14

Clinostats and Other Rotating Systems—Design, Function, and Limitations

Karl H. Hasenstein[1] and Jack J. W. A. van Loon[2]

[1]University of Louisiana at Lafayette, Lafayette, LA 70504, USA
[2]VUmc, VU-University, Amsterdam, The Netherlands

14.1 Introduction

Clinostats are rotational devices that have been in use ever since Julius Sachs invented a clockwork-driven device that rotated growing plants around their growth axis at the end of the 19th century [1]. The initial clinostat systems were mostly used in plant studies and rotated with a relatively slow frequency on the order of one rotation per couple of hours up to about 10 revolutions per minute. Seeds or adult plants were fixed to the clinostat within some semisolid or soil substrates. Although mostly used to simulate microgravity, there are some interesting adaptations of these systems made over the years. For instance, clinorotation was combined with centrifugation to generate a partial gravity in order to establish the gravity threshold of various systems [2–4]. In the 1960s, Briegleb [5] introduced the concept of a fast-rotating (on the order of 60 rpm) clinostat dedicated for liquid cell culture studies. Other improvements were implemented over time, and the most recent modification resulted in the so-called 3D clinostat [6] and the random positioning machine (RPM) [7, 8]. The widespread use of clinostats and the often-found notation that all it takes to eliminate gravitational effects on organism is to rotate them prompt this description of the purpose, goals, and limits of these devices. The most important message may well be the simple statement that clinostats, albeit intended for this purpose, do not simulate microgravity. The detailed assumptions and consequences of rotational movements in a static force field, such as Earth's gravity, are often overlooked and may result in questionable or downright incorrect statements. Since clinostats can also be used to mimic fractional gravitational loads, their usefulness goes beyond averaging the gravity vector over time; they can also be used to study the effects of hypogravity.

14.2 Traditional Use of Clinostats

The most important constraints have been recognized early on and were identified as centrifugal forces ($Z = r\omega^2$, product of radius and angular velocity), phase shifts of mobile particles as a consequence of rotation ($\tan \phi = -\omega$ m/f, the offset between the angular displacement of the rotating structure and the rotation of a cellular particle), and friction experienced by a mass as a result of rotation ($V_f = g/((f/m)^2 + \omega^2)^{1/2}$, the sedimentation of a particle as a function of its mass and the viscosity of the medium) [9]. In addition, the direction of rotation is relevant, and related concerns led to the development of random positioning machines whose sole purpose is to not generate constant forces in any particular direction. Let us consider the significance of each of these parameters.

14.3 Direction of Rotation

The intended averaging of the gravity vector can be accomplished by rotating an object such as a plant, bacterium, or (small) animal around a horizontal axis. However, the direction of the object is also important. While traditionally plants were rotated around their longitudinal axis (i.e., the shoot–root axis is positioned horizontally), gravity averaging is also possible by rotating the long (i.e., shoot–root) axis of a plant perpendicularly to a horizontal axis (aka vertical clinorotation). While the extended size of this axis limits studies to relatively short seedlings, studies have shown that plants are more sensitive to this latter type of rotation as the growth rate decreases more than after horizontal clinorotation [10]. Thus, the direction of rotation affects the physiology of biological objects, especially when the biological objects themselves are rotating such as tendrils or circumnutating stems [11].

14.4 Rate of Rotation

Rotating a physical object such as a growing plant regardless of the orientation relative to the horizontal axis of rotation shows that the radius is not constant. Horizontal rotation affects stems to a lesser extent than leaves that have a larger radius than stems. Thus, the centrifugal force varies within the organism based on the distance from the rotational axis. If a biological object (plant, seedling,

