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Inertial Shear Forces and the Use of Centrifuges in Gravity Research. What is the Proper Control?

Centrifuges are used for 1×g controls in space flight microgravity experiments and in ground based research. Using centrifugation as a tool to generate an Earth like acceleration introduces unwanted inertial shear forces to the sample. Depending on the centrifuge and the geometry of the experiment hardware used these shear forces contribute significantly to the total force acting on the cells or tissues. The inertial shear force artifact should be dealt with for future experiment hardware development for Shuttle and the International Space Station (ISS) as well as for the interpretation of previous spaceflight and on-ground research data. [DOI: 10.1115/1.1574521]

Introduction

When studying the impact of accelerations on living systems we compare a non- or hypo-gravity acceleration condition or a hyper-gravity acceleration with our reference of unit gravity.

To be able to draw any conclusions from spaceflight, i.e., microgravity or better microweight experiments, it is important to have a proper control group. To obtain the best control, we have to fully understand all possible influences, including artifacts, involved in such experiments.

What is this proper control? Consider comparing a sample exposed to spaceflight microgravity (μg) with a 1×g control. Based on Einstein's principle of equivalence, this 1×g control can either be a sample remaining on Earth or a sample that is put into a centrifuge rotating at 1×g on-board a free falling spacecraft. Since there are some important differences, i.e., experiment artifacts, for on-ground 1×g controls and either in-flight 1×g or in-flight μg samples, like launch vibrations, cosmic radiation and experiment lag time, the better control seems to be an on-board centrifuge.

For biological experiments it was mainly the Biorack facility [1], and later many others, that accommodated an on-board 1×g control. Such a configuration brings about important differences between ground and flight 1×g, one of them being inertial shear forces.

In this paper we try to identify the magnitude inertial shear forces may play in mainly cell biological research by evaluating on-board as well as on-ground, rotating systems. We describe numerical calculations to assess the relative contri-

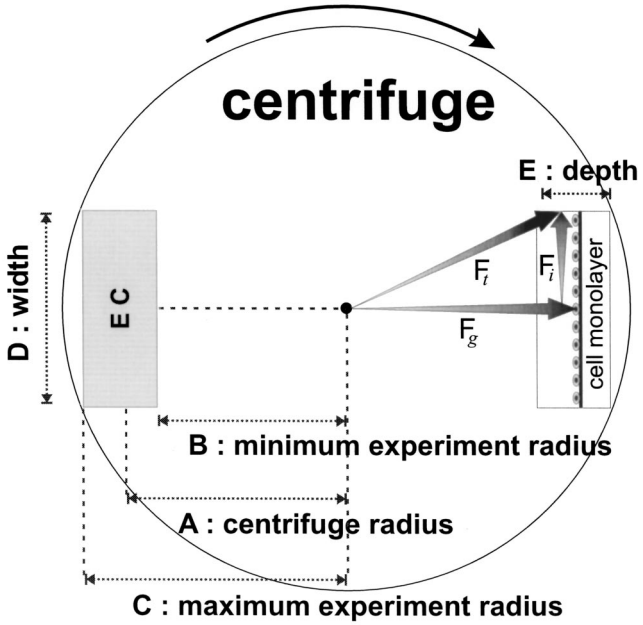

Fig. 1 Geometry of an Experiment Container (EC) accommodated on a centrifuge and forces within such a rotating system on board a spacecraft in free fall. The centrifuge radius, A, is defined as the distance from the center of rotation to the center of the EC. The minimum radius, B, is the distance from the center of rotation to the center-inner wall of the EC. The maximum radius, C, is the distance from the center of rotation to the outer wall of the EC. Width, D, is the maximum lateral width of an EC. E is EC depth. The force of gravity, F_g, increases radially from the center of centrifugation. The inertial shear force, F_i, increases laterally from the center of centrifugation as depicted in the right EC along a plane surface with a schematic monolayer of cells. F_i is the total resulting force.

