Chapter 2
Methods for Gravitational Biology Research

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Abstract To study the impact of gravity on living systems on the cellular up to the organismic level, a variety of experimental platforms are available for gravitational biology and biomedical research providing either an almost stimulus-free microgravity environment (near weightlessness) of different duration and boundary conditions. The spectrum of real-microgravity research platforms is complemented by devices which are used to either increase the gravity level (centrifuges) or modify the impact of gravity on biological systems (clinostats and random-positioning machines)—the so-called ground-based facilities. Rotating biological samples horizontally or in a two- or three-dimensional mode is often used to randomize the effect of gravity in the attempt to eliminate the gravity effect on sensing mechanisms and gravity-related responses. Sophisticated centrifuges have been designed allowing studies from cells up to humans, either on ground under hypergravity conditions ($>$ 1 g) or in space, where they offer the chance to stepwise increase the acceleration force from 0 g (microgravity) to 1 g or higher and *vice versa*. In such a way, centrifuges are used to determine threshold values of gravisensitivity and to unravel molecular and cellular mechanisms of gravity sensing and gravity-related responses. By using the whole spectrum of experimental platforms, gravitational biologists gain deep insight into gravity-related biological processes and continuously increase our knowledge of how gravity affects life on Earth.

Keywords Clinostat · Random Positioning Machine · Parabolic flight · ISS · Space shuttle · Satellite

2.1 Introduction

Gravity is a unique environmental stimulus, constantly acting, thus, having shaped life during evolution. Consequently, the question arises about its impact, how it affects fundamental physiological processes. Most organisms have developed a specific gravisensor system and use gravity for orientation, but gravity also generally affects physiological, cellular and molecular processes, both best investigated in the

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stimulus-free environment of space in the absence of gravity-induced phenomena such as sedimentation, buoyancy and convection.

Studies in microgravity opened new scientific perspectives and a new field of experimentation. We are now able to bring an organism in a very new and unique environment that it has not yet experienced before. What does this mean for the organism? Does it cause stress when you take away an environmental cue that was used for orientation? Does an organism experience less stress when a structure which has been under tension is now relaxing or which might have sedimented on membranes will now float in the absence of gravity? What does a three-dimensional free-floating environment mean for a cell which has before been attached to a layer? What is the effect of mechanical unloading with respect to function and differentiation? How quickly will a biological system respond, will it adapt, what is the minimum amount of gravitational acceleration necessary to initiate a gravity-related response? Are there sensitive windows in development during which an organism is more sensitive to changes of environmental parameters like gravity? In order to address these fundamental questions which also bear—as we will see later—application potentials, dedicated platforms for such research are needed.

Due to the presence of masses in our universe, it is impossible to achieve zero gravity (real weightlessness). Even on the ISS circling Earth at an altitude of about 350 km, the level of gravity is only 8% less than on the Earth's surface. It is the velocity of the space station that creates a centrifugal force which exactly compensates the centrifugal force of the gravitational pull of the Earth that results in a free-fall situation which we call microgravity (near weightlessness) due to some residual acceleration forces in a range of 10^{-2}–10^{-6} g.

Limited access to space flight and high costs motivated developments to achieve—to at least to some extent—microgravity conditions on ground. Today, ground-based studies have great importance in gravitational space biology and human physiology research. They increasingly contribute to our understanding of how biological systems (from cells to humans) sense gravity and to study the consequences when the influence of this fundamental force is lacking, to study the impact on health and signaling cascades, but also to study adaption mechanisms to this new environmental condition. In this chapter, we give an overview of available ground-based microgravity simulators and platforms providing increased gravity levels, rounded up by a comprehensive summary of platforms enabling unique experimentation in real microgravity. We will focus on the underlying principles, boundary conditions and the experimental possibilities.

