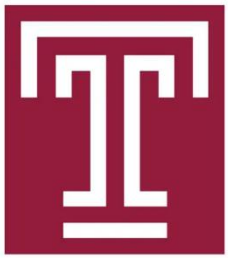

Temple University

College of Engineering

Bioengineering

Testing (only section)

Partial Gravity Bioreactor

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Test Method

To guarantee the validity of the results and decisions, specific tests must be conducted.

1. Inclination Angle Test

To attain the necessary gravitational conditions for the prototype—including Earth, Mars, Lunar, and Micro-gravity environments—the inclination angle of the plane is meticulously adjusted. Consequently, precise determination of the inclination angle corresponding to each specific gravitational type is of paramount importance. Furthermore, given that the inclination adjustment may be repeated multiple times, ensuring the repeatability and accuracy of the angle measurements is critical. An inclination angle test has been conducted to evaluate both the accuracy and repeatability of the adjustments. Due to the absence of standardized procedures for such tests, a protocol has been developed. As the prototype is anticipated to operate within an incubator during testing, environmental factors such as temperature and air quality are maintained at consistent levels, thereby minimizing errors and variability attributable to environmental fluctuations.

The inclination angle is measured from the incubator's horizontal surface to the inclination plane movement to the intended angle (the inclination angle). Measurements commenced from an initial inclination of 0 degrees, with recorded values taken incrementally across predefined target angles (0°, 5°, 10°, 20°, 45°, 90°). At each configuration, the tilt was adjusted using the tilt code in the motor connected to the rod. To ensure the rotation of the bioreactor does not affect or cause a lag, each angle was run at different RPM values (0 rpm, 5 rpm, 10 rpm, and 20 rpm). The side-view videos of the prototype were captured with a phone camera for all the runs. The videos were recorded transitioning from 0 degrees to each target angle and back to zero, to evaluate hysteresis through positive and negative rotations. Subsequently, MATLAB code was employed to process these videos, determining the angle between the incubator's horizontal reference plane and the inclined plane. The use of video recording, as opposed to static images, ensured a smooth transition between angles and maintained flow stability within the bioreactor. At each position, three measurements were taken to assess repeatability. A total of 144 runs will be run. The design of the inclination angle test experiment table is seen in Supplementary S T 1.

The 0 degrees for each run were tested and compared to one another to ensure that the 0 degrees had not shifted during the runs. All data were recorded in a single session with the phone stabilized by a sturdy object placed on a surface. The deviation was calculated by determining the difference between the intended angle and the measured angle. The standard deviation for each repetition was also calculated. Linearity was assessed by plotting the measured angles against the observed angles. The mean error and repeatability were calculated for each position. The total uncertainty was estimated by finding the variance using an R function. The acceptable ranges of errors are ±0.01° to 0.3°. Errors would include the vibration of the prototype, vibration of the incubator, vibration caused by the bioreactor, and the weight of the material inside the bioreactor. All the statistics will be run in R.

2. RPM Test

One of the most critical components of the prototype is to ensure that the particles are suspended within the fluid and subjected to the intended gravitational forces. Since the bioreactor is designed to rotate smoothly to maintain a steady flow and provide a stable environment for the particles, the RPM must remain constant. Furthermore, as the combination of RPM and inclination determines the effective gravity acting on the particles, the RPM must be precise. An RPM test will be conducted to verify accuracy and ensure a steady flow.

Using a wireless digital tachometer, the RPM of the rotating bioreactor will be measured at the specified RPMs of 0, 5, 10, 15, 20, and 30 RPM. For each RPM, five measurements will be recorded at 30-second intervals to verify the consistency of the RPM within the flow. To ensure the stability of the digital tachometer, a phone stand will be used to support the device, and the angle of the bioreactor will be maintained perpendicular to the horizontal surface. A total of 90 runs will be performed. The design of the RPM test experiment table is presented in Supplementary S T 2.

The measurements shall be documented utilizing JMP software. For each RPM, three trials will be conducted to evaluate repeatability. The mean, standard deviation, and variance will be calculated using R. The permissible error margins are within ±0.1 rpm. Linearity was assessed by plotting the measured RPM against the observed RPM. Errors considered may include vibrations from the prototype and bioreactor, as well as factors such as air quality, temperature, and the mass of materials within the bioreactor. All statistical analyses will be performed using R.