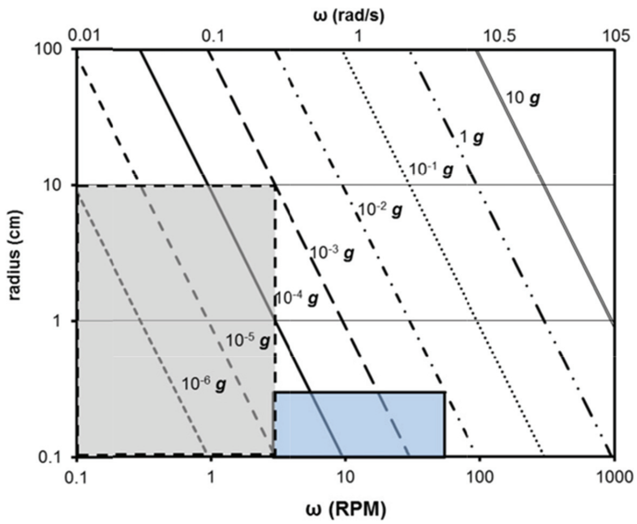

Figure 14.1 Log/log plot of radius and angular velocity (expressed as revolutions per minute and radians per second). The different lines define the centrifugal force induced by the respective rate of rotation. The rectangles exemplify usable dimensions and angular velocity ranges for slow-rotating (gray) and fast-rotating (blue) clinostats. The relative acceleration based on angular velocity and radius is shown as g-equivalent.

or other organism) is offset from the rotational axis, the centrifugal force is minimized (zero) only for structures that are aligned with the rotational axis. However, because gravity may be averaged but is never eliminated, elastic bending of the stem or leaves ensues and the center of rotation constantly shifts. Thus, the changing weight distribution causes bending stress and non-random mechanostimulation because structures such as leaves and petioles have a specific load-bearing design. A similar process might occur with mammalian cells [8]. However, simplifying the complexities of body structure and adjusting the rotational speed such that even larger dimensions are not exposed to more than the minimal centrifugal force require a careful consideration of applicable rotational speeds (Figure 14.1).

14.5 Fast- and Slow-Rotating Clinostats

While the slow-rotating clinostat simply considers the overall geometry and establishes a rotational regiment that fulfills predetermined conditions (e.g., centrifugal forces less than 10^{-3}g, Figure 14.1), the fast-rotating clinostat also considers the path of sedimentation in a fluid, typically an aqueous growth medium for small (<1 mm) organisms. In aqueous conditions, sedimentation and (slow) rotation result in appreciable side effects such as spiral combinations of movements stemming from sedimentation, centrifugation, and viscosity-dependent Coriolis force $g_f = 2\omega\varphi$, where g is gravity, ω is the angular velocity, and φ is the angle per unit time. When the frequency of rotation is increased, sedimentation of a particle will be less than the movement of the liquid, leading to a reduced radius that eventually becomes smaller than the size of the particle or a cell. Under those conditions, the rotation stabilizes the fluid around the particle, effectively eliminating gravity effects [5, 8, 12].

Related to the fast-rotating clinostat as introduced by Briegleb is the rotating wall vessel (RWV), which is mostly used in cell biology and tissue engineering [13]. Basically, the RWV is a relatively large (5–20 cm diameter) liquid-filled container that rotates around a horizontal axis at 10–20 rpm [14]. Samples within the container are prevented from settling by matching the rotation speed to the sedimentation velocity of the sample. This velocity depends on the specific density of the sample (cells, nodules, or others), their volume and shape, as well as the density and viscosity of the suspending medium. In the fast-rotating clinostat, cells rotate around their own center and experience no direct fluid shear force; in contrast, cells and tissues in the RWV are constantly falling within the fluid. The sedimentation velocity and direction combined with the rotation of the fluid generate spiral trajectories within the vessel [15]. The samples' motion relative to the fluid generates shear forces on a particle surface ranging from 180 to 320 mPa for 50-μm particles [14], up to 780 mPa for 300-μm spherical particles [16]. These are significant shear forces compared to physiologically relevant shear values, for example, endothelial cell responses that are on the order of 500–1000 mPa [17, 18]. Also, when the mass distribution within the particle is anisotropic, the particle may retain its orientation with respect to the gravity vector which is to be avoided if one wants to simulate microgravity.