2.2 Microgravity Simulators—Efforts to Mimic the Effects of Weightlessness

2D, 3D, fast and slow rotating clinostats, rotating wall vessel, magnetic levitator, Random Positioning Machine—if you are looking for devices in order to mimic the effects of weightlessness on ground you will find a catalogue of possibilities. How

do they differ, what is the method of choice, what is the best suitable facility for my scientific object and question?

The idea to alter the influence of gravity and study the impact on basic biological mechanisms is quite old and started experimentally at the end of the eighteenth century with plants due to their easy observable gravitropic responses. By putting them horizontally the restoration of their original growth direction by gravitropic responses becomes obvious—roots growing downwards and shoots growing upwards. If such kind of arrangement is equipped with a motor and the plant is rotated around an axis perpendicular to the gravity vector, the unidirectional influence of gravity is turned into an omnilateral stimulation which in many cases abolishes the gravitropic response. Under optimal conditions the gravitropic stimulus is neutralized, like in real microgravity, a situation called simulated weightlessness (microgravity). In both cases a plant will no longer show gravitropism, but what has happened with respect to the underlying gravisensory mechanism—is it permanently stimulated or does it receive no further input? We will come back to this point later. Such kind of experimental arrangement is called a clinostat.

A 2D clinostat has only a single rotation axis. Wolfgang Briegleb used the 2D clinostat principle for studying the effect of weightlessness on small plants and animals and cells. He postulated that speeding up and thus transforming a slow rotating clinostat, normally rotated with 1–2 rpm (revolutions per minute), into a fast-rotating one (in the range of 60–90 rpm) will optimize the simulation of weightlessness conditions (Briegleb 1988; Klaus et al. 1998). Furthermore, not only speed but also the effective radius (diameter) has to be considered. Under optimal conditions, the diameter of the sample containers is kept small (in the range of a few mm) and the objects are placed in the center of rotation in order to keep residual accelerations as minimal as possible. The latter concerns thresholds for gravity stimulus perception of the respective organism, which are in most cases not known. A 2D clinostat constantly runs in one direction inducing a static change of the gravity vector in relation to the sample. Sedimentation is thereby prevented and small bodies (e.g. single cells or statoliths within cells) describe floating circles in the media comparable to the floating conditions in real microgravity. The speed of rotation determines the circles' diameter; the faster the rotation, the smaller the circles; too fast rotation, however, results in radial accelerations. Let us transfer this idea to statoliths in roots or rhizoids and imagine their movements depending on the speed of rotations. Having done so, Hensel and Sievers (1980) demonstrated by morphological studies of slowly clinorotated roots (1–2 rpm) strong damages of the statocytes on the ultrastructural level, e.g. revealed by a considerable increase of the lytic compartment. They related these changes to the continuously changing direction of the gravity vector, which is different to the situation in real microgravity.

2D clinostats have been adapted to several experimental demands (for review see Brungs et al. 2016): clinostats for suspended or adherent organisms and cell cultures, for aquatic systems and in combination with online analyses using photomultipliers or microscopy (Fig. 2.1).

Assuming that two rotation axes provide more complex ways to average the influence of the gravity vector and simulate weightlessness more perfectly, 3D

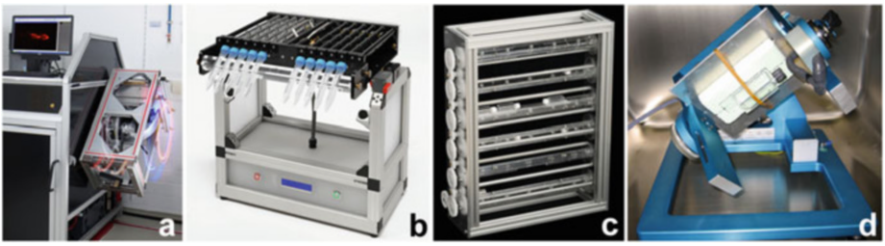

Fig. 2.1 Examples of ground-based facilities to simulate microgravity conditions: Various 2D clinostats based on the principle of fast and continuous clinorotation around one axis of rotation: (**a**) live-cell imaging fluorescence clinostat microscope, (**b**) pipette-based clinostat for the exposure of cell suspensions, (**c**) slide-flask clinostat for adherent cell cultures. Random-positioning machine mostly used in a random speed and random direction operational mode, resulting in a disorientation of the exposed samples (**d**)

