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

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INTRODUCTION

- **Microgravity:** a reduction in gravity to 10^{-4}-10^{-6} g. Offers a unique opportunity to isolate gravity as an experimental variable. Areas of application include evolutionary development, protein crystallization, colloid studies, etc. Platforms for microgravity studies include the International Space Station (ISS), nanosatellites, etc.
- Preparation for these experiments often involves Earth-based simulated microgravity, including the Rotary Cell Culture System (RCCS), shown below [1].

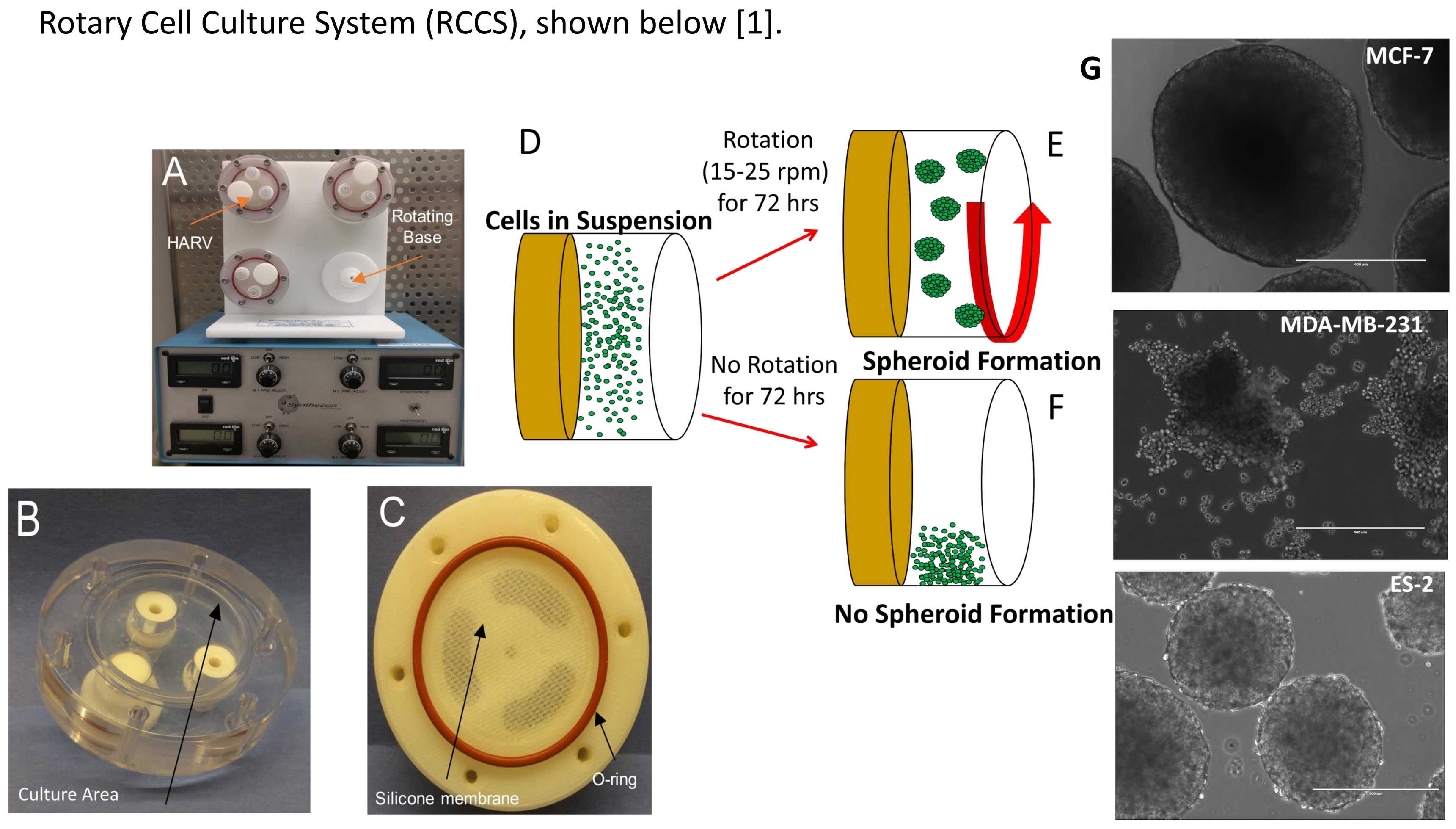

Figure 1. The RCCS and its schematic of operations. A) The main body of the RCCS with the controller (bottom box) and three High-Aspect Ratio Vessels (HARVs) attached to the rotating bases. B,C) The two main components of the high aspect-ration vessel (HARV), both the front with syringe, fill ports and culture area (B) and the back with a silicone membrane for oxygen exchange (C). D) A suspension of cells is place in the HARV and will either form spheroids over time if rotated (E) or the cells will pool at the bottom and not form spheroids if the rotation is absent (F). G) Examples of spheroids produced in the RCCS at a cell density of 1x10^5 cells/mL over 72 hrs with a ramped rotation starting at 15 rpm and increased by 5 rpm every 24 hrs up to 25 rpm. They includes breast (MCF-7, MDA-MB-231) and ovarian cancer cell lines (ES-2).

- Cells in suspension tend to form three-dimensional (3D) aggregates called spheroids. Microgravity promotes spheroid formation by removing sedimentation and allowing high degree of freedom in cell self-assembly. Spheroids are increasingly being adopted as tumor models in cancer studies.
