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Laboratory Simulations of Simultaneous Reduced Gravity and Ionizing Radiation Environments

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***Abstract*— A novel system has been developed to simulate the combined effects of reduced gravity and ionizing radiation present during spaceflight on biological and particulate samples. The miniature rotary cell culture system (mRCCS) was designed to synchronously rotate up to five independent vessels containing particulate samples suspended in fluid media, constructed using radiation tolerant, biocompatible, and vacuum compatible materials. Reduced gravity conditions were achieved when particles (*e.g.*, 200 μm polystyrene microcarrier beads with or without adhered cell clusters) were suspended inside the vessels moving near terminal velocity in viscous fluid media with densities matched to the suspended particles to achieve neutral buoyancy and minimal effective gravity. Variations in centripetal acceleration from slow rotation of the vessels limited reduced gravity environments from ~$2{\cdot}10^{-2}$ to <$1{\cdot}10^{-5}$ g, comparable to similar commercially available systems. The effective gravitational acceleration experienced by the suspended particles was calibrated by tracking of particles within the mRCCS systems vessels. The entire mRCCS apparatus can be used in a standalone configuration for independent reduced gravity simulations or can be introduced into the Utah State University's Space Survivability Test (SST) chamber for radiation exposure or simultaneous radiation exposure under reduced gravity. The SST chamber has a ~90 mCi ^{90}Sr source that emits 0.2 to 2.5 MeV β radiation. The combined mRCCS and SST chamber system can provide average effective dose rates for the suspended particles, controlled over a broad range (>900X) from ~3.7 mGy/day to 3.4 Gy/day by varying the source-to-sample distance and using varying slit width graphite shields. This system can provide stable, simultaneous space-like radiation and reduced gravity environments for experiments conducted on timescales of minutes to months.**

I. Introduction

One of the major challenges faced by astronauts during space missions is tackling the harsh space environment.[1] There are many factors that can affect their health, but two of the most serious are caused by simultaneous exposure to reduced gravity and increased levels of ionizing radiation.[2,3] On a cellular level, microgravity damages cellular DNA by increasing the production of harmful reactive oxygen species, while ionizing radiation damages DNA by creating double-stranded DNA (dsDNA) breaks.[4-6]

In vitro testing to model synchronous effects is critical, but it can be complex and expensive.[7,8] Therefore, to overcome these issues in testing the Materials Physics Group (MPG) at Utah State University (USU) designed a custom mini-rotary cell culture system (mRCCS) that can simulate reduced gravity in a vacuum laboratory environment and can be used either in a standalone configuration or in combination with an ionizing radiation source.

II. Environment Simulation Methods

A. Simulation of Reduced Gravity

Production of simulated reduced gravity is based on concepts used in the Synthecon rotary cell culture system, which is a standard cell culture system used by NASA.[9,10] The mRCCS was designed to accommodate up to five independent polycarbonate vessels that are synchronously rotated by a variable speed motor-driven chain. These vessels contain particulate samples that are suspended in fluid media, and were constructed using radiation tolerant, vacuum compatible materials, and biocompatible, and is autoclavable (see Fig. 1).

Reduced gravity conditions were achieved when suspended particles (*e.g.*, 200 μm polystyrene microcarrier beads with or without adhered cell clusters)[10,11] inside the vessels moved near terminal velocity at almost constant radii in viscous fluid media with densities matched to the suspended particles to achieve neutral buoyancy. As described by Stokes' law[12] suspended particles reach terminal settling velocity,

$$v_s = [2g\,(\rho_c - \rho)\,(R_{bead})^2] \,/\, 9\mu \qquad (1)$$

as they fall with near zero net forces from gravity, buoyancy, and viscous drag (see Fig. 2); here ρ_c is the density of the cell cluster, ρ is the density of the culture media, R_{bead} is the radius of the cell cluster on microcarrier beads, and μ is the dynamic viscosity of the culture media fluid.