clinostats and the Random Positioning Machines (RPM) have come into use. They are characterized and operated with two independently rotating frames mounted in a gimbal manner (Hoson et al. 1996; van Loon 2007). An algorithm controls the motors with respect to acceleration or directional changes. Commonly, 3D clinostats are continuously rotating but changing the velocity at random. In a RPM, not only the velocity but in addition also the direction of rotation is randomized.

Comparative studies—also in real microgravity—are necessary to understand the differences and to validate the quality of the simulation (Herranz et al. 2013). Here, we will give some examples to demonstrate this kind of approach.

To critically assess the assumption whether a second rotation axis and sophisticated modes of operation provide a more perfect simulation, Krause et al. (2018) studied the dynamics of the actin-dependent movements of statoliths in the rhizoids of *Chara* (cf. Chap. 4). The role of gravity in this process was already investigated in real microgravity in a MAXUS sounding rocket mission; thus, data for verification and validation were available and could be compared to data from 2D and 3D clinorotation. Fast rotational speeds in the range of 60–85 rpm in 2D and 3D modes resulted in a similar kinetics of statolith displacement as compared to real μg, while slower clinorotation (2–11 rpm) caused a reduced one. The addition of a second rotation axis clearly did not increase the quality of microgravity simulation, however, increased non-gravitational effects such as an increase in the level of vibration (with multiple potential side effects). Thus, for *Chara* rhizoids, fast 2D clinorotation is the most appropriate microgravity simulation method for investigating its graviperception mechanism.

Hauslage et al. (2017) visualized shear and hydrodynamic forces in various ground-based facilities by using dinoflagellates as bioassay and mechanosensitive reporter systems. *Pyrocystis noctiluca* populations were exposed on a Random Positioning Machine either operating as 2D clinostat (constant rotation around one axis with 60 rpm) or in a random positioning mode (two axes with random velocity and direction). Shear stress due to hydrodynamic forces leads to a deformation of

the cell membrane, induces intracellular signaling triggering an increase in cytosolic Ca^{2+} and in turn the reaction between luciferin and luciferase resulting in the emission of light. Thus, the signal intensity provides valuable information about the shear stress induced by the different microgravity simulation methods. The data show that exposure on the RPM resulted in a higher mechanical stress for the dinoflagellates than during constant clinorotation (Fig. 2.2). This proved 2D clinorotation as a low shear stress environment.

Rotating wall vessels (RWVs) or rotating bioreactors are further methods which are frequently being used to neutralize sedimentation in aquatic systems. Although these methods have been widely used for studying cell cultures, protists and other small aquatic organisms, its ability to mimic weightlessness still has to be demonstrated by comparative studies in real microgravity (Schwarz et al. 1992).

Magnetic levitation is offered as a further approach to achieve microgravity in a ground laboratory. However, in the case of biology, the effects of the magnetic