- The RCCS simulates microgravity by creating a low-shear stress environment, mimicking the lack of sedimentation in actual microgravity. This low shear also falls within the range expected in physiological conditions (often <0.1-10 dPa) [2]. However, cell lines form a variety of different spheroid sizes, which could affect the experienced shear stress and the consistency/reliability of the experiments.
- Space-based experiments are long and expensive ventures; dependable simulated results are crucial.

***Experimental Challenge:** How can we ensure consistent, low shear stress across spheroids of various sizes?

***Proposed Solution:** Model fluid and particle dynamics using the COMSOL ® Multiphysics 5.3 Computational Fluid Dynamics (CFD) and Particle Tracing modules to show how adjusting rotational speed and media viscosity can improve experimental consistency.

Computational Methods

-Single Phase Flow (spf) studies under laminar conditions and based off the Navier-Stokes equation. The size of the HARV was constant so the variables were the rotating speed and the viscosity of the media. Rotation speeds for the RCCS are 15 and 25 rpm and the rotating domain was defined in the clockwise direction along the z-axis to mimic the horizontal orientation of HARVs on the RCCS.

-For Particle Tracing, *p*article sizes were chosen based on both our own experimental results and what has been previously published. The particle mass was calculated based on the volume of the spheroid and an assumed density of 1.04 g/cm^3 [6].

Equation 1

$$\rho(u \cdot \nabla)u = \nabla \cdot [-p\mathrm{I} + \mu(\nabla u + (\nabla u)^T)] + F + \rho g$$

$$\rho \nabla \cdot (u) = 0$$

Equation 2 [3]