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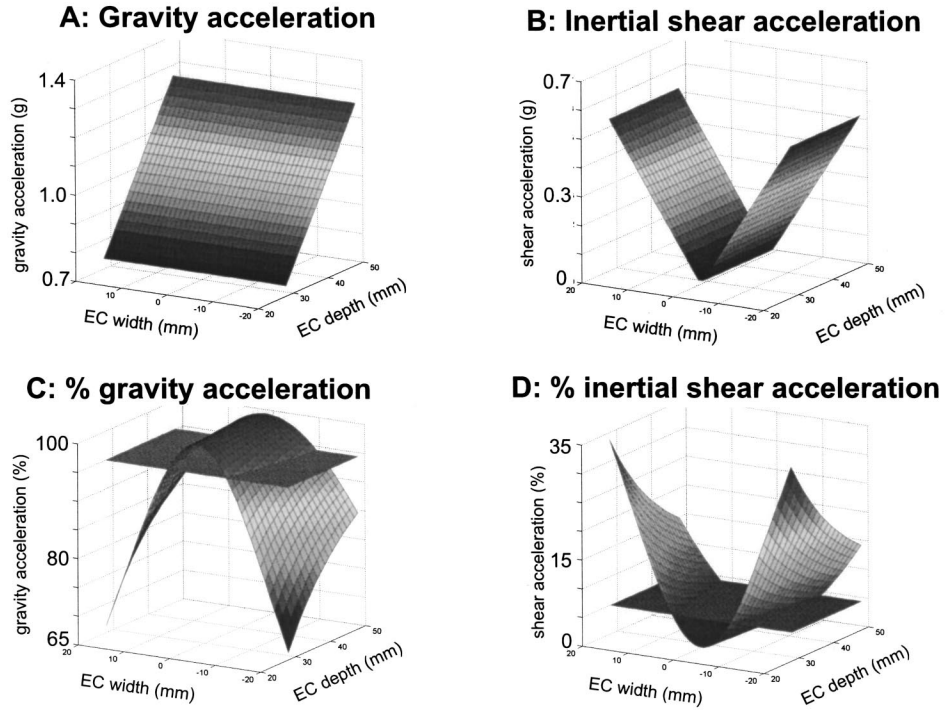

Fig. 2 Graphical representation of gravity and inertial shear accelerations as they will be generated in a Type-I EC accommodated on the small centrifuge of the Biopack facility. A: Gravity accelerations. B: Inertial shear. C: Percentage gravity acceleration of total acceleration. The horizontal plate indicates an arbitrary level of 95% gravity acceleration. D: Percentage shear acceleration over total acceleration. All values below the arbitrary plain division indicate the surface area within an experiment container where less than 5% of the total acceleration generates inertial shear.

bution of inertial shear force and provide a simple numerical model of a cell exposed to the mechanical conditions inside a centrifuge.

Methods and Calculations

Shear forces can be brought about by inertia (inertial shear) and/or fluid flow (fluid shear). In cell biology fluid shear is an important physiological phenomenon and most common in blood vessels where endothelial cells are exposed to blood flows. Endothelial cells experience a shear stress in the order of 0.1–0.5 and 0.6–4.0 Pa in venous and arterial vessels, respectively [2]. Not only the cardiovascular system but also the mechano-sensing and adaptation of bone is most likely governed by fluid shear forces around osteocytes [3,4]. Inertial shear forces, on the other hand, are mostly generated in materials exposed to accelerations. In cells both fluid shear stress and inertial shear stress will generate

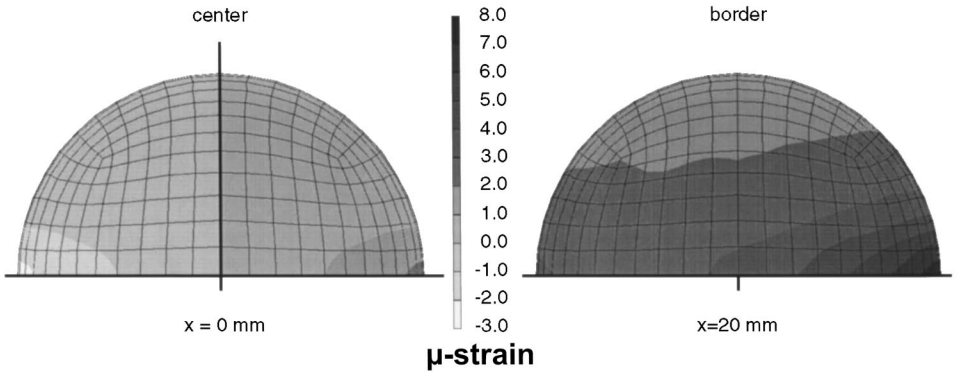

Fig. 3 Shear strains calculated from a finite element model for an idealized homogeneous isotropic cell accelerated in the center plane (center) of a Type-I experiment container in the Biopack small centrifuge running at 1×g, versus a similar cell located at x = 20 mm from the center plane (border). The absolute deformation of the cell is small, but the peak shear strain in the eccentric cell is more than three times higher than in the cell in the central position (7.98 μstrain vs. 2.37 μstrain, respectively). The related peak shear stress in the eccentrically located cell (0.027 Pa) is likely large enough to provoke a biological response.