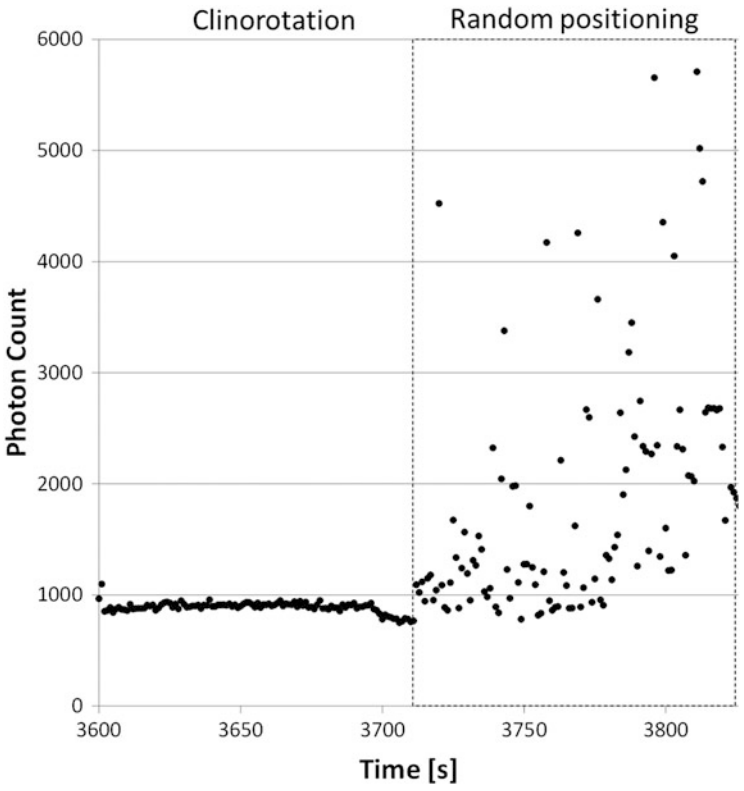

Fig. 2.2 The capacity of bioluminescence as a result of a mechanical stimulation of dinoflagellates was used as bioassay. Exposure of *Pyrocystis noctiluca* on a rotating device, either operated as 2D clinostat (constantly running around one axis) or as random positioning machine (rotating around two axes at a random speed and random direction mode) revealed a differential stress response indicated by the number of photons emitted, modified after Hauslage et al. (2017)

field itself have to be considered, which had a strong impact on the alignment of gravitactic organisms such a *Euglena* and *Paramecium* (Hemmersbach et al. 2014) and mask the expected behavior known from experiments in real microgravity (Häder et al. 1990; Hemmersbach-Krause et al. 1993) (cf. Chap. 3).

2.3 Centrifuges—The Benefit of Hypergravity in Gravitational Biology Research

Centrifugation is an experimental approach to artificially and experimentally increase the influence of gravitational acceleration greater than the one normally acting on the surface of Earth (1 g). Hypergravity is a tool to obtain new insights into gravity-related molecular and physiological mechanisms. Centrifugation experiments are widely used as controls to study the effects of launch, reentry and landing accelerations of space vehicles like rocket payloads and reentry satellites, which allows discriminating between these effects and the one of the microgravity conditions. Furthermore, the potential of hypergravity as a countermeasure method against negative physiological adaption processes of the human body to long-term microgravity, such as a strong bone and muscle loss and decrease in cardiac functioning, is under investigation.

In gravitational biology, different centrifuge designs are being used, operating at low speed and thus physiological range up to 20 g, different to usual laboratory centrifuges, where high accelerations are applied for separation during sample preparation. Custom-built desktop centrifuges are appropriate to culture cells and developing plants or (small) animals, while larger samples, increased hardware or instruments for online analyses demand large centrifuges (for review see Frett et al. 2016). Centrifuges used in space provide an appropriate in-flight 1-g reference control in close vicinity to the samples which are kept under microgravity. Consequently, experimental as well as control samples experience identical environmental conditions such as vibration and radiation besides gravity, which facilitates the identification of gravity-related responses. Centrifuges in space are also in use for determination of thresholds of gravity-related processes. Examples are the STATEX hardware including a centrifuge for the performance of an experiment addressing the development of the vestibular system of toads and fish under microgravity (Neubert et al. 1996). NIZEMI (Niedergeschwindigkeits-Zentrifugen-Mikroskop), a slow rotating centrifuge microscope operated in space during the IML2 mission in 1994 and later on ground (Friedrich et al. 1996) clearly demonstrated the existence of thresholds in plants and microorganisms in the range of 0.1–0.3 g.