$$WSS = \sqrt{\tau^2{}_{xy} + \tau^2{}_{yz} + \tau^2{}_{xz}}$$

Volume: 10 mL

Diameter: 4.5 cm

Height: 0.63 cm

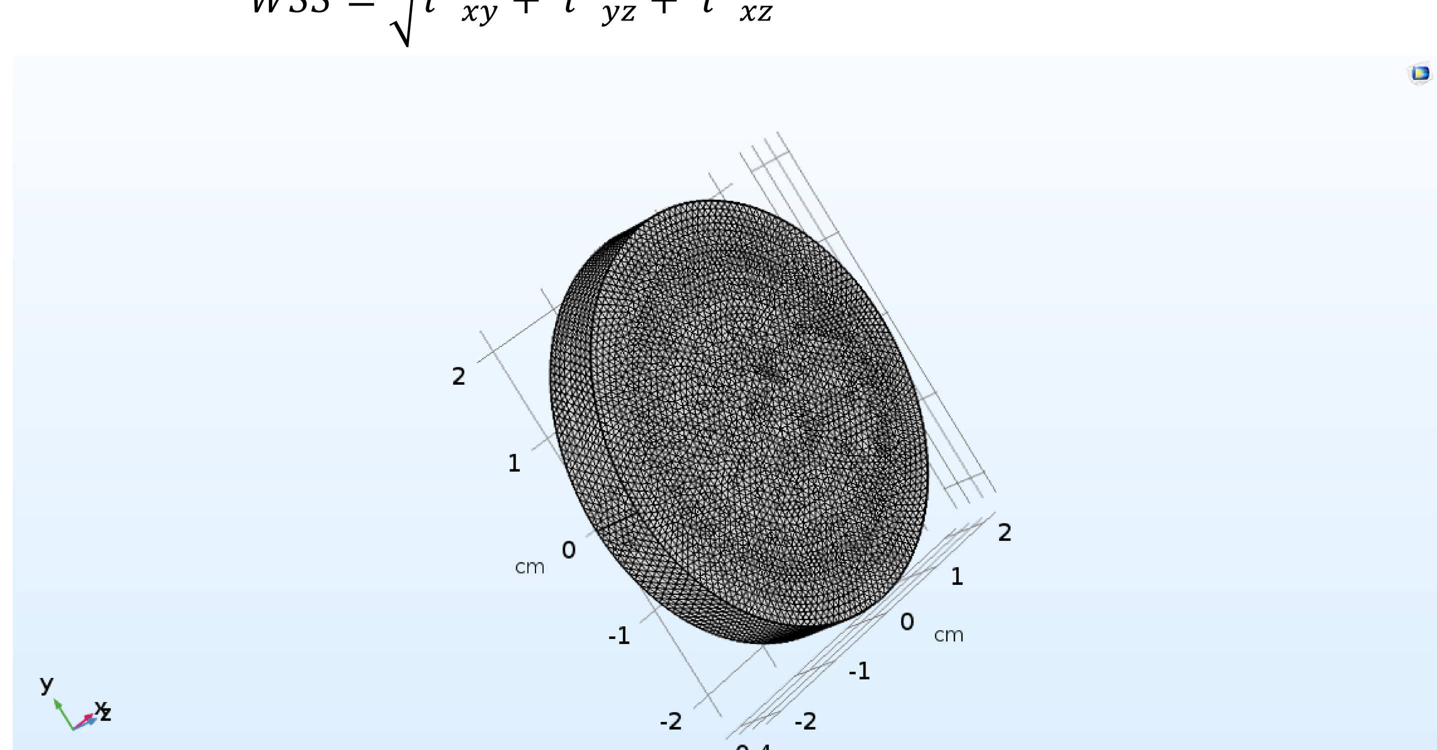

Figure 2. COMSOL drawing of HARV culture area with fine triangular meshing.

