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Modification of a commercial cell sorter to support efficient and reliable preparation of ALDH-bright cells for clinical use

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Background

Cell populations manufactured by conventional commercial cell sorters have been safely infused into patients, but reliably sterilizing these instruments remains challenging. We are developing clinical protocols involving use of ALDH bright cells manufactured by cell sorting in patients. However, we encountered problems when we attempted to reliably sterilize the FACSAria® cell sorter using standard methods.

Results

We have identified and modified potential sources of microbial contamination in several FACSAria systems. We added new filter systems to the sheath and sample air lines, to the wet cart fluid supply, and to the sample line. Sheath was provided from an external sterile, disposable bag through sterile disposable tubing sets. The plenum reservoirs were modified in several ways to allow efficient decontamination of internal surfaces. A new bubble filter assembly was added and one valve was eliminated from the sample pathway to improve flow cell sterilization. A new cleaning and sterilization protocol was developed and validated. All cell products manufactured using the modified instrument and validated cleaning protocol have met lot release criteria for prevention of microbial contamination and safe clinical use.

Discussion

The instrument modification and cleaning protocol described enable reliable manufacture of ALDH bright cell populations that are suitable for clinical trials. We have manufactured nineteen consecutive samples that meet all clinical release criteria in an on-going Phase 1 human trial.

Keywords

aldehyde dehydrogenase (ALDH), ALDH bright cells, cell therapy, clinical trial, flow cytometry, sterile cell sorting, umbilical cord blood.

Introduction

We are developing cell therapies in which populations of human stem and progenitor cells expressing high levels of the enzyme aldehyde dehydrogenase (ALDH) are infused into patients. These cell populations are prepared by sorting cells with low side-scatter (SSC^{lo}) and high ALDH ($ALDH^{br}$, for ALDH bright) activity. ALDH activity is measured in intact cells using the ALDESORT™ (Aldagen Inc., Durham, NC, USA) substrate for the enzyme. When reacted with this substrate, intact $ALDH^{br}$ SSC^{lo} cells exhibit a high green fluorescence signal, compared with a control sample in which the enzyme is inhibited [1]. Characteristic $ALDH^{br}$ SSC^{lo} cell populations sorted from human UC blood [1–4], peripheral blood [5] and BM [6] are highly enriched in many stem and progenitor cell activities and have considerable potential in cell therapy. We have initiated clinical trials using these cells and will present the details of these investigations elsewhere. In this paper, we describe changes in the air and fluid systems of the FACSAria® (BD Biosciences, San Jose, CA, USA) cell sorter that facilitate sorting of sterile and potent $ALDH^{br}$ SSC^{lo} cells for clinical use.

Although sorted cell populations have been safely infused into patients [7–11], reliably sterilizing the cell sorter in a time frame compatible with clinical operations remains a critical concern in cell processing [12,13],

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especially when a new type of instrument is used. Early in the development of our clinical methods, we encountered difficulty in efficiently and reliably sterilizing the FACSAria cell sorter with standard protocols using dilute bleach or ethanol solutions. The instrument showed persistent microbial contamination that was difficult to eliminate. Consequently, we modified the instrument as described here. These modifications have permitted us to sort cell populations routinely that meet our sterility and other release criteria for clinical trials.

Methods

Human UC blood cells (UCB) were obtained with informed consent using protocols approved by appropriate institutional review boards. Partially purified UCB were reacted with the ALDESORT substrate and washed according to the manufacturer's specifications. We also used apheresis products from G-CSF-mobilized PBSC from normal volunteers purchased from Cambrex Inc. (Walkersville, MD, USA) for some early development work. UCB samples were then treated with Ab-coated immunomagnetic beads to reduce cell numbers for cell sorting. Details of the protocols used for each type of cell population will be presented elsewhere in connection with the reports of the cell processing used for clinical trials. The strategy used to identify and sort $ALDH^{br}$ SSC^{lo} cells was basically identical to what has been described in detail elsewhere [1,6]. Briefly, signals from nucleated cells were gated on the basis of forward vs. side scatter, and a sort region containing $ALDH^{br}$ SSC^{lo} cells was identified on a green fluorescence vs. side scatter plot. We defined the sort region by comparison with a control sample in which ALDH activity was inhibited by diethylaminobenzaldehyde.

UCB was sorted on a FACSAria in a dedicated sorter room with positive pressure and HEPA-filtered air, at the Duke Pediatric Bone Marrow Transplant Program (Duke University Medical Center, Durham, NC, USA). Access to the room was controlled, and air-borne contaminants were regularly monitored. The facility met all local requirements for patient sample processing. The instrument was operated at 55 PSI sheath pressure; sorts were performed using the default 'Purity' mode. The instrument was equipped with a vacuum-based aerosol containment system provided as an option by the manufacturer for operator safety. The sorter operator used sterile gloves and universal precautions, including a surgical mask. Cells were sorted at 10 000 events/s $\pm$ 3000 into approximately 2 mL CellGro stem cell growth medium (CellGenix, Freiburg, Germany).

To develop a protocol for reliable sterile sorting, the air and fluid systems of the FACSAria were evaluated systematically. Sheath fluids were provided directly from closed, bagged sources. Fluidic pathway components were tested individually and subsequently modified to bypass or remove components likely to retain microbial contaminants, to allow better cleaning in place of others, and to simplify component replacement that must be done between patients in the clinical environment. Additional consideration was given to cost, ease of use and quality control. Tubing sets with traceable components and disposable filters were developed. Protocols were developed with ease of use in mind using these components for reliable sterile sort set-up.

The yield, purity, functionality and sterility of sorted cells prepared by each protocol were measured. Viability of sorted cells was determined by trypan blue exclusion, and hematopoietic stem cell activity was measured by colony-forming cell assays (CFC; Methocult H4434®, Stem Cell Technologies, Vancouver, Canada). Samples of the sheath stream and sorted cell samples were tested for microbial contamination using the clinical BacT/ALERT® (bioMérieux, Durham, NC, USA) automated detection system; each sample was tested using both the SA and SN culture bottles for detection of both aerobic and anaerobic bacterial and fungal contamination. Samples were also tested for mycoplasma contamination by polymerase chain reaction (PCR) by a commercial service (Clongen, Germantown, MD, USA). Endotoxin content was measured using the *Limulus* amebocyte lysate assay (Cambrex, Walkersville, MD, USA).

Results

The FACSAria has an automated fluidics system that provides fluid exchange and basic cleaning cycles with minimal operator manipulation. However, the fluidics system valves and plenum reservoirs are not completely flooded with cleaning chemicals during the built-in cleaning cycles. These components constitute potential niches for persistent microbial contamination, and we had intermittent failures in obtaining sterile sorts during our early development efforts. We tried several methods to clean the FACSAria 'in place' in order to give consistently sterile sorts without modifying the instrument. These included using the built-in 'aseptic sort set-up' and using