1-g reference centrifuges and threshold centrifuges are also operated in the drop tower or parabolic flights of airplanes and rockets (TEXUS, MAXUS) as well as in the ESA's European Modular Cultivation System EMCS and ESA's BIOLAB, both facilities aboard the ISS and designed to carry out experiments in biomedical

research (Brinckmann 2005). The fact that thresholds for gravity-related responses exist, as revealed by centrifuge experiments in space, indicate physiological rather than a pure passive mechanism of a response which was e.g. a long-lasting debate in case of gravitaxis of protists (unicellular organisms) (see Chap. 3). Furthermore, it shows that we cannot just simply extrapolate data obtained in hypergravity to "0 g" and predict the result. Large centrifuges such as the Short Arm Human Centrifuge at DLR meet the demands of life science researchers for complex training and biomedical examinations under hypergravity conditions. Such a large centrifuge system provides a great platform for biomedical and neurophysiological research, but is also used by cell biologists, who started using it to operate various instruments like a life cell imaging microscope under hypergravity conditions.

With respect to exploration and the need of closed biological life support systems, plants and (lower) animals might become essential parts of bioregenerative life support systems in order to provide nutrients and energy on long-term missions or on other planets. It is therefore of great importance to investigate the effects of lunar (ca. 0.16 g) or Martian (ca. 0.38 g) gravity on plant/animal metabolism, growth, proliferation and development as well as on human beings. Centrifugation in space provides these conditions. EU:CROPIS, a compact satellite scheduled to fly in 2018, will provide lunar and Martian gravity conditions for 6 months each to study the impact of these gravity levels on the performance of a biological life support system, further equipped with a special trickling filter unit for urine degradation, a food production unit and a *Euglena* compartment for oxygen production (cf. Chap. 8).

2.4 From Drop Tower to ISS—Biology in Free Fall

Several excellent experimental platforms offer real microgravity conditions for gravitational biology research: drop towers, parabolic flights, sounding rocket flights, satellites and the International Space Station ISS (Fig. 2.3). They differ in the time of microgravity provided and the quality of microgravity that can be achieved. Thanks to the excellent cooperation between the scientists, technicians and astronauts highly sophisticated research hardware has been developed and operated in space. After several decades of space biology research, nowadays, a huge amount of experience is available for performing biological experiments on cells, tissues, and organisms including human beings. Cultivation and fixation, life support systems as well as online microscopic and kinetic studies are daily work for the astronauts on the ISS. This chapter briefly describes the platforms that provide real microgravity conditions with special focus on their characteristics and boundary conditions. Some of them even provide opportunities for student experiments such as the "Drop, Fly, and Spin your Thesis" or the REXUS/BEXUS student rocket and balloon program at Esrange near Kiruna in Northern Sweden, jointly organized by the Germany DLR Space Administration and the Swedish National Space Board.