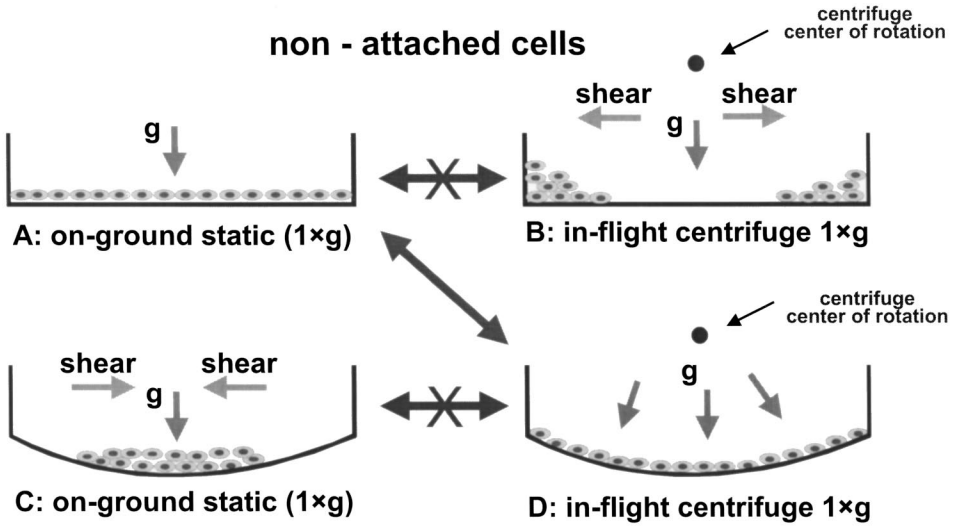

Fig. 4 The distribution of non-adherent cells in a 1×g static on-ground centrifuge (A and C) or on a 1×g on-board centrifuge (B and D), in sample chambers of different surface geometry. Note that the mark for 'center of rotation' and the curvature of the chamber are for clarity of the drawing not on the same scale.

cell deformation, i.e., strain. In centrifuges an essential difference between inertial shear force, F_i, and force of gravity, F_g, is that inertial shear acts perpendicular to the gravity acceleration vector. (See Fig. 1.)

The magnitudes of the total acceleration vector and its components gravitational acceleration and inertial acceleration are calculated using standard trigonometry and the dimensions and rpm's of the different facilities. As an example we focus on the dimensions of a standard Biopack Type-I experiment container (EC), which has a maximum width of 40 mm, a depth of 20 mm and a mean distance to the center of rotation of 77.4 mm. Gravitational and shear accelerations for each location on the EC are calculated. (See Fig. 2.)

To predict cellular stresses and strains a simple finite element model (FEM) of a single cell is developed in MARC/MENTAT. The geometry of the model is again based on the Biopack Type-I EC and it is assumed that the total bottom of the EC consisted of culture surface, covered with adherent cells. Since accelerations are laterally symmetrical, only one half of the container width is considered. The model cell is positioned in the center plane of the container and at the edge of the culture surface, in this geometry at 20 mm from the center of rotation. (See Fig. 3.) The cell is modeled as half a perfect sphere with 3,456 brick (8-node) shaped elements connected at 3,927 nodes. The total degrees of freedom of the problem DOF=11,781. The height of the cell is set to 5 μm, the base diameter is 10 μm. The upper surface of the cell is allowed to move freely, while the cell base is fixed. The cell is considered as a continuum and material properties are modeled with a nearly incompressible Neo-Hookean material law. The Poissons ratio was set to 0.449.

At present no clear values for the moduli of cells have been defined. Nonetheless, homogeneous values have been estimated for simple modeling purposes, and they range from 2.8 kPa for bovine chondrocytes [5] to 12 kPa for TB/C3 hybridoma cells [6]. There are also significant modulus variations within the cell that may range from 0.5 up to 84 kPa [7,8]. For this study a modulus of 10^4 Nm^{-2} (10 kPa) has been applied as a first approximation of the elastic properties. Initially, the model is exposed to an angular velocity of 153.43 rpm, typical for the Biopack centrifuge spinning at 1.0×g in the center of the cell layer surface. The maximum deformation in shear strain was calculated using a cell specific density of 1050 kg/m^3.

