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EXPERT REVIEW

Remote Controlled Autonomous Microgravity Lab Platforms for Drug Research in Space

Shimon Amselem [1,2]

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ABSTRACT Research conducted in microgravity conditions has the potential to yield new therapeutics, as advances can be achieved in the absence of phenomena such as sedimentation, hydrostatic pressure and thermally-induced convection. The outcomes of such studies can significantly contribute to many scientific and technological fields, including drug discovery. This article reviews the existing traditional microgravity platforms as well as emerging ideas for enabling microgravity research focusing on SpacePharma's innovative autonomous remote-controlled microgravity labs that can be launched to space aboard nanosatellites to perform drug research in orbit. The scientific literature is reviewed and examples of life science fields that have benefited from studies in microgravity conditions are given. These include the use of microgravity environment for chemical applications (protein crystallization, drug polymorphism, self-assembly of biomolecules), pharmaceutical studies (microencapsulation, drug delivery systems, behavior and stability of colloidal formulations, antibiotic drug resistance), and biological research, including accelerated models for aging, investigation of bacterial virulence , tissue engineering using organ-on-chips in space, enhanced stem cells proliferation and differentiation.

KEY WORDS lab-on-chips · microgravity research · nanosatellites · organ-on-chips · parabolic flights

Guest Editors: Sara Eyal and Hartmut Derendort

✉ Shimon Amselem
amselem.shimon@space4p.com

[1] SpacePharma
Herzeliya, Israel

[2] SpacePharma
1st Aba Even Av., Herzliya Pituach, Israel & Rue l'Armeratte 3
2950 Courgenay, Switzerland

ABBREVIATIONS

2D	Two-dimensional
3D	Three-dimensional
API	Active pharmaceutical ingredients
CASIS	Center for the Advancement of Science in Space
CNES	Centre National d'Etudes Spatiales
CS	Colloidal systems
CSA	Canadian Space Agency
DLR	Deutschen Zentrums für Luft- und Raumfahrt
ESA	European Space Agency
GH	Growth hormone
hBTSCs	Human biliary tree stem/progenitor cells
hMSC	Human mesenchymal stem cells
ISRO	Indian Space Research Organization
ISS	International Space Station
JAXA	Japan Aerospace Exploration Agency
LRRK2	Leucine-rich repeat kinase 2
MEPS-II	Microencapsulation electrostatic processing system-II
MG	Microgravity
MRSA	Methicillin-resistant *Staphylococcus aureus*
NASA	National Aeronautics and Space Administration
NCATS	National Center for Advancing Translational Sciences
NIH	National Institutes of Health
OOC	Organ-On-Chip
RPM	Random positioning machine
RWV	Rotating wall vessel
SMG	Simulated microgravity
SPAD	SpacePharma advanced microgravity lab
SPmgLab	SpacePharma microgravity lab
TH	Tyrosine hydroxylase.

INTRODUCTION

The microgravity environment of space provides unique conditions for better understanding of physiologic and pathologic processes and has a substantial scientific, technological and commercial potential. Studying the physical chemistry of macromolecules in reduced-gravity environments enables research in the absence of gravity-induced surface constraints, convection, shear forces, sedimentation/stratification, and hydrostatic pressure. This results in much higher-resolution, 3D maps of the structure of drugs, vaccines and binding sites (1,2). Biological systems have also been shown to be modulated in space; under conditions of microgravity, aging and pathological processes may be accelerated (3–7). In addition, bacterial virulence, pathogenicity and resistance to antibiotics have been shown to increase in space (8). Hence, the knowledge gained through microgravity research can facilitate drug screening and improve drug design, delivery, and storage, thereby contributing to the development of new technologies and therapeutic products (9,10).

Given that the commercialization of space involves the pharmaceutical industry, the use of microgravity as a research tool in life sciences is expected to expend in the near future. Biopharma companies have a clear incentive to use the free-fall environment as a catalyst for accelerated models of disease onset and progression. Drug companies have already been performing drug research on accelerated models for osteoporosis and muscle atrophy, protein crystallization, vaccine development, colloidal formulations and other fields of research (9).

In this article the traditionally available microgravity platforms as well as emerging microgravity enabling tools for drug research are reviewed. A special emphasis is put on novel miniaturized, unmanned, remote-controlled microgravity lab platforms based on microfluidics and lab-on-chips that have been recently launched successfully to space on nanosatellites. Key life science fields that can significantly benefit from using these platforms are described.