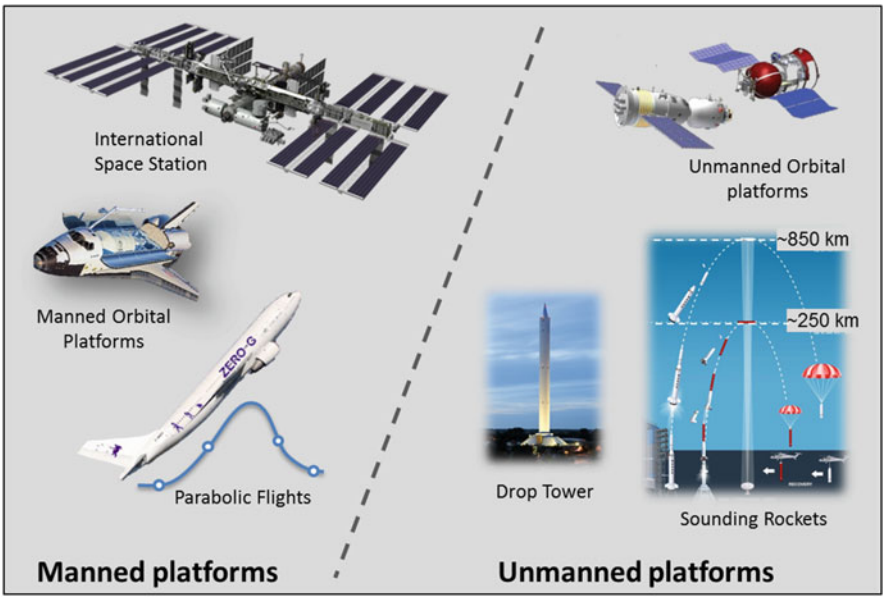

Fig. 2.3 Manned and unmanned Platforms providing real microgravity conditions

2.4.1 Drop Tower

A high quality of microgravity of about 10^{-6} g is achieved during the 110 m free fall in the vacuum tube of the drop tower at the Zentrum für Angewandte Raumfahrttechnologie und Mikrogravitation (ZARM) in Bremen, Germany. Favorable features are the daily accessibility, multiple launches and drops per week, thereby, guaranteeing a sufficient amount of replicates and a flexible experiment adaptation. Microgravity period of 4.74 s or even 9.3 s, the latter by using a catapult system, provide ample time for a surprisingly great number of fast cell biological and physiological processes (Könemann et al. 2015). Although an experiment time of 4.74 s is rather short, the fact that no hypergravity phase is involved is of great advantage especially for sensitive and quickly responding processes.

Using the catapult, however, unavoidably comes with an initial phase of high accelerations of up to 30 g for 0.26 s, to propel the drop capsule up into the evacuated tower shaft. These boundary conditions have to be taken into account and their effects on the biological sample have to be carefully assessed by control experiments. Biologists are frequently using the drop tower e.g. to study fast molecular, cellular and physiological responses of cells and organisms after the offset of gravity.

2.4.2 Parabolic Plane Flights

Microgravity times of several minutes are provided by very special parabolic flight maneuvers with airplanes. The term parabolic flight describes a flight maneuver that enables an aircraft, rocket or spacecraft to follow a free-fall ballistic Keplerian trajectory (Ruyters and Friedrich 2006b; Pletser et al. 2015). Parabolic flights of aircrafts have become a working horse for training astronauts, hardware testing and preparation for ISS experiments as well as for stand-alone biomedical and psychological experiments on human subjects and a broad variety of biological experiments.

Since 2015, the Airbus A310 ZERO-G, which replaced the old A300 Zero-G aircraft, is the largest aircraft for European microgravity research (Pletser et al. 2015). It is operated by Novespace, a subsidiary of the French National Space Center (CNES), with the European Space Agency ESA and DLR, the German Space Administration, as frequent customers. Usually, a parabolic flight campaign consists of 31 parabolas flown at each of the three consecutive flight days. In total, 93 microgravity (μg) phases of approximately 22 s add up to 10 min of μg with a residual acceleration of about 10^{-2} g. The parabolic flight maneuvers can be flown in such a way that partial g-levels in the range of lunar (0.16 g) and Martian (0.38 g) gravity are achieved for approx. 22 s (Pletser et al. 2012). Of great advantage for scientists is the fact that the investigator can bring basically his own familiar—even bigger—lab equipment on board and can perform the experiment himself during the flight, thus, being able to monitor and operate his experiment, change experimental parameters or the experimental set-up during the flight. A major disadvantage might be the flight profile consisting of alternating phases of hypergravity phases of up to 1.8 g, microgravity and 1 g acceleration in between the parabolic flight phases. A careful assessment of the results and proper control experiments are necessary to distinguish clearly between microgravity-induced effects, hypergravity effects and the effects of vibrations. Nevertheless, these microgravity periods are sufficient to address numerous questions in the area of gravitational biology ranging from the impact of microgravity on the cellular level, impacts on physiological parameters and the behavior of organisms up to biomedical and neurobiological studies on human subjects in microgravity.