Results & Discussion

The resultant gravity and shear accelerations for a typical centrifuge is shown in Fig. 2.

Since the acceleration is linearly proportional to the centrifuge radius, the gravity acceleration calculated over the total EC volume ranges from 0.737 to 1.263×g (Fig. 2A). It is obvious from Fig. 2B that the inertial shear acceleration increases laterally from minimum, in the central axes of rotation, towards the outer limits of the experiment container, ranging from zero to more than 0.526×g. The percentile contribution of gravity acceleration ranges from some 100 to 66%.

Adherent cells attached to a flat surface will experience a larger inertial shear force, F_i, when located further from the point where the radius is perpendicular to the surface (See Fig. 1). This effect is enhanced in smaller radii centrifuges. Inertial shear acceleration for adherent cells results in cell deformation, i.e., strain.

Figure 3 shows the deformed mesh and resulting shear strains of the cell model attached to a surface rotating at a speed of 153.43 rpm (1×g) in an EC in the Biopack facility small centrifuge. Cells are hardly deformed under these conditions with a vertical deformation <2 μstrain. However, shear strains increase by a factor of more than three when the cell is located at the outer edge of the surface as compared to the center (7.98 μstrain vs. 2.37 μstrain). The peak shear stresses in cells at the outer area are 0.027 Pa.

For non-adherent cells the situation is slightly different. Here the cells do not experience inertial shear strain, as they do not attach to the substrate. However, cells in a 1×g in-flight centrifuge arrange themselves differently in the sample volume than they would on Earth. Free moving particles in a rotating system will move to the area of highest acceleration. When we consider a homogeneous suspension of cells and place this, on-ground, in a flat bottomed dish the cells will distribute evenly over the surface area as shown in Fig. 4A. When we apply 1×g to the same dish in an on-board centrifuge the cells will, due to the inertial shear force, move to the EC outer edges and pile up onto each other. (See Fig. 4B.)

The way to avoid inertial shear accelerations in the on-board centrifuge would be to apply a curved sample surface with exactly the same curvature as the centrifuge radius (See Fig. 4D). However, the same curved geometry will lead to 'cell piling' in the on-ground 1×g condition (Fig. 4C). At present the custom set-up

for spaceflight experiments is to have identical hardware for on-ground, in-flight 1×g and microgravity samples. When we want to eliminate the shear acceleration artifact this scenario has to be revised. Ground 1×g hardware should have a flat surface as in Fig. 4A while the in-flight 1×g hardware should have a curved surface as drawn in Fig. 4D.

Some experimental set-ups also include an on-ground control centrifuge. Taken into account Earth's gravity and the centrifuge rotation this generates an acceleration, in the center reference point, of $\sqrt{2}\times$g. Since the ECs in such facilities are fixed and can not 'swing-out' the resultant acceleration vector is 45° from the horizontal. When we want to eliminate the inertial shear forces under these circumstances one has to manufacture and apply some complex hyperbolic surface.

Since the inertial shear force phenomenon has not been addressed earlier there is no actual data on possible differences of response in cells positioned at various sample locations. There is, however, the possibility to compare the 1×g ground with the 1×g in-flight results.

Biochemical data is blurred by its nature of sample collection and will average out any possible geometrical effects. A possible hint for differences would be a changed morphology (e.g., cytoskeleton orientation) of cells at different surface areas, but no papers indicate the exact location of a cell within the sample. However, there are some papers that identify a difference between ground 1×g and in-flight 1×g results. Driss-Ecole et al. and Yu et al. describe a difference in mitotic index of lentil roots cortical cells in ground compared to flight samples [9,10]. Schmitt et al. [11] studied the distribution of PK-C in leukocytes. This study showed differences between samples on a centrifuge, either on-ground or in-flight, and non-rotated groups. Although the authors argue that these differences might be resulting from launch effects, cosmic radiation or a pre-exposure of in-flight centrifuge samples to microgravity, it is possible that centrifuge inertial shear artifacts might have caused these differences.

Pross and Kiefer describe a decrease in repair capacity of radiation damaged yeast cells in the two centrifuge groups (in-flight 1×g and on-ground $\sqrt{2}\times$g) compared to the non-centrifuge samples [12]. Since, besides possible vibrations, all other artifacts between in-flight 1×g and on- ground 1×g seems of little effect in this experiment, it might well be that inertial shear forces have caused these differences. This would imply that cellular DNA repair processes in these cells are suppressed by the mechanical force of inertial shear. Since, due to Earth's gravity, the on-ground centrifuge generated higher shear accelerations compared to the in-flight centrifuge it is interesting to note that the repair process in the ground centrifuge is always below that in the in-flight centrifuge group.