The effective gravitational acceleration applied to particles was calibrated through particle tracking measurements of suspended particles within the mRCCS system vessels at various rotation speeds (see Fig. 3). Rotation calibration of the instrument was accomplished using different sized suspended particle clusters in one of the vessels completely filled with different density and viscosity liquids without any bubbles (Table 1). Microbeads of size ~200 μm were placed inside the vessel which was sealed using a 10 μm thick transparent membrane. The vessel was rotated from 1 rpm to 55 rpm and

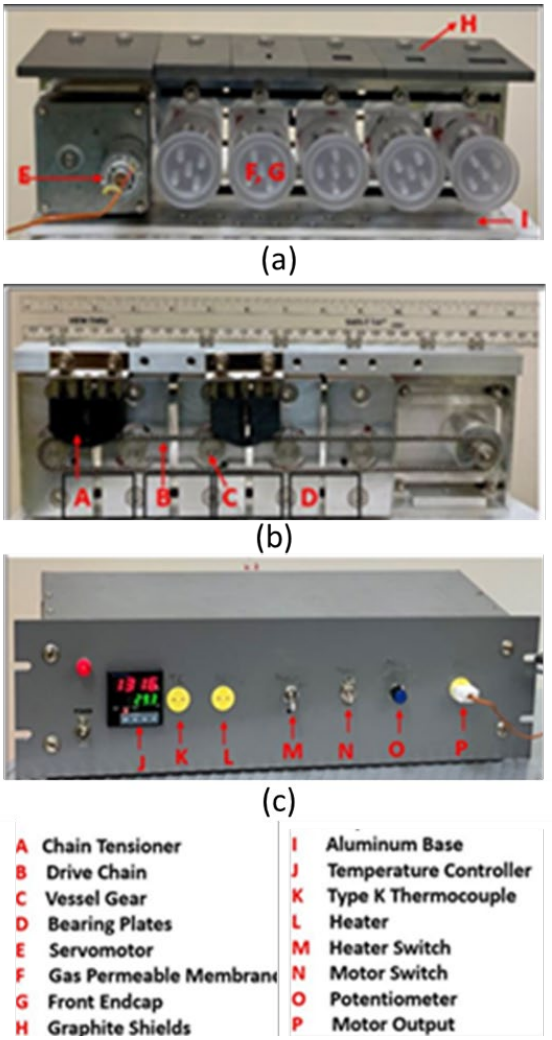

Fig. 1. Mini-RCCS system (mRCCS). (a) Front view. (b) Rear view with variable slit widths of graphite plates. (c) mRCCS controller.

the rotation of the beads was recorded at 30 frames per second using a high-definition camera with a macro lens. The tracking and data analysis of these microbeads were performed using Pasco Capstone™ software.[13]

A measure of the effective gravity experienced in the mRCCS is the difference in the low radial centripetal acceleration applied to cell clusters within the vessel. Radial acceleration characterizes variations in effective gravity experienced when microcarrier beads were at the top or bottom of the rotation with centripetal acceleration anti-parallel or parallel to the gravity vector. Radial acceleration or

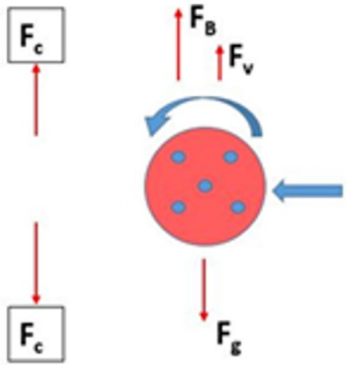

Fig. 2 Free body diagram of balanced external forces of buoyancy, F_B, and gravity, F_g, from Stokes' law and centripetal forces, F_c.

effective gravitational acceleration in units of g is calculated using,

$$a_{eff} = (1.12 \cdot 10^{-3})\, \bar{R}_{particle} \left({}^{\omega_{vessel}}/_{1000}\right)^2 \quad (2)$$

where $\bar{R}_{particle}$ is the mean radius of the suspended particles in mm and ω_{vessel} is the angular velocity of the rotary system in rpm. Figure 4 shows the radius of a typical microcarrier bead cluster as a function of time over more than 4 revolutions of the vessel. A sine function fit to the data has an amplitude 0.022 mm about a mean radius of 14.62 mm with an angular velocity of 126 rpm, equivalent to a residual acceleration of ±(0.39±0.06) μg (see Table 1).

