Analyzing Fluid Shear Stress in the RCCS: Applications for 3D Cell Culture in Simulated Microgravity

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Abstract

In the decades since humans first went to space, there has been growing interest in utilizing microgravity to improve our understanding in many scientific fields (1). Applied fields have included evolutionary development, cell mechanics and protein crystallization (2-4). Microgravity offers an unprecedented opportunity for the isolation of gravity as an experimental variable to elucidate cellular pathways, behaviors and attributes otherwise inaccessible that might be applied to benefit areas such as drug development, disease studies and tissue engineering. Among the most notable observations, in terms of impact on human health, is that expressions of metastatic markers were reduced in several kinds of cancer cell lines upon exposure to microgravity (5, 6). However, it is not yet known if the overall metastatic/cancerous potential of these cells in vivo is decreased. Understanding the mechanisms/pathways behind such changes could lead to discovery of new therapeutic signaling pathways and their potential targets for delaying or preventing cancer metastasis.

The Rotary Cell Culture System (RCCS) developed by NASA and produced by Synthecon™, shown in Figure 1 (7), is one of the available means of simulating microgravity on Earth. The horizontally-oriented high aspect ratio vessels (HARVs) create a low-shear stress environment for three-dimensional (3D) culture of cells, producing cell aggregates called spheroids (8). 3D cell culture is an increasingly prominent tool for tumor biology, among other fields, as spheroids are commonly used as tumor models for breast cancer, among others (9), and are specifically a crucial part of transcoelomic metastasis in ovarian cancer (10).

The forces acting on cells/spheroids within HARVs have been analyzed mathematically before but most studies focused on specific assumed parameters, typically rotation speed and aggregate size (11). The Computational Fluid Dynamics (CFD) module and Particle Tracing module of COMSOL® Multiphysics can be used to analyze the shear stresses produced in HARVs and what is experienced by spheroids. A simulation of fluid velocity and shear stress for a HARV is shown in Figure 2. When accounting for spheroids, different cells form spheroids of varying sizes, resulting in ranging levels of shear stress. Examples of breast and ovarian cancer spheroids produced in the RCCS under identical rotating conditions are shown in Figure 3. Given the varied sizes of different spheroids, the shear stress can be adjusted by modifying the rotation speed and the viscosity of the culture media. This will help maintain the consistency of simulated microgravity experiments

across different cell types. Such adjustments would also be needed if the HARV's geometry is modified in a custom design for more specialized experiments such as adding sub-chambers, or when spheroid size is further altered in increasingly sophisticated models including co-culture.

Microgravity promotes spheroid formation, providing new experimental possibilities for a familiar model. COMSOL® can be used to optimize the parameters of the RCCS's environment and produce cancer spheroids for running biological assays for invasion, drug resistance, etc. Improvement of these studies for on-ground simulated microgravity experiments will also lead to improved design/reproducibility for space-based experiments on platforms such as the International Space Station (ISS).

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Figures used in the abstract

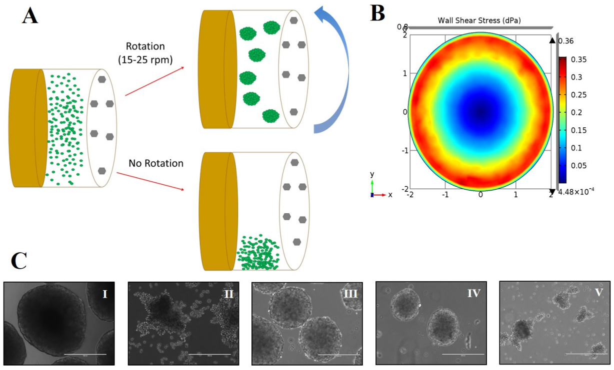

Figure 1: A) Experimental illustration. Cells are seeded in the HARV and rotated at 15-25 rpm for at least 72 hours to generate spheroids. Without rotation the cells pool at the bottom and fail to form spheroids. B) Simulation of shear stress in HARV at 15 rpm. C) Examples of spheroids produced in the RCCS. Breast cancer spheroids include MCF-7 (I) and MDA-MB-231 (II). Ovarian cancer spheroids include ES-2 (III), OVCA420 (IV) and OVCA433 (V). Scale bar: 400 μm.