At a (sub-) cellular level the force of gravity seems, compared to the three other basic forces in nature quite insignificant. Non-gravity related phenomena like thermal noise (kT) or chemical energies are orders of magnitude larger than a 1×g acceleration see also [13–19]. One main difference of inertial shear force compared to phenomena like Brownian motion is that inertial shear force is continuously acting in the same direction. The present concepts for cell 'gravisensing' are thought to be related to mechanisms like reaction-diffusion [19–21], stochastic resonance [22,23] or 'time averaging of a constant stimulus' (D. Kondepudi, Wake Forest Univ., personal communication). Considering these proposed mechanisms it might well be that the same applies for possible effects of inertial shear accelerations.

In this study the vertical deformation is 1.4 μstrain, and in the edges of the cell shear strains appear in the order of 2.4 μstrain (Fig. 3, left). For a cell placed at the edge of the culture surface (Fig. 3, right), the vertical deformation remains small (1.6 μstrain), but the shear strain is more than three times higher than in the central position (8.0 μstrain). The model demonstrates that the force of 1×g can affect cell shape only marginal. Assuming a Young's modulus of 10 kPa, an additional shear stress is introduced of about 0.027 Pa .

From various impeller stirred fermenter studies Van der Pol and Tramper concluded that for animal cells, cell damage and cell death was found in the range of shear stresses from 0.5–200 Pa [24]. The fluid shear stress in bone is calculated to be 0.8–3 Pa [3]. In an in vitro study using adherent endothelial cells exposed to 1.2 Pa steady shear stress generated by a fluid flow it was shown that cells will reorient themselves along the direction of flow [25]. Cultures of BHK-21 cells grown on microcarrier beads in the NASA designed low shear integrated rotating wall vessel (IRWV) show increased levels of glucose utilization, alkaline phosphatase, alanine transaminase, asparagine transaminase and lactate dehydrogenase at fluid shear stresses of 0.092 Pa as compared to 0.051 Pa. A difference in fluid shear stress of only 0.041 Pa [26]. In our calculations the peak shear stress is 0.027 Pa. It might be argued that, this level of inertial shear might have a significant effect on cell behavior.

It is not only the centrifuges and experiment hardware geometry that provokes inhomogeneous acceleration profiles within centrifuges. Also the samples themselves like e.g., adult plants or mammalian tissue constructs might be shaped such that there are undesired internal shear forces that cannot be overcome.

It is well know that shoots and roots will grow more randomly and circumnutate differently in real microgravity [9,10,27,28] and in simulated microgravity [29,30]. In a typical ISS plant research facility, like the European Modular Cultivation System (EMCS), the radius of centrifugation in the center of the experiment container is 200 mm. When we consider *Arabidopsis thaliana* grown in this facility the gravity variation over an adult plant is 0.6–1.4×g while the lateral inertial shear force ranges from 0 to 0.15×g.

When such a structurally unbalanced plant is transferred from a microgravity environment into a centrifuge to study subsequent gravitropic responses the plant will, besides gravity, experience a lateral shear force within its structure. A small deviation of the stem or leaves from an exact alignment along the line of radial acceleration will result in forces generated within the plant that are different from that on Earth. It might be expected that this will be partially, or fully, compensated by the plant's active internal gravitropic response but this is a completely different and more complex field of forces and responses compared to the on-ground situation. This makes the interpretation of the effect of 'gravity' on a plant in such a system very difficult.

Conclusion

In conclusion we can state that in most spaceflight facilities for biological microgravity research the magnitude of inertial shear force compared to the gravity acceleration component is considerable and can not be neglected. For the various spaceflight facilities used in ISS and Shuttle the percentage inertial shear force may ranges from zero to more than 99%. For 2D sample structures, like a cell monolayer, this artifact can be overcome by shaping the cell substrate parallel to the centrifuge radius. There is no possibility to eliminate the inertial shear force artifact from relatively larger, 3D, structures accommodated in a centrifuge. The relative influence of inertial shear force may be limited by using large radii centrifuges such as the Centrifuge Accommodation Module as is currently foreseen for the ISS.

The inertial shear force artifact should be dealt with in future missions experiment hardware development as well as for the interpretation of previous spaceflight and on-ground data.

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