B. Simulation of Radiation Exposure

The entirety of the apparatus can be used in a standalone configuration for separate microgravity experiments or can be introduced into the USU Space Survivability Test (SST) chamber for radiation exposure (see Fig. 5).[14,15] The SST chamber has a ~90 mCi ^{90}Sr source that emits 0.2 to 2.5 MeV β radiation, with an average penetration depth in water of ~5 mm.[16] This high vacuum system is particularly well suited for cost effective tests of multiple small-scale components or materials samples over prolonged exposure to simulate space environmental components. The SST chamber simulates several critical characteristics of the space environment: electron flux, VUV/UV/Vis/NIR photon flux, beta and VUV ionizing radiation, temperature, and neutral gas environments. Further details of the SST chamber and the energy ranges of SST electron and ionizing radiation sources are provided in Refs. 14 and 15.

Table 1 Rotation speeds and accelerations of particles suspended in different density liquids.

Fluid	Density (g/cm^3)	Angular Speed (rpm)	Average Radius (mm)	Radius Amplitude (mm)	Average Centripetal Acceleration (μg)	Residual Acceleration (μg)
Isopropyl Alcohol	0.79±0.01	45.7±0.3	3.17±0.02	0.24±0.02	56±1	±0.56±0.06
Water	0.997±0.003	41.5±0.3	5.50±0.02	0.34±0.02	98±2	±0.66±0.06
Cell Media	0.98±0.02	41.3±0.3	3.12±0.02	0.35±0.02	55±1	±0.67±0.06
Salt Water	1.18±0.02	42.2±0.3	11.50±0.02	0.01±0.02	205±3	±0.03±0.06

Fig 3 Microcarrier bead cluster tracking inside the media using Pasco Capstone™ software.

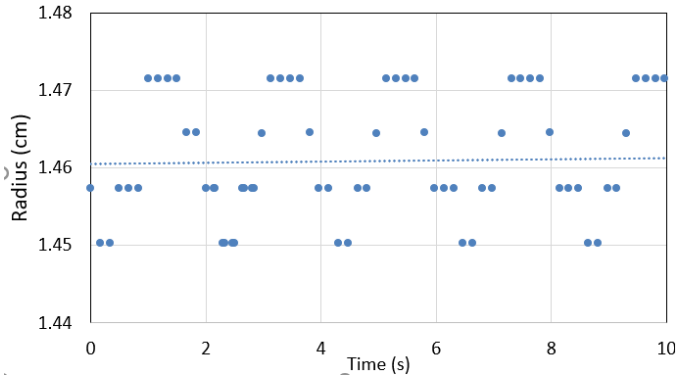

Fig 4 Radius of typical microcarrier bead cluster as a function of time over more than 4 revolutions. Fit to the data is a standard sine function.

