Regulatory T Cell Activation in Simulated Microgravity Using a Multi-axis 3D printed Rotary Cell Culture System

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Introduction: Long term space exploration and commercialized space travel are becoming increasingly popular ideas as advancements in space technology make the journey safer for all, regardless of their occupation and training. However, recent observations show that over half of returning astronauts experience infections, colds, or the reactivation of dormant viruses within a week of returning to Earth[1]. Microgravity (μG) has been shown to impact immune cell function, specifically by dampening $CD4^+$ and $CD8^+$ T cell responses and in enhancing regulatory T cell (Treg) activation[2].

In order to study the stimuli driving these microgravity effects in greater detail, we need cell culture systems which allow us to mimic microgravity on earth. Researchers can use rotary cell culture systems (RCCS) to simulate μG. The current gold standard RCCS is a horizontally rotated cylinder with a coaxial oxygenator in the center. The cylinder is rotated clockwise until it reaches terminal velocity where hydrodynamic forces of shear, centrifugal force, and Coriolis force are counterbalanced by gravitational force[3]. These devices are expensive to purchase and are typically single axis devices that allow cells to move in a predetermined motion. Here, we show a novel 3D printed multi-axis RCCS to simulate μG while eliminating predetermined cellular motion, which is inferred to resemble cells within the human body in space. We are studying Treg activation within our simulated μG environment in the presence of different stimulatory agents to explore the effect of microgravity on Treg behavior.

Figure 1. CAD design of multi-axis RCCS.

Methods: We used CAD to design, and 3D print an RCCS consisting of three gimbal rings and a series of gears for 360° rotation. A 12V DC gearbox motor is attached to the largest ring as well as a speed controller which rotates the external ring at a specified angular velocity. This induces random rotation of the inner rings, promoting the counterbalance of gravitational force with shear forces, centrifugal force, and Coriolis force. The inner-most ring is designed with a clip to hold a 50ml falcon tube. Cells are grown within the 50ml falcon tube with an air permeable lid to allow for passive diffusion of O_2 and CO_2. Using this system, CD4+ cells are cultured and differentiated into FOXP3 Treg cells (using anti-CD3, anti-CD28, IL2) and then exposed to 1G or simulated μG within the multi-axis RCCS. Flow cytometry assays are performed to evaluate Treg function and assessment of CD25, CD69, STAT5 and cytokine secretion is evaluated.

Results: The forces acting upon the cells within the falcon tube in the RCCS are the normal force, gravitational force, and the fictitious forces (Coriolis and centrifugal). Therefore, $\boldsymbol{F_N + mg + F_{fict} = 0}$. Microgravity is achieved when $\boldsymbol{F_{fict} = -mg}$ causing $\boldsymbol{F_N \approx 0}$. $\boldsymbol{F_{fict}}$ is a combination of the Coriolis and centrifugal force, determined by $\boldsymbol{\vec{F}_{fict} = 2m\vec{r} \times \vec{\Omega} + m(\vec{\Omega} \times \vec{r}) \times \vec{\Omega}}$. Angular velocities of each rotational plane are optimized by solving the Euler's equations,

$$\textbf{[Euler's equations]} = \begin{aligned} \boldsymbol{\Gamma_1} &= \boldsymbol{\lambda_1\dot{\theta} - (\lambda_2 - \lambda_3)\phi\psi} \\ \boldsymbol{\Gamma_2} &= \boldsymbol{\lambda_2\dot{\phi} - (\lambda_3 - \lambda_1)\psi\theta} \\ \boldsymbol{\Gamma_3} &= \boldsymbol{\lambda_3\dot{\psi} - (\lambda_1 - \lambda_2)\theta\phi} \end{aligned}$$

where $\boldsymbol{\dot{\theta}}$, $\boldsymbol{\dot{\phi}}$, and $\boldsymbol{\dot{\psi}}$ are the angular velocities of rotation with respect to time of the x, y, and z plane respectively. A linear combination of velocities are substituted into $\boldsymbol{\vec{\Omega}}$ to solve for the $\boldsymbol{\dot{\theta}}$, $\boldsymbol{\dot{\phi}}$, and $\boldsymbol{\dot{\psi}}$ values necessary to minimize the difference between $\boldsymbol{F_{fict}}$ and **mg** to successfully achieve microgravity.

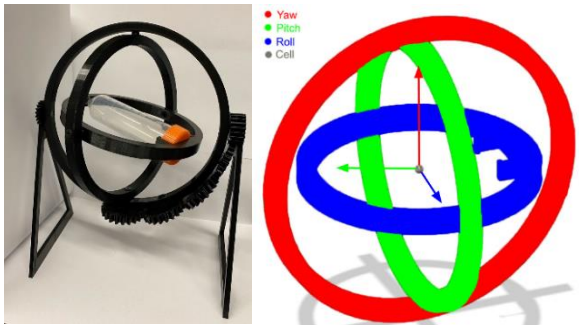

Figure 2. 3D printed/assembled RCCS and force vectors experienced by cells within cell culture system.

Conclusions: We have developed CAD designs for a low-cost, open-source multi-axis RCCS which can be 3D printed. This system is able to achieve μG when the angular velocity of all rotational planes are optimized to counterbalance gravitational force. The normal force acting upon the cells is minimized causing them to experience microgravity. We have used this system to explore the effect of microgravity on T cell function, and in particular, Treg differentiation, showing that Treg function is altered in microgravity.

References: [1] Rooney BV. Fro Microbiol. 2019; 10:16. [2] Spatz JM. Sci Rep. 2021; 11:11872. [3] Synthecon, Low shear Micro Gravity, White paper 267