RESULTS

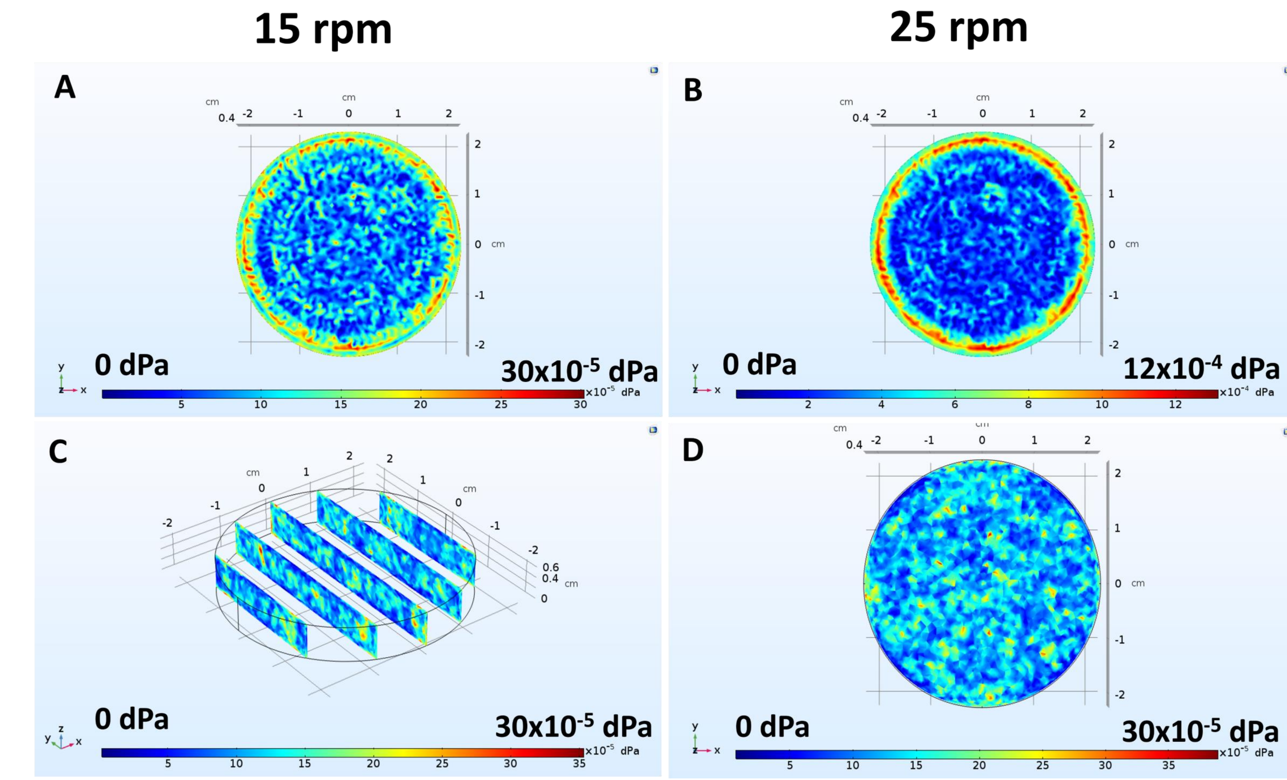

Figure 3. Baseline fluid shear stress at the top of a HARV at 15 (A) and 25 rpm (B) at a viscosity of 0.75 cP, mimicking cell culture media. The highest shear stress is at the edges of the HARV. C) Cross-sectional slices of shear stress distribution at 15 rpm. D) Fluid shear stress in the middle of the HARV at 15 rpm.

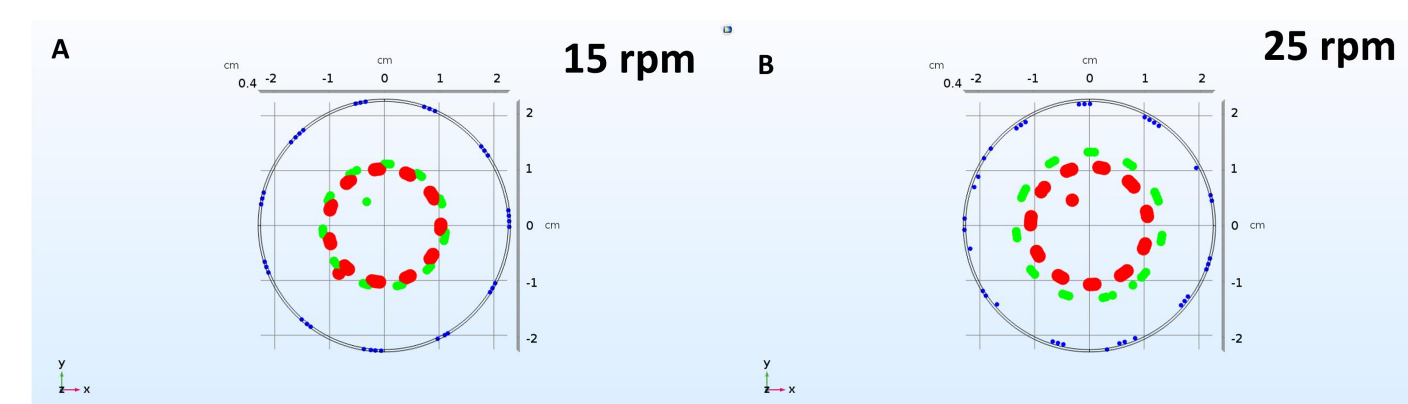

Figure 4. Particle Tracing of spheroids at 15 (A) and 25 rpm (B) at a viscosity of 0.75 cP. Particles have 50 (blue), 100 (green) and 200 (red) µm radii. In both cases the smallest spheroids are readily deflected to the outer edges of the HARVs while the larger, heavier particles tend towards the middle. There is greater deviation, however, in the 15 rpm trace (A) compared to the 25 rpm (B) where the 100 µm particles are pushed away from the center under higher rotation speed.