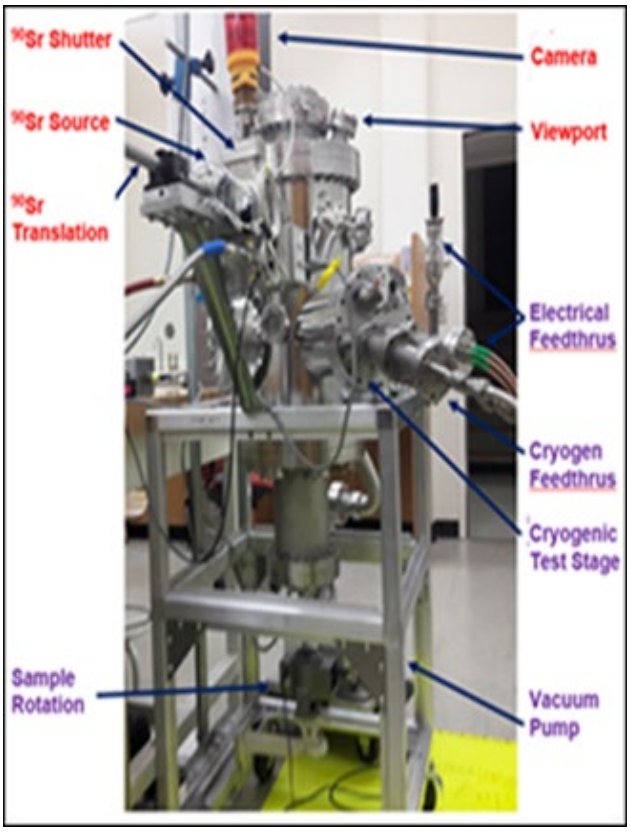

Fig.5. Space Survivability Test Chamber

III. Results and Discussion

The operational range of rotation speeds for microbeads in viscous fluid media with densities ~1 g/cm^3 was determined to be from 6 rpm to 42 rpm, while maintaining near circular trajectories within the mRCCS vessels (see Fig. 3). Variations in centripetal acceleration from slow rotation of the vessels limited reduced gravity environments from ~$1\cdot10^{-5}$ g to ~$2\cdot10^{-2}$ g, which is comparable to similar commercially available systems.[9,10,11] The effective gravity experienced by suspended particles in the mRCCS, measured as the range of the low radial centripetal accelerations when microcarrier beads were at the top or bottom of the vessel rotation, was <$1\cdot10^{-5}$ g. Further analysis of rotation calibration needs to be done for different sized suspended particles with varying drag coefficients and liquids of various densities and viscosities to gauge their effects on the simulated reduced gravity.

The combined mRCCS and SST chamber system can provide average effective β radiation dose rates for the suspended particles from ~3.7 mGy/day to 3.4 Gy/day, controlled over a broad range (>900X) by varying the source-to-sample distance and using graphite shields with varying slit widths.[17] The range of achievable total dose equivalents of ~10 mSv to >3 Sv, using the mRCCS in the SST chamber for practical experiment durations, spans a range of exposures typical of manned spaceflight missions, including >10-yr ISS, lunar and martian missions and NASA astronaut career limits.[11] It also allows relatively low exposure rates comparable to those from solar wind and galactic cosmic background radiation in interplanetary space.

IV. Conclusions

The two main objectives of this project were realized. The first one was to develop a novel, versatile, cost-effective, open-source system to model cellular damage from exposure to reduced gravity conditions and ionizing radiation present during long-term spaceflight. This system described here can provide stable, simultaneous space-like radiation and reduced gravity environments for experiments conducted on timescales of minutes to months at significantly less costs than other available systems.

The second objective was to simultaneous model reduced gravity and ionizing radiation in an *in vitro* space environment. Previous studies have shown that most research groups have applied ionizing radiation and microgravity simulation asynchronously or individually.[10,18,19] However, asynchronous modeling does not fully replicate the long-term effects of space environment.[20,21,22] The mini-RCCS can be used to simulate microgravity at the same level as commercially available systems alone or the combined effects

of microgravity and ionizing radiation to better understand the pathways leading to cellular damage.

Initial experiments have focused on understanding cellular damage due to the effects of radiation and reduced gravity on cardio and neurological cell clusters. Experiments are in progress to study C2C12 mice muscle cells grown on the microcarrier beads.[10] These experiments are designed to evaluate the impact of reduced gravity and ionizing radiation on these mice muscle cells both synchronously and asynchronously, with a long-term goal of evaluating biological reagents and chemical methods to mitigate the effects.

However, an environment where long-term gravitational forces can be minimized also allows for study of questions related more directly to spacecraft charging. These might include the study of electrostatic interactions between particles with very small internal charges (induced perhaps from electron or photo yields), in experiments similar to the seminal Millikan oil drop experiment.

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