Actin Signatures: New Technologies for Assessment of Human Circulating Blood Cells

Brian B. Hashemi,
National Space Biomedical Research Institute

Duane L. Pierson, *Johnson Space Center*
Mark Ott, *Johnson Space Center*

Studies of human immune cells in tissue culture have demonstrated dramatic alterations in the responsiveness of cells during spaceflight and in ground-based models. Atopic and autoimmune diseases may occur due to long-term spaceflight effects on immune-regulatory pathways or specific immune cells. This primary risk area is influenced by contributing risk factors such as radiation exposure, microgravity isolation, and stress, all of which have a powerful effect upon the cells of the immune system such as T-cells, Neutrophils, and Monocytes. The Actin Signature technology developed in this research provides a new surrogate marker of immune function that can allow for the monitoring of immune cells during a long-duration spaceflight.

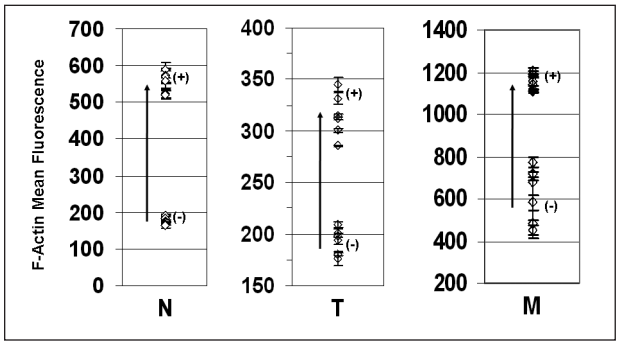

Fig. 1. F-actin measurements in whole blood samples.

The cell's cytoskeleton plays a crucial role in signal transduction and activation response of all human cells and is involved in many aspects of cellular function including signal transduction, orchestration of mechanical forces, and functional response of cells. In vitro spaceflight experiments with human immune cells have demonstrated that cell cytoskeletal function can be significantly inhibited in microgravity culture. However, no rapid technologies are available for accurately measuring the adaptation and functional response of immune cells in whole blood samples. The technologies developed in this research use individual circulating blood cells as biosensors reporting on the adaptation and response of immune cells to spaceflight, and they provide a new surrogate biomarker for monitoring the adaptation and response of human circulating white blood cells.

The actin cytoskeleton system forms a key structural and functional element of every human cell. In the past, measurements of F-actin in circulating immune cells were limited due to: 1) the need to isolate cells from human blood samples prior to analysis requiring a large number of manipulations; and 2) the variability in measurements caused by the sensitivity of the cytoskeleton to these manipulations. An important achievement of this investigation is the development of new methods for controlling data variability and accurate measurement of F-actin in whole blood samples without the need for purification of cells. This approach enables simultaneous analysis of F-actin responses of multiple cell types (e.g., T-lymphocytes, Neutrophils, and Monocytes) in a small amount of donor blood sample.

Data obtained from a large number of test subjects demonstrate the utility of this technology for the simultaneous measurement of F-actin levels in multiple cell types using as little as 90 µl of blood sample. Moreover, the activation response of each cell type can be quantified using stimulatory molecules that specifically activate each cell type through a specific surface receptor (figure 1). Through this query mechanism, each cell type is evaluated based on six parameters that represent the status of the actin cytoskeleton, and other indexes representing the cell's size and granularity, as well as their responses to activation. Data collected from multiple cell types in a single blood sample are then compiled to form the information-rich Actin Signature of the donor representing the collective status of his/her circulating white blood cells.

The Actin Signatures developed in this research are a surrogate marker of immune function that allows rapid monitoring of immune cells using a small amount of donor blood sample. We applied this technology to a new in vitro model of circulating blood cells developed in the NASA-developed High Aspect Ratio Vessel (HARV) bioreactors (figure 2). This new whole blood tissue culture model enables the culture of human circulating white blood cells for extended periods of time up to 96 hours without isolation of cells from blood samples. We used this model to evaluate the use of Actin Signatures for assessing the adaptation and response of immune cells to