14.6 The Clinostat Dimension

Tradition subdivides clinostats into 1D, 2D, and 3D (three-dimensional) systems. Of course since even the 1D clinostat operates as a three-dimensional system, the traditional naming is totally incorrect. Rather than referring to dimensions, clinostats may be better described by the number of rotating axes. Thus, a one-axis (or 1D) clinostat rotates around one axis. The orientation of this axis determines its properties. Under the correct conditions (dimension and rotational speed), it compensates or averages the vectorial character of gravity. In a vertical position, it moves an object but the gravity vector is consistently experienced toward the basal (Earth-facing) side of the object. Positioning the axis at an angle in between the horizontal and vertical position results in a net proportion of Earth's gravity such that the experienced or virtual gravity g_v is equal to the sin α (the angle from the horizontal) times g. A simulation of Moon's gravity (1/6 g) therefore requires an angle of about 10 degrees; Mars' gravity ($\sim$0.37 g) could be simulated by tilting the axis by 22 degrees. The advantage of using a single-axis clinostat lies in its simplicity of establishing virtual conditions.

Things become considerably more complicated when more than one axis is employed. However, a true rendition of all spatial orientation requires rotation around the three axes of space; adapting aviation terminology, pitch, yaw, and roll represent rotation around the horizontal (*x*) axis, the vertical (*y*) axis, and the *z* axis, respectively. This definition only applies when the rotation center is located inside the object of interest. Under these conditions, movement around the three axes is described by the rotation matrix $R^* = Rx(\alpha)\ Ry(\beta)\ Rz(\gamma)$, where α, β, and γ represent pitch, yaw, and roll angles.

$$Rx(\alpha) = \begin{pmatrix} 1 & 0 & 0 \\ 0 & \cos\alpha & -\sin\alpha \\ 0 & \sin\alpha & \cos\alpha \end{pmatrix},\ Ry(\beta) = \begin{pmatrix} \cos\beta & 0 & \sin\beta \\ 0 & 1 & 0 \\ -\sin\beta & 0 & \cos\beta \end{pmatrix},$$

$$Rz(\gamma) = \begin{pmatrix} \cos\gamma & -\sin\gamma & 0 \\ \sin\gamma & \cos\gamma & 0 \\ 0 & 0 & 1 \end{pmatrix}$$

This complete set of operation is in contrast to the two axes used in the so-called 3D clinostat or the random positioning machine (RPM). So what is the justification of calling a two-axis system a 3D clinostat? The simplest explanation relates to the ability to observe the entire three-dimensional space by moving, for example, a camera (or head) around only two axes (up/down and left/right). This concept was illustrated using a "ball RPM," which consists of a sphere resting on three support points, one of which consists of a wheel that drives the sphere by rotating in one or several directions (e.g., vertical or horizontal). The remaining support points are passive but omnidirectionally rotating spheres [7]. Alternatively, two drive wheels rotate around the normal axis of the sphere at different rates. Either system can rotate the ball in one or more planes, thus simulating a single-axis clinostat or an RPM. Regardless of the drive mechanisms, the distance between the surface of the sphere and the rotational axis varies and thus centrifugal accelerations can change.

Engaging a second drive or rotating the single drive wheel adds motion around a second axis and the superposition leads to a movement that depends on the relative angular velocity. If both drives operate synchronously, the plane of rotation tilts by the cross product of $Rx(\alpha)$ and $Ry(\beta)$. If the drives operate asynchronously, the movement of the sphere becomes complex. If the speed of the drivers differs but is constant, a phase angle results, and the motion of the sphere relative to the axis of rotation and the angular velocity change. Of course, as long as the sequence of rotational changes is known, nothing about the motion is random and the motion is reproducible. True randomness requires changes in the velocity of the drives that are not reproducible. However, it is doubtful whether such subtle modifications can be perceived by biological systems on the background of constant motion. Therefore, the "random positioning machine" might better be renamed "variable positioning machine."