2.4.3 Sounding Rockets and Suborbital Platforms

Microgravity in the range of minutes is provided by parabolic flights of rockets like MASER, TEXUS and MAXUS (Ruyters and Friedrich 2006a; Seibert and Battrick 2006). Today, sounding rockets are frequently used in microgravity research all over the world. In Germany, the TEXUS Sounding Rocket Programme (Technologische EXperimente Unter Schwerelosigkeit) started 1977 and 56 TEXUS rockets have

been launched until 2018. In 1990, the first European sounding rocket MAXUS was launched, followed by the first MiniTEXUS rocket in 1993. With Mapheus (Materialphysikalische Experimente unter Schwerelosigkeit), DLR started another rocket program in 2009. The research rockets are launched from the European rocket launch site ESRANGE near Kiruna in Northern Sweden. On its ballistic flight, microgravity conditions in a range of 10^{-4} g last for about 3.5 min on a MiniTEXUS flight, about 6 min on a TEXUS/Mapheus flight and about 13 min on a MAXUS flight. The payload part of the rocket consisting of the Experiment Modules and the Recovery and Service System decends on a parachute and samples are transported back to the science labs at the launch site within 1–2 h by helicopter. Scientists can directly monitor and control their experiments via telecommanding. Especially appreciated by biologists is the late access allowing a loading of samples into the payload only a few hours before launch and early retrieval of samples after landing. Hence, a broad spectrum of basic biological and biomedical research was performed with a variety of organisms, in most cases accompanied by centrifugation control experiments to assess the influence of launch vibrations and accelerations in a range of 6–12 g.

Alternatively, longer and more flat parabolas with a suborbital trajectory up to an altitude of 100 km can be flown providing a continuous microgravity environment for about 3 min. With such suborbital platforms, several US companies are aiming to make microgravity experience available to space tourists. Such a commercial platform might also add to the spectrum of microgravity research platforms; however, the reliability and acceptability for space biology experiments still needs to be demonstrated. Some considerations on this topic have been published e.g. by Karmali and Shelhamer (2010).

2.4.4 Orbital Platforms—Space Shuttle, Satellites and the International Space Station

Long-term effects of microgravity in the range of weeks or months can only be studied in low Earth orbit on board of satellites and human-tended space laboratories such as NASA's Space shuttles (which retired in 2011), Russian space stations and the International Space Station ISS. In 1957 the dog Laika was the first animal astronaut that surrounded Earth onboard a Sputnik-2 satellite. Laika did not survive but two other dogs following in Sputnik-5 provided evidence that living organisms can survive in low Earth orbit. Since then, numerous Russian Bion satellites and American biosatellites housed a great variety of animals like snails, worms, spiders, bees, frogs, fish, birds, mice and rats and all kinds of higher plants, fungi, lichens, mosses, ferns and microorganisms. Even complex live support systems have been developed and tested, which will increasingly become important

to complement physico-chemical life-support systems on future long-term space missions (cf. Chap. 9).

Satellites provided research platforms to study the effects of space conditions, mainly weightlessness and radiation, on living organisms. Encouraged by the first results, in 1961 Jurij Gagarin became the first human astronaut flying and surviving in low Earth orbit 300–400 km above the ground. Today, human beings have been working and living in space well protected from the harsh conditions of space—vacuum, extreme temperatures and high radiation exposure—in different kinds of return capsules, in Saljut and Kosmos space stations, Skylab, Space Shuttles, the Russian space station MIR and more recently in the Chinese space lab Tiangong. Microgravity research in space in the field of human physiology, neuroscience, animal physiology, plant biology, radiation biology, astrobiology, exobiology and microbiology always was and will be very much relying on international cooperation, mostly unimpressed by short-lived political issues.

