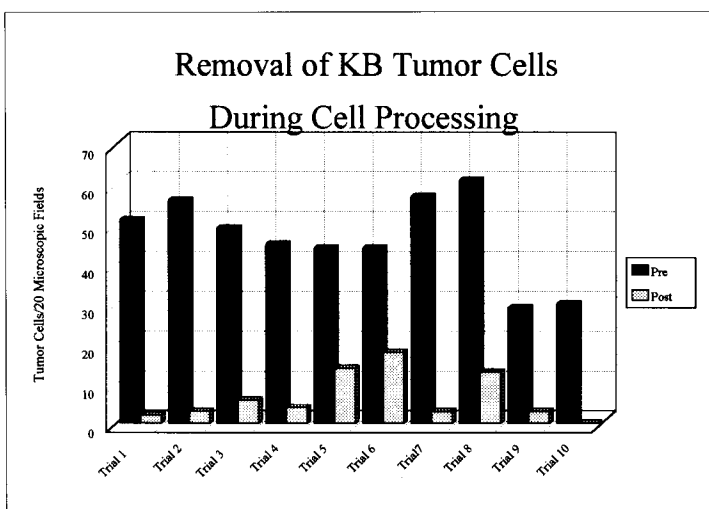
Numbers represent tumor cells/20 microscopic fields

Table 2: Percentage of KB tumor cells removed

Trial	Pre-Cell Salvaging Cells/20 Fields	Post-Cell Salvaging Cells/20 Fields	Removal Efficiency
1	51	2	96%
2	56	3	95%
3	49	6	88%
4	45	4	91%
5	44	14	68%
6	44	18	59%
7	57	3	95%
8	61	13	79%
9	29	3	89%
10	30	0	100%

* Mean Removal Efficiency = 86% ± 13%

Figure 1: Pre and post tumor cell counts



*p<0.001 (pre to post for each trial), p=NS between trials

c Minitab, Inc. State College, PA 16801

d Harvard Graphics, Software Publishing Co., Mountain View, CA 94039-7210

system showed a removal efficiency of 55% – 76% using human vulva carcinoma cells (A431). Of the approximate 12% of tumor cells that made it through the system, 62% of these cells were deemed nonviable on the basis of trypan blue exclusion (11). Trypan blue exclusion is a test for cellular membrane integrity and does not take into account programmed cell death. In this study by Karczewski, et al., three in-line filters were used that ranged from 20 μm to 170 μm , and the processing of the washed samples was done in two stages (complete density gradient separation and filter enumeration), which may have altered cellular recovery. Yaw and associates failed to prove that sepsis or spread of neoplasm is associated with autotransfusion, but they did find cytologic evidence of neoplasm in the final product of autotransfusion in a case report (9). It appears that, regardless of the cell salvaging system used, there is no device that offers 100% removal efficiency of tumor cells from salvaged blood. However, removal efficiencies have improved with advances in technology and research methods.

A review of the literature and the results of this study show that there is no perfect salvaging system on the market for the complete removal of malignant cells. The decision to autotransfuse processed/washed blood must focus upon risk of metastasis and patient benefit. Since circulating cancer cells are routinely found in the blood of a surgical cancer patient with metastatic disease, the risk may be negligible (12). Intraoperative autotransfusion has been widely used in major urologic surgery at the University of Florida since July 1984, with no evidence of tumor dissemination or decreased survival in 20 patients receiving intraoperative autotransfusion (13). The study described above used autotransfusion for three types of genitourinary malignancy with varying cell types and sizes. The predicted overall survival and risk of recurrence in patients that have been autotransfused intraoperatively compare favorably with patients who have not been subjected to intraoperative autotransfusion (14, 15). This research supports Weiss and his "metastatic inefficiency" theory that states that as few as 0.01%-0.000001% of disseminated cells may form metastatic lesions, therefore yielding a low-seeding potential of tumor cells (1).

Together these findings suggest that the use of intraoperative blood salvaging and processing may have a negligible to minor role in the dissemination of tumor cells in the surgical cancer patient. The most important advantage of autologous blood is that it is safer and carries no risk of infectious disease, such as viral hepatitis, HIV, cytomegalovirus, and the human T-cell lymphotropic virus. Since many surgical cancer patients are having surgery as a palliative treatment and not as a cure, the use of autotransfused blood should be reconsidered as an alternative to homologous blood.

Before many clinicians will choose autotransfused blood over homologous blood, further research must be done to determine the removal efficiency of the cell processing device on various cancer cell types. Post-processing viability of various cell types, as well as retrospective studies, are needed to determine which tumor cell types are more apt to seed a malignancy if reinfused by autotransfusion.

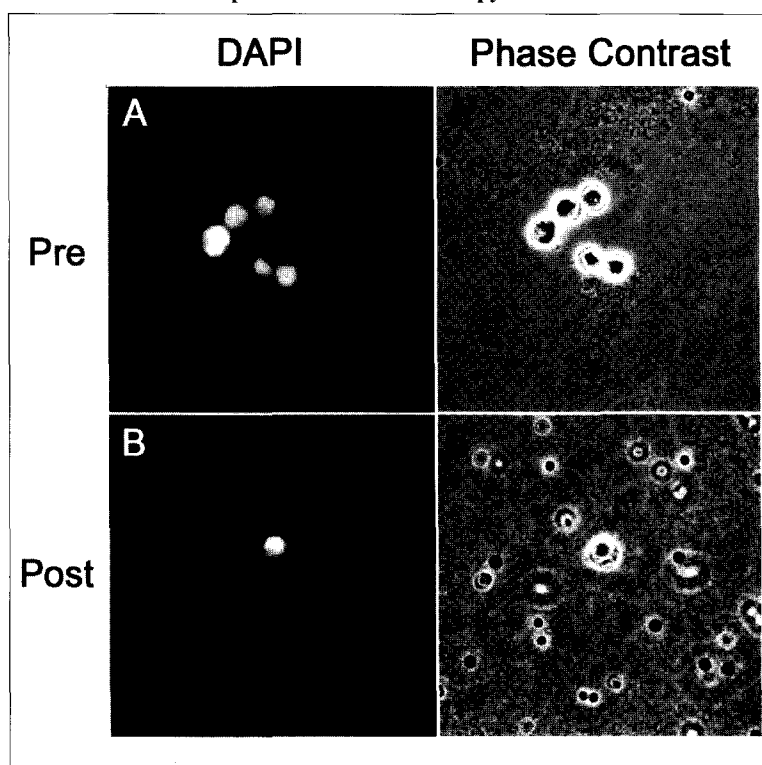
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Figure 2: (A) DAPI labeled KB cells in a pre washed specimen. (A') The same KB cells under phase contrast to show the relationship of KB cells to red blood cells. (B) DAPI labeled KB cells found in a post washed specimen. (B') The same post washed cells under phase contrast microscopy.



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