Different ratios of angular velocity lead to well-known Lissajous figures projected onto the surface of the sphere with the position of the trace = A $\sin(\omega_0 t)$ + B $\cos(\omega_0 t)$. The phase shift between these two parameters determines the "tilt" of a point on the sphere surface but any rotation will be uniform; thus, different levels of gravity can be obtained based on the extent of the phase shift; zero corresponding to a one-axis clinostat and a phase shift of 10 degrees simulates Moon's gravity, as explained above. If the frequencies change, seemingly random patterns emerge (Figure 14.2).

Current developments also look into generating partial gravity in 3D rotating systems like an RPM. Dutch Space (Leiden, the Netherlands) presented a software-controlled partial g RPM (European Low Gravity Research

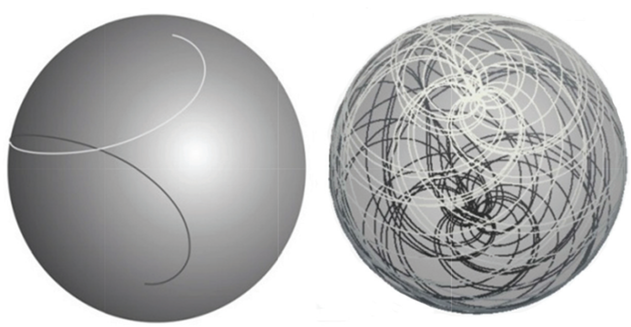

Figure 14.2 Projected traces of a surface point on a sphere that rotates with the same frequency for two perpendicular axes (left). Changing the frequency of one axis produces a distribution that covers the entire surface of the sphere. Calculations were performed after Kaurov [20].

Association in Vatican City in 2013) and a group from Switzerland published a comparable design [19].

14.7 Configurations of Axes

The previous example refers to two axes that rotate around a single point. However, the arrangement of rotational axes can be more complicated. For a single axis, no modifications are possible. Two rotational axes can be configured according to the description of the hypothetical sphere above; that is, they are part of a gimbal suspension. A second mode of arranging two axes consists of arranging the second axis perpendicular to the first axis of rotation. Such a device (Figure 14.3) has been implemented to examine the acceleration sensitivity of shoots and roots [4]. The relative rotation of the vertical axis of two wheels is used to turn "spokes" that extend from the center of the axis (Figure 14.3). This arrangement allows the entire system to function as a centrifuge if both upper and lower wheels rotate at the same angular velocity. If the lower wheel remains fixed and the top gear rotates, the experimental chambers that comprise the spokes rotate around their horizontal axis and are thus clinorotated as explained above for the 1-axis clinostat. Any additional movement by the lower wheel rotates the clinorotating chambers. This setup was used to determine the acceleration threshold of roots and shoots to about 10^{-3} and 10^{-4} g, respectively [4].

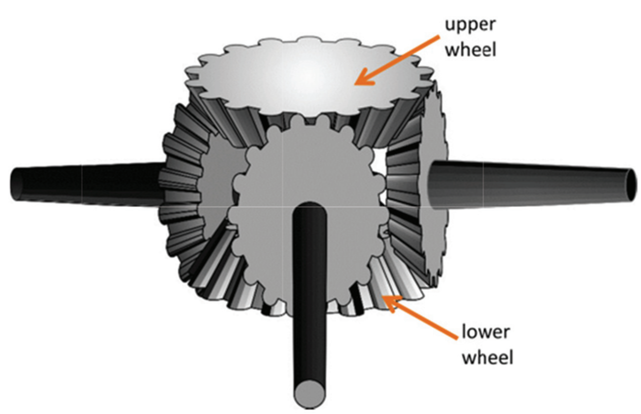

Figure 14.3 Drawing of a gearhead that translates the relative motion of a vertical shaft into a rotational motion of lateral axes. If the two center wheels rotate at the same rate, the horizontal axes function as a centrifuge and only yaw rotation applies. If the horizontal wheels spin at unequal rates, the lateral axes rotate and can drive a 1D clinostat with variable yaw and roll.