The International Space Station is the largest international science and technology project ever undertaken representing the by far largest microgravity research platform that ever existed involving the United States, Russia, Japan, Canada and 10 member states of the European Space Agency including Germany, France and Italy. Based on the political decision for a symbol of international peaceful cooperation in the Low Earth orbit, all Space Station partners have invested greatly in this unique endeavor. Although the various space agencies may emphasize different goals and research objectives in the use of the ISS, they are all unified in the (1) recognition of the ISS as an education platform to encourage, inspire and ultimately motivate today's youth to pursue careers in math, science and engineering, (2) advancement of knowledge in all areas of human physiology, biology, material and physical sciences in a very unique radiation, microgravity and isolation environment and (3) translation of that knowledge to health, advanced product developments, socio-economic and environmental benefits to our lives on Earth.

For a general description and information on the ISS check the following DLR, ESA, NASA websites. A list of all research facilities onboard ISS is continuously updated at http://www.dlr.de/dlr/desktopdefault.aspx/tabid-10301/460_read-534/#/gallery/503, http://www.esa.int/Our_Activities/Human_Spaceflight/International_Space_Station, https://www.nasa.gov/mission_pages/station/main/index.html. The assembly of the ISS started in 1998 and was completed in 2010 providing pressurized modules developed by the NASA (USA), Roscosmos (Russia), JAXA (Japan) and ESA (Europe) and external platforms for science, technology demonstrations, education and a test bed for human space exploration beyond the low Earth orbit.

A great number of various types of specific racks offer experimental conditions and equipment for systematic studies in long-term microgravity. NASA provides utilization statistics and a history of research projects at the following website: http://www.nasa.gov/pdf/695701main_Current_ISS_Utilization_Statistics.pdf. Updates

on ISS activities, research and accomplishments can be found at: http://www.nasa.gov/mission_pages/station/main/index.html. For more detailed information on European participation and facts about the ISS please check: http://www.esa.int/Our_Activities/Human_Spaceflight/International_Space_Station/About_the_International_Space_Station. The Erasmus Experiment Archive is ESA's database for European funded or co-funded experiments not only on ISS but also on other microgravity platforms and in microgravity ground-based facilities: http://eea.spaceflight.esa.int/portal.

Due to the fact that spaceflight-related projects are costly and research opportunities are scarce, great efforts are undertaken to coordinate scientific utilization of the ISS in a most efficient way by coordination through international and bilateral working groups consisting of the Space Station partners and other leading space agencies like DLR (Germany), CNES (France) and ASI (Italy).

Since the ISS is the only available platform of its kind with regard to humans as subjects for health-related and fundamental biological research, the long-term microgravity, isolation and radiation environment, sophisticated research facilities with significant power and data resources, highly efficient and extensive utilization and exploitation of this unique research platform are essential for the next decade—and are mandatory for preparing human exploratory missions to Moon and Mars and beyond.

2.5 Conclusions

In the last decades, our knowledge in the field of gravitational biology has made considerable progress thanks to an increasing number of microgravity platforms providing almost stimulus-free environments of different quality and duration. Microgravity research opportunities, however, are still rare, costly and require a complex organization, preparation and in most cases highly specific experiment hardware for habitation, cultivation, fixation and sample analyses—well adapted to the respective platform. Various microgravity simulation methods complementing the real microgravity platforms have been invented for gravitational biology research aiming to neutralize the effects of gravity on biological systems and alter gravity conditions on ground. These methods are valuable tools for stand-alone experiments, for proving new concepts and hypotheses, preparing microgravity experiments, verifying microgravity results and testing hardware. However, thoroughly assessing all kinds of side effects and boundary conditions is required for each biological sample. With the availability of space stations like the ISS and future stations in low Earth orbit and beyond, the way has been paved for long-term experimentation in microgravity yielding great opportunities for unraveling the impact of gravity on life on Earth and preparing humans to explore the solar system.

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