Phase 2 Project Summary

Firm: CFD Research Corporation
Contract Number: NNX09CB76C
Project Title: Non-Destructive Detection and Separation of Radiation Damaged Cells in Miniaturized, Inexpensive Device

Identification and Significance of Innovation:
NASA has identified a need for rapid, efficient and non-destructive detection and isolation of radiation damaged cells. Classical identification methods (hybridization) consume the sample, limiting downstream analyses. Conventional flow cytometers, while very fast and powerful, have several disadvantages in sorting radiation damaged cells. The cytometer action ("jet-in-air") can by itself induce cellular damage. Furthermore, cytometers are complex, bulky, expensive systems limiting access to core facilities. There is currently no device/methodology for rapid, gentle and non-destructive selection and screening of radiation damaged cells deployable in a research lab and later in space.

Technical Objectives and Work Plan:
In response to this challenge, and in collaboration with radiation biologists at Temple University, we propose to develop a simple and low-cost, miniaturized device for automated, non-destructive detection and sorting of radiation damaged cells. Our approach features several novel elements. First, novel cell surface markers (indicative of DNA damage) are utilized for identification/tagging of radiation damage. Next, dielectrophoretic (DEP) forces are exploited to continuously separate tagged cells, by the application of merely a modest voltage signal. Finally, a simple (non-optical) and robust electrical means of cell counting is implemented to provide a quantitative measure of damaged cell population. Specific Phase 2 objectives were (from Phase 2 proposal):
(1) Identify surface markers associated with radiation-induced damage. Endothelial cells will be irradiated (dose of 1 cGy to 10 Gy) followed by gene expression studies to identify characteristic expression levels and patterns. Specific, sensitive and robust surface biomarkers will be downselected and verified using fluorescent antibody assays. Effort on this task was led by Temple University.
(2) Demonstrate automated separation and counting of radiation-damaged cells using DEP-based cell sorter and impedance based cell counter. Effort on this task was led by CFD Research Corporation.

Technical Accomplishments:
Building on the Phase I study, we successfully identified and validated surface biomarkers of radiation damage using a combination of wholoe genome studies, RT-PCR, FACS and comet assays. Primary endothelial cells (HUVECs) and an endothelial cell line (HMEC-1) were used as candidates for evaluating biomarkers of low dose radiation damage. Both microparticle and nanoparticle based decoration of targeted cells were optimized for use in the DEP sorter. Computational Fluid Dynamics (CFD) based modeling was used to adapt the DEP sorter design followed by fabrication using lithography process. DEP spectra of particles and endothelial cells in buffers were characterized to yield the optimal buffer for use in the DEP sorting experiments. The fabricated prototype was used to demonstrate sorting of particle decorated and native endothelial cells. Finally, proof of concept of cell counting was demonstrated using an impedance based methodology.

NASA Application(s):
The end product of the proposed effort will be a first-of-its-kind, commercially available, compact, low-cost, integrated device for sorting of radiation damaged cells. This device will greatly aid in NASA's efforts to minimize radiation hazard, and develop countermeasures, enabled by fundamental understanding of radiation biological effects at the molecular and cellular level. The device will be of direct use to NASA's ground-based research facilities and amenable for space deployment as well (in-situ gene expression studies). In addition, the technology can benefit research efforts focused on other space-induced biological phenomena such as bone loss, immune modulation, and oxidative stress among others.

Non-NASA Commercial Application(s):
It is also expected that the developed technology will find ready applications in the following civilian markets: (a) Pharmaceutical and Drug Discovery Companies, (b) Pre-clinical and Clinical Researchers (in

particular stem cell and oncology researchers), and (c) Hospital & Health Site Monitoring (for nuclear medicine, immune ex-vivo treatments).

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