The creative arrangement of axes can be applied to the variable positioning system. If the lateral axes in Figure 14.3 contain a two-gimbal-supported suspension, a constant yaw acceleration is possible that averages all other motions relative to g. The constant yaw rotation generates a centrifugal force that is superimposed onto (1D) clinorotation. The effect of the angular velocity allows for the determination of a g-threshold value [4].

The data in Figure 14.1 indicate that the clinostatting of plants should not induce effects that are related to residual gravitational effects as long as the radius of rotation is less than the calculated values. However, recent studies clearly indicate that the rate of rotation over the magnitude of an octave (10-fold increase in frequency) affects induced curvature after gravistimulation. Brassica roots that were horizontally placed for 5 min and monitored for two hours of clinorotation between 0.5 and 5 rpm showed stronger curvature at higher frequency although the effective radius was less than 3 cm [10]. This observation, in addition to many others [21–25], indicates that the clinostat-associated mechanostimulation exerts largely unknown effects that prevent labeling clinorotation even in its most sophisticated form "microgravity simulation." The effects of mechanical unloading that are the hallmark of free-fall and orbital conditions do not apply to clinorotation. Nonetheless, the fascinating possibilities of manipulating organisms relative to the gravity vector for various times and under various conditions are bound to shed light on important aspects of sensory biology.

Acknowledgement

This work was supported by NASA grant NNX10AP91G to KHH and ESA grant TEC-MMG/2012/263 and Netherlands Space Office NSO/NWO grant to JvL.

References

[1] von Sachs, J. "Über Ausschliessung der geotropischen und heliotropischen Krümmungen wärend des Wachsthums." *Würzburger Arbeiten* 2 (1879): 209–225.

[2] Galland, P., H. Finger, and Y. Wallacher. "Gravitropism in Phycomyces: Threshold Determination on a Clinostat Centrifuge." *The Journal of Plant Physiology* 161 (2004): 733–739.

[3] Laurinavicius, R., D Svegzdiene, B Buchen, and A. Sievers. "Determination of the Threshold Acceleration for the Gravitropic

Stimulation of Cress Roots and Hypocotyls." *Advances in Space Research* 21 (1998): 1203–1207.

[4] Shen-Miller, J., R. Hinchman, and S.A. Gordon. "Thresholds for Georesponse to Acceleration in Gravity-Compensated Avena Seedlings." *Plant Physiology* 43 (1968): 338–344.

[5] Briegleb, W. "Ein Modell zur Schwerelosigkeits-Simulation an Mikroorganismen." *Naturwissenschaften* 54 (1967): 167–167.

[6] Hoson, T., S. Kamisaka, Y. Masuda, M. Yamashita, and B. Buchen. "Evaluation of the Three-Dimensional Clinostat as a Simulator of Weightlessness." *Planta* 203 (1997): S187–S197.

[7] Borst, A.G., and J. van Loon. "Technology and Developments for the Random Positioning Machine, RPM." *Microgravity Science and Technology* 21 (2009): 287–292.

[8] van Loon, J.J.W.A. "Some History and Use of the Random Positioning Machine, RPM, in Gravity Related Research." *Advances in Space Research* 39 (2007): 1161–1165.

[9] Albrecht-Buehler, G. "The Simulation of Microgravity Conditions on the Ground." *ASGSB Bulletin* 5 (1992): 3–10.

[10] John, S.P., and K.H. Hasenstein. "Effects of Mechanostimulation on Gravitropism and Signal Persistence in Flax Roots." *Plant Signaling & Behavior* 6 (2011): 1–6.