3. Validation of Size

Given the constraints in the prototype's dimensions, the size is a critical parameter requiring precise measurement. Accordingly, the dimensions of the prototype will be measured three times weekly over two weeks after its construction. Temperature will be recorded during each measurement session. For each day of data collection, three measurements will be conducted to assess repeatability. These measurements will encompass length, width, and height at 0- and 90-degree angles. The mean, standard deviation, and variance will be computed using R. The measurements will be deemed acceptable if they are below the specified threshold, $21 \times 17 \times 14\ in$, which corresponds to the measurements of the internal space within the incubator.

4. Validation of Electrical Safety

Regarding the safety of the electrical component, the International Electrotechnical Commission (IEC), specifically IEC 60204-1, which delineates the general requirements for electrical machinery equipment, will serve as the basis for safety validation. According to 'Safety of Machinery - Electrical Equipment of Machines - Part 1: General Requirements', a protective bonding circuit is mandated to ensure that all exposed metallic parts of the machinery are properly bonded and grounded [1]. This requirement encompasses electrical enclosures and wiring, which should be systematically organized, clearly labeled, and adequately protected, with cables rated appropriately for the voltage and current they conduct [1].

Compliance with EN 61010-2-030, Measuring Circuits and Test Equipment Safety Test, ensures adherence to the standard [2]. A multimeter will be employed to verify the current and voltage flow through components, ensuring their compliance. Furthermore, the visual inspection of wiring will be conducted to assess its integrity. The validation process will be performed weekly to guarantee user safety and the stability of electrical circuits. No statistical testing will be conducted.

5. Validation of Fluid Dynamics Using Alginate Beads

To validate the partial-gravity bioreactor and fluid dynamics, alginate beads can be added to visualize the motion of particles. Developed from a calcium chloride solution, they have similar densities to that of a cell. Due to the increased size of alginate beads, the bioreactor would have to run at 15 rpm [3]. This validation will directly influence whether it is possible to culture cells within the bioreactor.

Alginate beads are produced by dropwise adding a (w/v) aqueous mix of 0.75% sodium alginate, 1% polyethylene oxide, and 2% powdered charcoal (or fluorescence dye) for color into a stirred 2% calcium chloride bath [3]. The shear values were obtained using a computer modeling program (ANSYS). The position of the bead is measured using MATLAB code. A video of three trials for the alginate beads in each gravitational condition will be recorded using a camera setup of the prototype. At the beginning of each trial, an image of the alginate bead will be taken, with another picture taken at the end of a 30-minute simulated run. Using ImageJ, the circularity of the beads will be calculated using the total perimeter and the area in the images taken [3]. Measuring the circularity would ensure the flow is steady and does not affect the particles. The measurements will be calculated three times to ensure repeatability. The mean, standard deviation, and variation of each gravitational condition will be calculated using R. The circulatory is expected to be closer to 1, showing little to no change in the dimensions of the beads.

6. Cytotoxic Direct Contact Test

With the bioreactor being built from scratch, the materials that are in contact with the cells should be tested to ensure they are biocompatible and do not produce any leaches. This ensures that the cells are in their most favorable environment and conditions. According to the ISO 10993-5: Tests for In Vitro Cytotoxicity, three tests could be run [4]. It ensures that the substance used would not cause deformation or lysis of the cells beneath or around it. It is evaluated microscopically and then graded. The protocol is the following [5]:

- L929 cells (ATCC No. CCL1, NCTC clone 929 (connective tissue mouse), clone of strain L(DSMZ)) are grown at 37 °C in a 5% (v/v) CO2 incubator in DMEM supplemented with 10% (v/v) fetal bovine serum
- A 30mg sample of the material was placed into an empty well of a six-well culture plate.
- The cells were seeded into the well at a density of 50,000 cells per well.
- The plate was incubated at 37 °C for 24 hours
- The visual cytotoxicity was visualized using a microscope
- The score was assigned according to a 5-point scale, ranging from 1 to 5, with 1 showing cytotoxicity present and 5=no cytotoxicity. Figure 1 shows the visualization of the scale.

- To ensure the scores are accurate, three different wells will be tested.

The positive prediction is that the item would meet requirements if the test score of the response to the item were not greater than grade 2 (mildly reactive) [6].