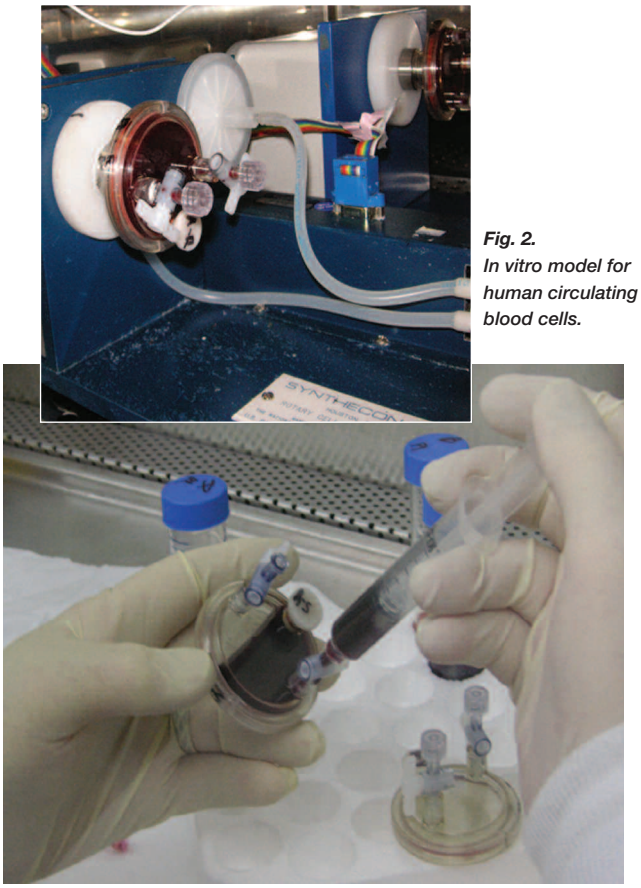

Fig. 2.
In vitro model for human circulating blood cells.

the HARV bioreactor. Other applications of this whole blood in vitro model include new approaches for assessing bacterial and viral infections in human blood samples using HARV bioreactors.

The utility of this technology was demonstrated with whole blood bacterial infections in tissue culture. Figure 3 represents the Actin Signature of a human blood sample infected with the Salmonella bacterium. Of the 18 signature parameters representing three cell types, six parameters were not responsive to Salmonella during the 90-minute infection time course, and three parameters are very responsive to the infection. Actin Signatures from a number of studies demonstrate unique responses in parameters that represent the response of white blood cells to various types of infections and environmental factors.

This technology is providing new tools for monitoring immune cells and their functional responses in whole blood samples. The use of Actin Signatures on long-duration spaceflight missions can provide early detection of cellular damage in flight crews prior to clinical manifestations. Earth-based applications of this technology include new diagnostic tools for the clinical assessment of human immune cells and monitoring of disease evolution in patients. Two domestic and one international patents are pending for Actin Signatures and their clinical applications.

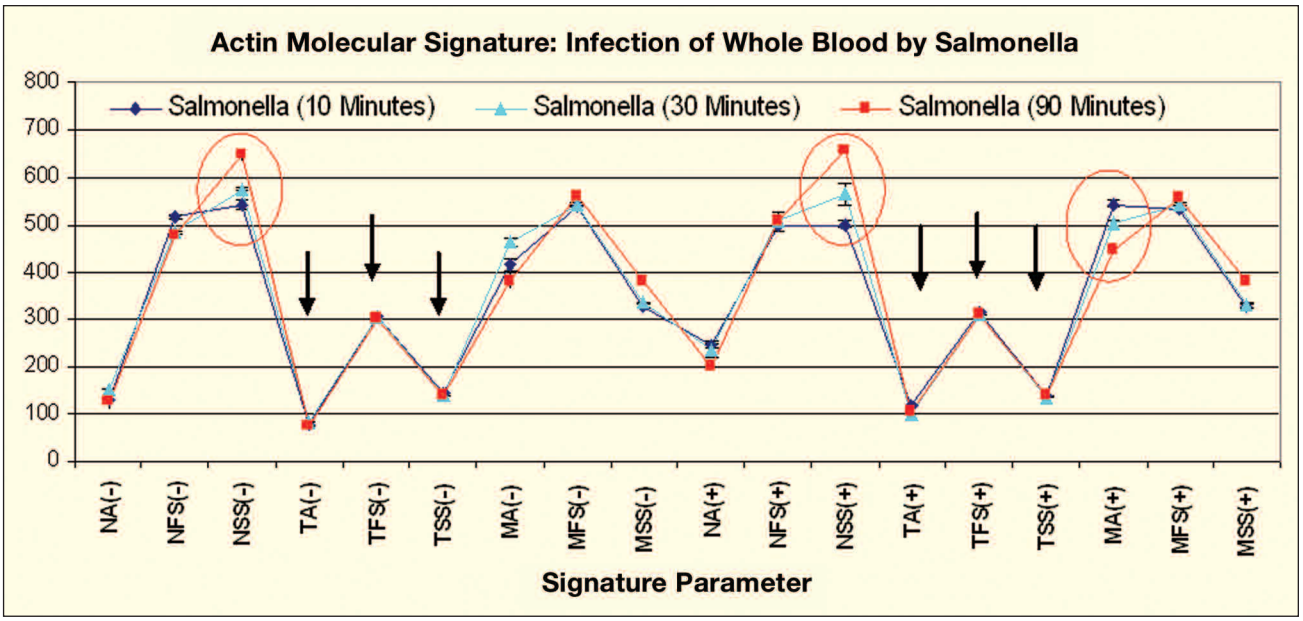

Fig. 3. Actin Signatures for Salmonella infection time course.