[11] Israelsson, D., and A. Johnsson. "A Theory for Circumnutations in Helianthus annuus." *Physiologia Plantarum* 20 (1967): 957–976.

[12] Briegleb, W. "Ein Beitrag zur Frage physiologischer Schwerelosigchkeit." *DVL Cologne* (1967): 7–42.

[13] Schwarz, R.P., T.J. Goodwin, and D.A.Wolf. "Cell Culture for Three-Dimensional Modeling in Rotating-Wall Vessels: An Application of Simulated Microgravity." *Journal of Tissue Culture Methods* 14, no. 2 (1992): 51–57.

[14] Liu, T., X. Li, X. Sun, X. Ma, and Z. Cui. "Analysis on Forces and Movement of Cultivated Particles in a Rotating Wall Vessel Bioreactor." *Biochemical Engineering Journal* 18, no. 2 (2004): 97.

[15] Hammond, T.G., and J.M. Hammond. "Optimized Suspension Culture: The Rotating-Wall Vessel." *American Journal of Physiology* 281(1) (2001): F12.

[16] Nauman, E.A., C.M. Ott, E. Sander, D.L. Tucker, D. Pierson, J.W. Wilson, and C.A. Nickerson. "Novel Quantitative Biosystem for Modeling Physiological Fluid Shear Stress on Cells." *Applied and Environmental Microbiology* 73, no. 3 (2007): 699.

[17] Galie, P.A., D.H. Nguyen, C.K. Choi, D.M. Cohen, P.A. Janmey, and C.S. Chen. "Fluid Shear Stress Threshold Regulates Angiogenic Sprouting." *Proceedings of the National Academy of Sciences of the United States of America* 111, no. 22 (2014): 7968–7973.

[18] Zeng Y., Y. Shen, X.L. Huang, X.J. Liu, and X.H. Liu. "Roles of Mechanical Force and CXCR1/CXCR2 in Shear-Stress-Induced Endothelial Cell Migration." *European Biophysics Journal* 41, no. 1 (2012): 13–25.

[19] Wuest, S.L., S. Richard, I. Walther, R. Furrer, R. Anderegg, J. Sekler, and M. Egli. "A Novel Microgravity Simulator Applicable for Three-Dimensional cell Culturing." *Microgravity Science and Technology* 26 (2014): 77–88.

[20] Kaurov, V. "Lissajous Patterns on a Sphere Surface." http://demonstrations.wolfram.com/LissajousPatternsOnASphereSurface/WolframDemonstrations Project Published: July 1, 2011.

[21] Anken, R.H., U. Baur, and R. Hilbig. "Clinorotation Increases the Growth of Utricular Otoliths of Developing Cichlid Fish." *Microgravity Science and Technology* 22 (2010): 151–154.

[22] Barjaktarovic, Z., A. Nordheim, T. Lamkemeyer, C. Fladerer, J. Madlung, and R. Hampp. "Time-Course of Changes in Amounts of Specific Proteins Upon Exposure to Hyper-g, 2-D Clinorotation, and 3-D Random Positioning of Arabidopsis cell cultures." *Journal of Experimental Botany* 58 (2007): 4357–4363.

[23] De Micco, V., M. Scala, and G. Aronne. "Effects of Simulated Microgravity on Male Gametophyte of Prunus, Pyrus, and Brassica species." *Protoplasma* 228 (2006): 121–126.

[24] Wei, N., C. Tan, B. Qi, Y. Zhang, GX Xu, and H.Q. Zheng. "Changes in Gravitational Forces Induce the Modification of Arabidopsis Thaliana Silique Pedicel Positioning." *Journal of Experimental Botany* 61 (2010): 3875–3884.

[25] Zyablova, N.V., Y.A. Berkovich, A.N. Erokhin, and A.Y. Skripnikov. "The gravitropic and phototropic responses of wheat grown in a space greenhouse prototype with hemispherical planting surface." *Advances in Space Research* 46 (2010): 1273–1279.