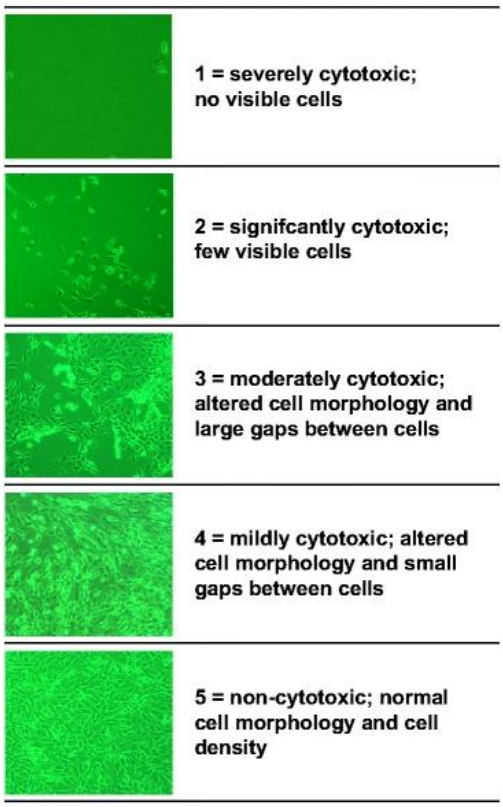

Figure 1. Cytotoxicity Scale Visualization

The figure shows the scheme for assigning visual cytotoxicity scores to biomaterials.

References

1) "ISO 13850:2015," ISO. Available: https://www.iso.org/standard/59970.html

2) "IEC 61010-2-030:2023," IEC. Available: https://webstore.iec.ch/en/publication/75915

3) M. A. Phelan, A. L. Gianforcaro, J. A. Gerstenhaber, and P. I. Lelkes, "An Air Bubble-Isolating Rotating Wall Vessel Bioreactor for Improved Spheroid/Organoid Formation," Tissue Engineering Part C Methods, vol. 25, no. 8, pp. 479–488, Jul. 2019, doi: 10.1089/ten.tec.2019.0088. Available: https://pmc.ncbi.nlm.nih.gov/articles/PMC6686703/?term=%22Tissue%20Eng%20Part%20C%20Methods%22%5Bjour%5D#SD1

4) "ISO 10993-5:2009," ISO. Available: https://www.iso.org/standard/36406.html

5) S. K. Bhatia and A. B. Yetter, "Correlation of Visual In Vitro Cytotoxicity Ratings of Biomaterials With Quantitative In Vitro Cell Viability Measurements," Cell Biology and Toxicology, vol. 24, no. 4, pp. 315–319, Oct. 2007, doi: 10.1007/s10565-007-9040-z. Available: https://doi.org/10.1007/s10565-007-9040-z

6) "Direct Cell Contact Test - Eurofins Medical Device Testing," Eurofins Scientific. Available: https://www.eurofins.com/medical-device/testing/cytotoxicity-testing/direct-cell-contact-test/

Supplementary Data

Supplementary ST 1: Design of Inclination Angle Test Experiment (for 1 Trial)

Direction	RPM	0	5	10	20
	Angle				
Forward	0	00F	50F	100F	200F
	5	05F	55F	105F	205F
	10	010F	510F	1010F	2010F
	20	020F	520F	1020F	2020F
	45	045F	545F	1045F	2045F
	90	090F	590F	1090F	2090F
Reverse	0	00R	50R	100R	200R
	5	05R	55R	105R	205R
	10	010R	510R	1010R	2010R
	20	020R	520R	1020R	2020R
	45	045R	545R	1045R	2045R
	90	090R	590R	1090R	2090R

The table shows the design of the experiment for the inclination angle test. There are three factors: RPM, Angle, and Direction. The levels are the following: RPM (4 levels), Direction (2 levels), and Angle (6 levels). There are 48 runs for each trial, and since there are three trials, there is a total of 144 runs.

Supplementary ST 2: Design of RPM Test Experiment (for 1 Trial)

Time	0	30s	1min	1:30min	2min
RPM					
0	00	0.50	10	1.50	20
5	05	0.55	15	1.55	25
10	010	0.510	110	1.510	210
15	020	0.515	115	1.515	215
20	020	0.520	120	1.520	220
30	030	0.530	130	1.530	230

The table shows the design of the experiment for the RPM test. There are two factors: RPM and Time. The levels are the following: Time (5 levels) and RPM (6 levels). There are 30 runs for each trial, and since there are three trials, there is a total of 90 runs.