THE INFLUENCE OF MICROGRAVITY ON ASTRONAUT HEALTH: GLOBAL STUDY OF MICROGRAVITY EFFECTS ON HUMAN STEM CELLS. E. Blaber[1,2], H. Marcal[3], L.J.R. Foster[2], and B.P. Burns[1,2] [1]*Australian Centre for Astrobiology,* [2]*School of Biotechnology and Biomolecular Sciences, and* [3]*The Graduate School of Biomedical Engineering, The University of New South Wales, Sydney, NSW, Australia. email: brendan.burns@unsw.edu.au*

Background: The hostile environment of space is known to have an impact on human physiology, and astronauts are faced with several health risks during both short and long duration spaceflight. Some of these health problems include bone loss, muscle atrophy, cardiac dysrhythmias, and altered orientation. This year marks the 40th anniversary of our first steps on the moon, and within decades it is hoped that human kind will have established a settlement on Mars. The bioastronautics field has emerged in life sciences to ensure the health and safety of humans during space travel by addressing the medical issues encountered. As humans advance to exploratory planetary missions, their ability to survive is dependent on the ability to adapt to and/or counteract the hostile environment of space which is known to have an impact on human physiology. One of the major issues encountered in space is the absence of gravity. Although a wide range of research has been conducted into the systemic effects of microgravity on human physiology, an area that has received insufficient attention is the effect of microgravity on cellular function. To gain a comprehensive understanding of these issues, the effects microgravity has on the human body at a cellular level need to be analysed as life's basic processes are conducted at this stage.

Aims: The aim of the present study was to employ a global approach to examine the effect of microgravity on a stem cell line. These are the cells that all other cells emanate from and ultimately regenerate organs in the human body, and thus an ideal platform to begin to understand the effects of microgravity at the cellular level.

Methods: A NASA rotating-wall vessel (RWV) bioreactor was used to simulate microgravity (SMG) conditions. The RWV bioreactor is a NASA-designed tissue culture vessel which is used to simulate microgravity whilst reducing the shear and turbulence that is associated with impeller driven and stirred bioreactors. The system is based on two important design principles: solid body rotation about a horizontal axis, and oxygenation by active or passive diffusion through a silicone rubber membrane. By incorporating the design principles of solid body rotation and membrane oxygenation, the RWV is an extremely low shear, low turbulence, microgravity environment, thus allowing for the culture of high density three-dimensional mammalian cells. The effect of microgravity was analysed on a human embryonic stem cell line (NCCIT) that has the potential to differentiate into all three germ cell lineages. This study utilised an expression proteomic approach (2D gel electrophoresis and Fourier-Transform mass spectrometry) to generate a comparative expression profile of cells grown under SMG conditions. Additionally, scanning electron microscopy (SEM) was used to analyse the morphological adaptations of cells to SMG conditions and control conditions.

Results: Generation of 2D gel protein maps enabled the visualisation of a vastly different proteomic profile of NCCIT cells grown under SMG compared to control samples. Additionally, results demonstrated that 64% of proteins identified from cells grown in simulated microgravity were not found in control samples. Isolated proteins were categorised based on their sub-cellular location and then organised into 17 functional categories. These included proteins involved in cell regulation and signal transduction. Specifically this study found the presence of a particular protein involved in the degradation of bone under SMG conditions as well as several proteins known to effect intracellular calcium levels. Additionally several proteins identified in SMG were found to be associated with cell motility and cell division as well as impacting physiological systems such as the immune system and the musculoskeletal system. Proteins with antioxidant functions were found to be decreased in SMG, thus indicating that microgravity imposes oxidative stress on these cells. As the functional units of a cell are proteins, these results suggest that microgravity has a significant impact on cell function.

Conclusions: To the best of our knowledge, this is the first study conducted that has investigated the effect of SMG on an embryonic stem cell line and demonstrated a significant alteration in human cell function as a result of growth in microgravity conditions. Specific proteins were identified and linked to pathways that are affected by microgravity, and these proteins can then be tentatively correlated to observed health problems encountered in microgravity. This research is critical to both space science and cell biology due to its vast potential to decipher the effect of microgravity on humans at a cellular level as well as regeneration of tissues in the human body, and to use these alterations to solve ground based medical problems. These preliminary findings points to potential detrimental effects to the health of astronauts and therefore may also have detrimental effects on the success of a mission.