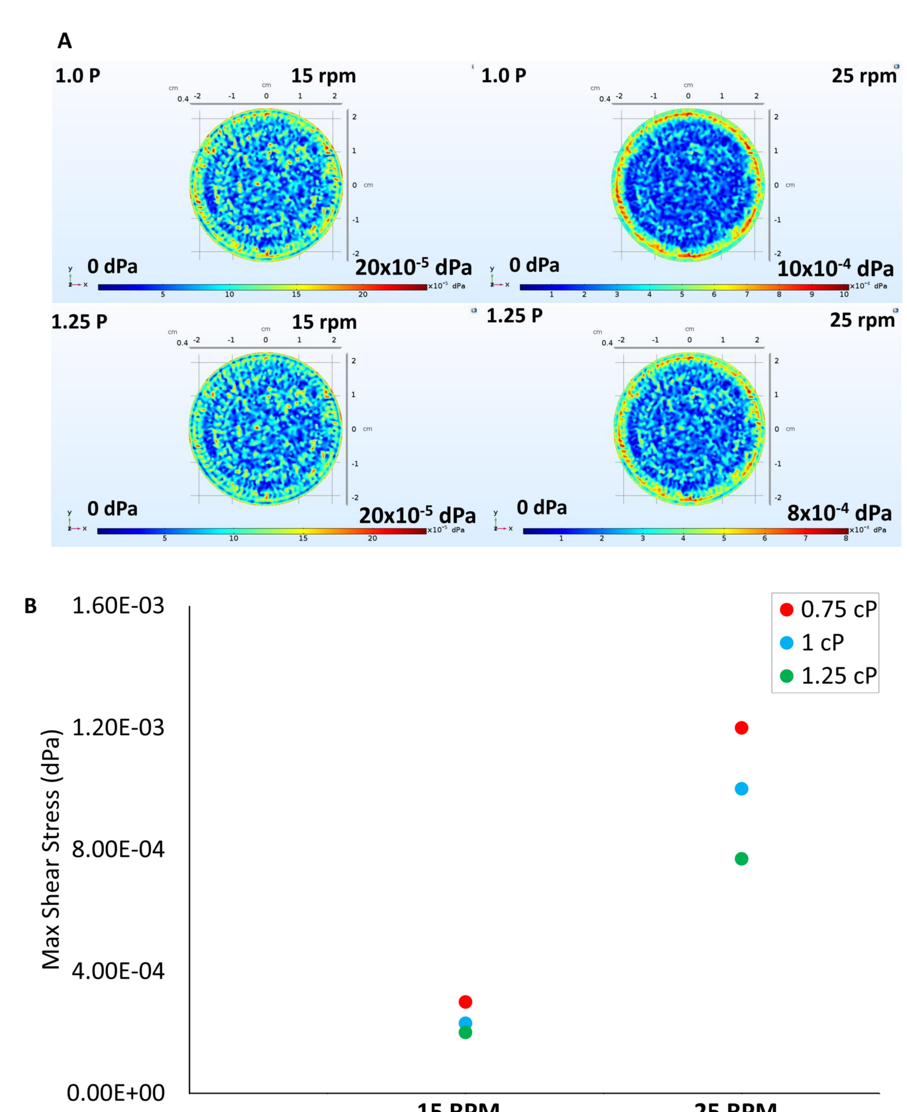

Figure 5. The Effects of Viscosity on Shear Stress. A) CFD simulations of shear stresses at both 15 rpm and 25 rpm for both 1.0 and 1.25 cP at the top of the HARV. The range of maximum shear stress is graphed in B. There is comparatively little difference between the two viscosities as opposed to the two rotation speeds.

