

Korus™: Transforming Monocyte Based Cell Therapy Manufacturing via Elutriation

INTRODUCTION

Monocyte derived immunotherapies represent a promising approach to treating a variety of human cancers and other diseases. These therapies include monocyte derived dendritic cell vaccines, engineered CAR-macrophages, among others. Existing manual and semi-manual methods of monocyte isolation provide a source of manufacturing inefficiency and impaired quality. Invetech has developed the Korus platform to address these issues during manufacturing by providing a fully automated, closed counterflow centrifuge system that can enrich monocyte populations from a full leukopak in less than an hour.

Herein we present data for the efficient and effective enrichment and concentration of a monocyte cell population, starting with a healthy donor leukopak.

Methods

Experimental Design

In order to obtain an enriched monocyte population from a single donor, a fresh leukopak was diluted in elutriation buffer and processed using the Monocyte Elutriation protocol on Korus. Input, lymphocyte fraction, and harvest samples were analyzed for cell number and composition. Following loading of input material into the chamber, the lymphocyte fraction is elutriated into our "transfer fraction" bag using 1000 mL of elutriation buffer. Cells remaining in the chamber containing the enriched monocyte fraction, are then transferred into the harvest bag. The harvest bag contains concentrated monocytes in a 150 mL volume, limiting the need for further processing before the next stage of a cell manufacturing process.

Analytics

Cell analytics were performed as following. Cell enumeration and viability was quantified using CBC ABX Micros 60 (Horiba) hematology analyzer and NucleoCounter® NC-200™ (ChemoMetec®) cell counter. Cell populations were further analyzed using Agilent NovoCyte® 3000 flow cytometer and markers were identified using the flow cytometry panel in Table 1.

Cell Population Subset	Antibody
Total WBC population	Anti-CD45
Monocytes	Anti-CD14
Lymphocytes	Anti-CD3, Anti-CD19, Anti-CD16, Anti-CD20, Anti-CD56

Table 1: Flow cytometry panel antibody composition for labeling a subset of White Blood Cells (WBC)s. Data acquisition and analysis were performed using an Agilent NovoCyte® 3000 flow cytometer.

Elutriation Buffer

HBSS (Hanks' Balanced Salt Solution) with 1% HSA (Human Serum Albumin).

Key Parameters

Centrifuge speed: 2800 RPM
Elutriation volume: 1000 mL

Apheresis Elutriation

Input material consisting of approximately 8 billion WBCs was diluted in HBSS with 1% HSA to 20 X 10⁶ cells/mL. Apheresis was aseptically connected to a Korus Single Use Set by Terumo TSCD® II. Korus parameters were configured as per Invetech's monocyte elutriation protocol with a harvest output volume set to 150 mL.

Calculations

$Recovery = Output\ Cells \div Input\ Cells$

$Purity = Target\ Cells \div All\ Viable\ Cells$

Results

Monocyte Elutriation Performance

Monocyte enrichment performance using the monocyte elutriation protocol on Korus is shown in Figure 1. Monocyte recovery in the harvest averaged $88.6 \pm 5.8\%$ with average purity of $75 \pm 4.98\%$ CD14 positive cells. Platelets (PLTs) and Red Blood Cells (RBCs) were efficiently depleted with an average depletion of $99.7 \pm 0.07\%$ and $90.1 \pm 8.5\%$ respectively. The harvested monocyte enriched fraction had an increase in viability on average of $0.47 \pm 0.33\%$ relative to the input material. The full run time from loading the input bag to completion of the run was 35 minutes.

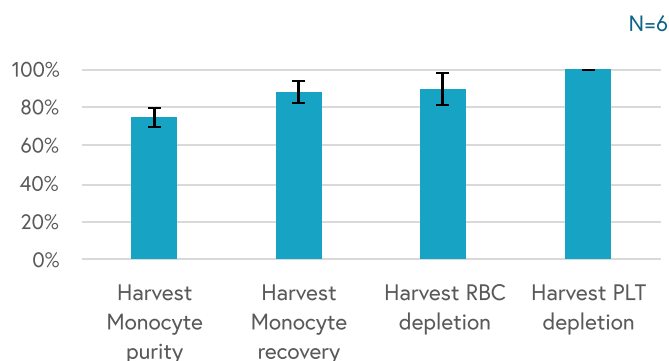


Figure 1: Monocyte elutriation performance of Korus. Error bars indicate standard deviation.

Elutriated Lymphocyte Fraction

The Korus elutriation protocol and single use set enables the user to capture the elutriated lymphocyte fraction in a single bag if needed for additional process needs. Analysis of the recovered lymphocyte fraction is shown in Figure 2. Lymphocyte recovery averaged $86.9 \pm 7.9\%$ with average purity of $92.3 \pm 2.48\%$. Platelets and RBCs were depleted with an average depletion of $77.5 \pm 3.9\%$ and $47 \pm 10.7\%$ respectively, relative to the input material.

N=6

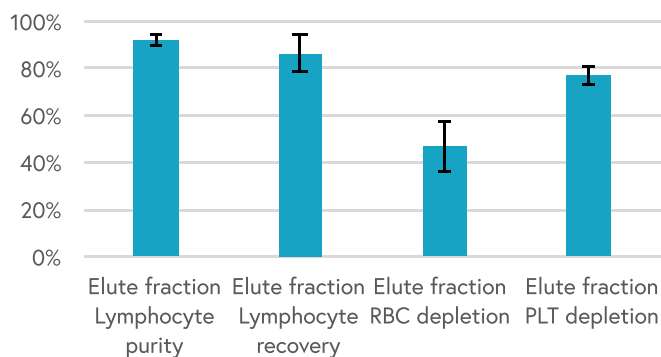


Figure 2: Elutriated lymphocyte fraction using the monocyte elutriation protocol. Error bars indicate standard deviation.

CONCLUSIONS

Korus can successfully produce an enriched monocyte population with high purity, recovery, and viability starting with fresh apheresis material using a commonly available elutriation buffer. The Korus monocyte elutriation process also enables the operator to capture an enriched lymphocyte fraction simultaneously from the same donor and process if needed.

This Korus elutriation protocol can be configured by the operator to achieve the desired level of harvest purity and optimized to specific process requirements and then locked down for GMP manufacturing. This process transforms cell therapy manufacturing using elutriation, a gentler way to separate and wash cells, while maintaining viability. It offers better overall process performance through higher recovery and purity of target cells.

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FURTHER INFORMATION

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