

PROTEOMIC RETRIEVAL FROM NUCLEIC ACID DEPLETED SPACE-FLOWN HUMAN CELLS

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Cells grown in space or in any hostile environment with a necessity for preservation are subject to decomposition and loss of valuable scientific information. To maximize the science return from flight samples, an optimized method was developed to recover protein from samples that had been stored for long periods of time at refrigerated temperatures. This technique also allows multiple analyses on a single cellular sample when frozen. Our group specifically designed this for use with samples from the International Space Station (ISS) (Love, et al., 2003) at the same time that a different technique was established by others (Rodrigo, et al., 2002). This work extends our initial work, using additional antibodies and cell lines, as well as corroborating with histological staining.

Cell cultures were grown in American Fluoroseal bags (TCM) either on the ISS or in flight hardware on the ground, and treated at regular intervals with an RNA stabilizing agent (RNAlater®, Ambion, Austin, TX) or a formalin solution. The samples were refrigerated for 3 months. RNA was purified using an RNeasy® kit (Qiagen) and the remaining RNA free supernatant was precipitated with 5% trichloroacetic acid. The precipitate was dissolved in SDS running buffer and tested for protein content using a bicinchoninic acid assay (Sigma, Dallas). Equal loads of protein were placed on SDS-PAGE gels and transferred using Western Blotting techniques (Towbin, et al., 1979). Protein on the blots from preserved cells were analyzed using horseradish peroxidase antibodies, with an ECL+ fluorescent stain (Amersham, Piscataway, NJ). The Storm imager and Imagequant software (Amersham) quantified the pixel volumes for rectangles of equal size. Human renal cortical epithelial (HRCE) cells (Cubano, et al., 2004; Cowger, et al., 2002) grown onboard the ISS during Increment 3 and in ground control cultures exhibited similar immunoreactivity profiles for antibodies to the Vitamin D receptor (VDR) (Fig 1), the beta isoform of protein kinase C (PKCβ) (Fig 2), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Fig 3). The ground (Grd) and flight (Flt) samples are presented on the graphs using an untreated control to normalize the data. A

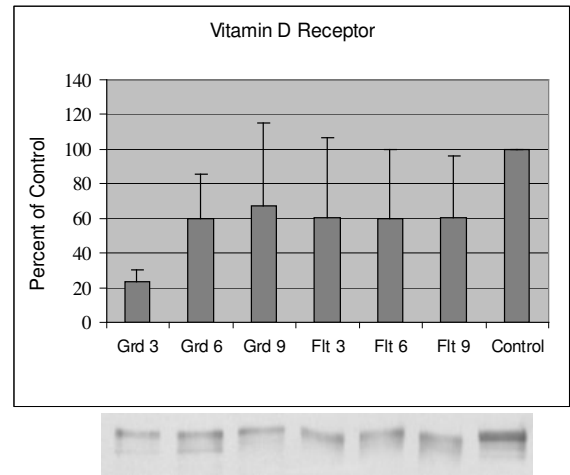


Figure 1 - Vitamin D Receptor (VDR) antibody stained blots, prepared with equal loading of protein (5 µg/lane), measured a protein with a molecular weight of 60-64kD. Samples are from ISS Flt samples from days 3, 6 and 9 and the Grd matched controls from the same days. An example of one of the blots is shown below the graph, which is an average of four blots.

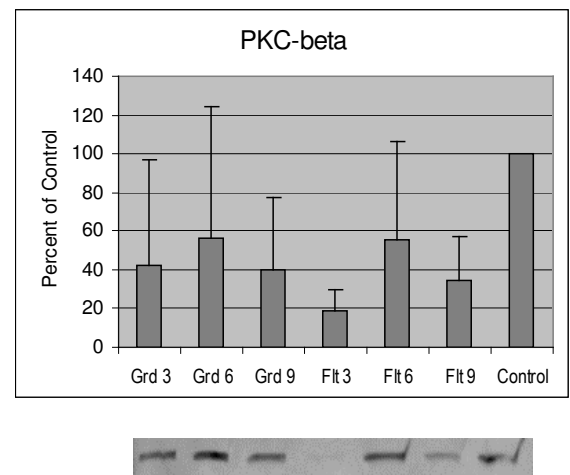


Figure 2 - Protein Kinase CβII (PKCβII) antibody stained blot, prepared with 6 µg/lane of protein, measured a protein with a molecular weight of 80 kD. The graph is an average of four blots with an example below the graph.

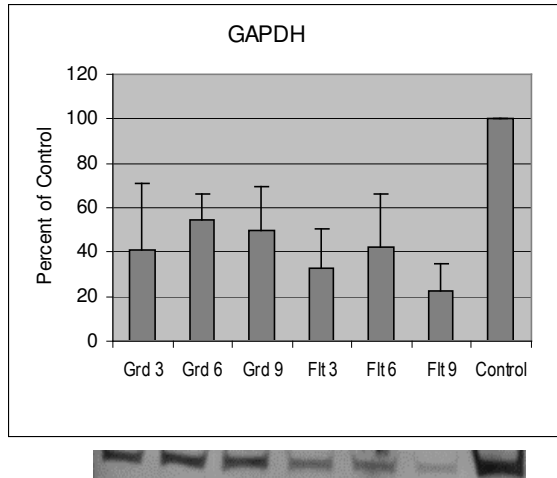


Figure 3 - Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody stained blot, prepared with 6 µg/lane of protein per lane, measured a protein with a molecular weight of 42-44 kD. The graph is the average of three blots with an example below the graph.

Student's t-test was used to compare the ground and flight samples at each time point, days 3, 6 and 9. There was no significant difference between Flt and Grd samples at any time point ($p > 0.05$).

Parallel immunohistochemical studies on formalin-fixed flight and ground cultures also showed positive immunostaining for VDR (Fig 4) as well as other biomarkers (not shown here). These results are consistent with data from antigenic recovery experiments performed on human mixed Müllerian tumor cells cultured in microgravity (Hammond, et al., 2005) as well as data from cells grown using different culture methods (Cubano, et al., 2004). Although on a protein per protein basis, the preserved cells demonstrated slightly less antigenic protein than untreated cells (control), there was a high percentage of recovery of antigenic protein using three different antibodies in both the ground and flight samples.

These studies demonstrate that quantitative proteomic information can be acquired from samples stored in less than optimum circumstances, such as long term storage at refrigerated temperatures with different cell lines, using multiple antibodies. Since space flight experiments are often executed under resource constraints, this is a valuable addition to our knowledge of how to best utilize our space samples and to other work done in extreme environments such as in undeveloped countries where biospecimens are kept in suboptimal conditions.

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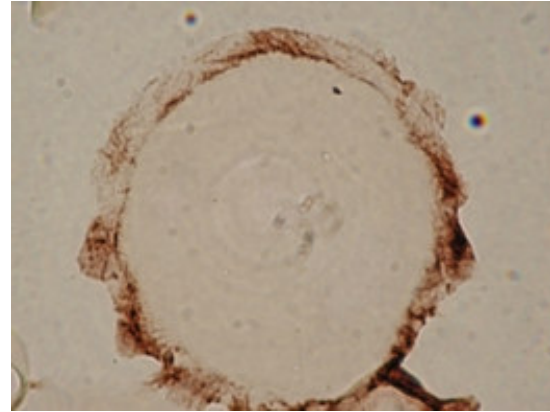


Figure 4 - Light photomicrograph (600x) of Vitamin D receptor immunoreactivity in sectioned formalin fixed HRCE cells cultured on Cytodex-3 microcarrier beads in microgravity for 12 days during ISS Increment 4.

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