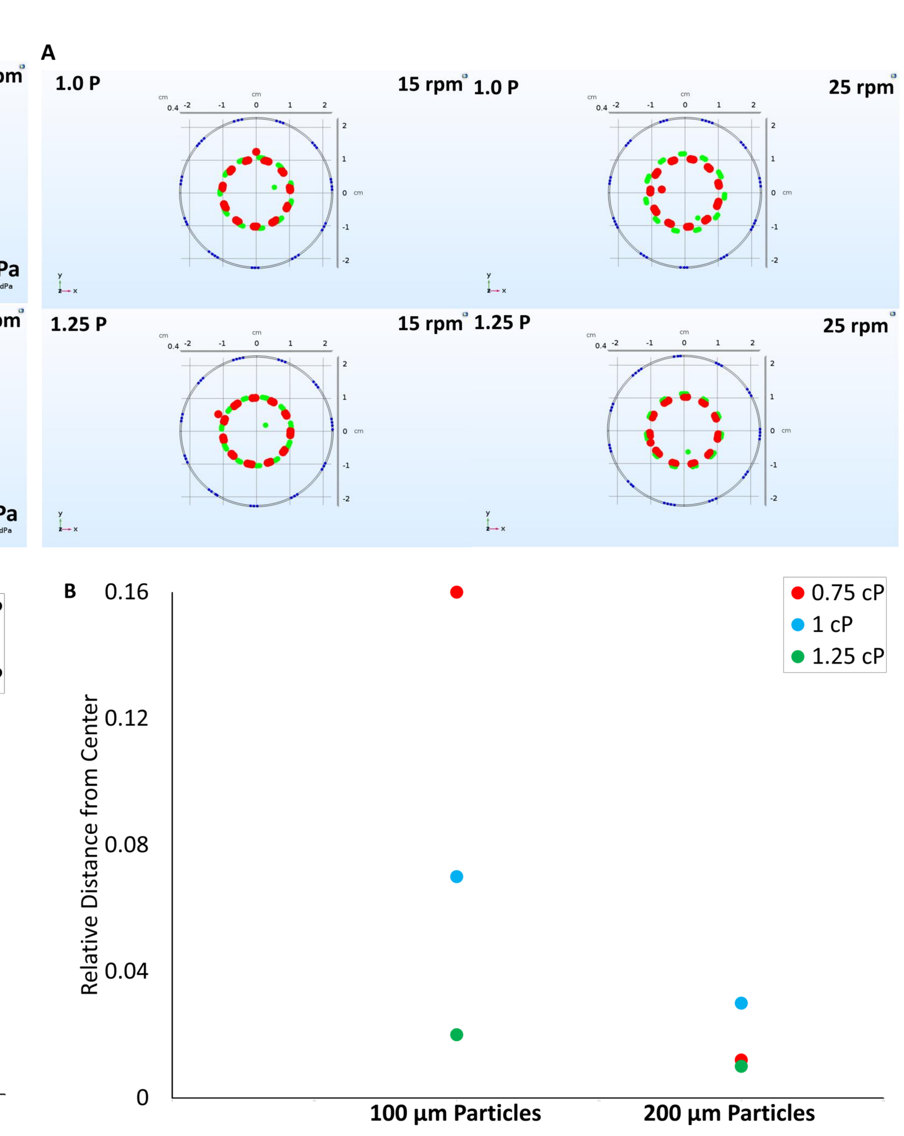

Figure 6. **Figure 8.** The Effects of Viscosity on Spheroid Position. A) Particle tracing simulations of spheroid position at both 15 rpm and 25 rpm for both 1.0 and 1.25 P. The relative distance from the center at 25 rpm is quantified in B. The differences between positions of 50 and 200 µm spheroids are minor but for 100 µm the deviation from the middle at 25 rpm is considerably restored by increasing the viscosity.

Conclusions

CFD experiments show the low shear stress within HARVs. In addition, particle tracing models how the spheroids behave under that shear stress, with higher speeds pushing mid-sized spheroids towards the edge. To ensure consistency of shear stress across spheroid diameters, modulating the viscosity can align the spheroids closer to the middle, lowering the shear stress. This will be further verified and refined in the future with more simulations modeling the changes in shear stress over longer culture times as the spheroid size gradually changes. RCCS experiments with methyl cellulose-supplemented media will also be used to verify the simulation results.

Acknowledgments

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