TRADITIONAL MICROGRAVITY PLATFORMS

Microgravity research has been dominated by a limited number of solutions: ground simulators, drop towers, parabolic flights, sounding rockets, short-duration orbital platforms (*e.g.* dedicated Foton capsules flights), and long-duration orbital platforms, mainly the International Space station (ISS). On Earth, brief courses of free falls, *e.g.* by using parabolic airplane flights and drop towers, can generate short-term approximate weightlessness. However, prolonged periods of microgravity can be achieved only in space, for example, on satellites and space stations (11).

The methodology of achieving microgravity conditions for scientific experimentation depends on the type of research as well as the desired level of gravity and duration of the study. The following sections provide an overview of the currently available microgravity platforms.

Random Positioning Machine

The random positioning machine (RPM) is a two-axis version of a clinostat which has been used for microgravity simulation and hardware testing (12). A typical RPM system comprises two independently motor-driven frames (Fig. 1a) that constantly reorient the samples within the inner frame. The average trajectory over time of the gravity vector is randomly distributed across directions and is thus expected to converge towards zero. The microgravity is in the range of 10^{-2}-10^{-3} g. The RPM is typically applied to processes on the timescale of hours or longer, including mammalian cells behavior in microgravity (Table I).

RPMs can reproduce effects that have been observed in space. However, some studies yielded cellular effects ranging from those obtained in real microgravity to those of the ground control conditions (13). Hence, the RPM serves as an ideal and important preliminary, ground-based microgravity screening tool prior to conducting live science experiments in space. Advances in RPM engineering make it suitable for novel applications, *e.g.*, 3D cell culturing and tissue engineering (13).

Rotating Wall Vessel

The rotating wall vessel (RWV) is an additional ground-based simulator of microgravity that has been utilized by NASA since the early 1990s. The RWV consists of a chamber that rotates around an axle and its vessel can contain culture medium and cells (Fig. 1b). As the rotation velocity of the fully filled vessel increases, relative fluid motion gradually halts (14). The rotation of the media carries cells that begin falling toward the vessel bottom back upward thereby keeping them suspended in an orbital path. Hence, the cells can attach to each other to form 3D cultures but do not attach to the chamber walls because they are subjected to a continuous free fall (15). The RWV bioreactor effectively simulates two key aspects of the microgravity culture environment: 1) a continuous suspension condition and 2) an environment of minimized turbulence and shearing forces. The RWV bioreactor has been increasingly used in studies of microbial responses (16) (Table I).

Drop Towers

Drop towers are vertical structures that allow free fall of payloads to generate microgravity conditions, the duration of

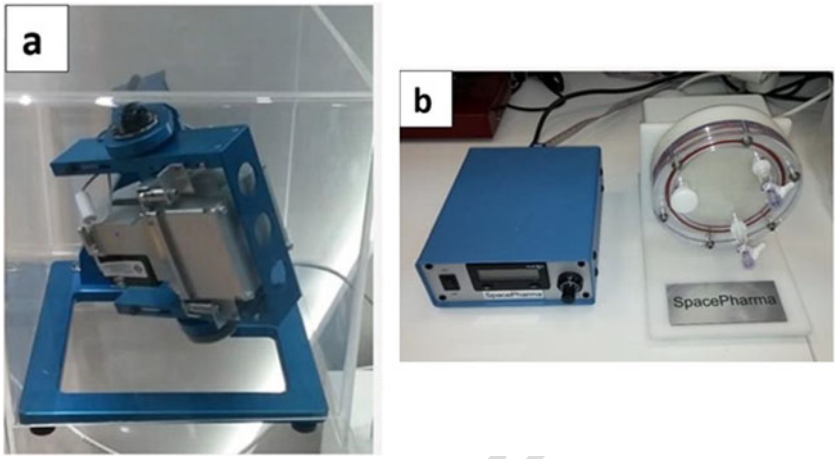

Fig. 1 Microgravity Ground Simulators. (**a**) A Random Positioning Machine (RPM) holding a SPmgLab microgravity lab. (**b**) A Rotating Wall Vessel (RWV).

which is determined by the tower height. The 10^{-3} g microgravity level that could initially be achieved has been improved to the current level of 10^{-5} g of most drop towers using techniques to counter the effect of acceleration (11).

Several countries have constructed drop towers to enable microgravity experiments on Earth. The two drop towers of the USA (24 m and 142 m) are located at the Lewis Research Center, Cleveland, and provide microgravity for 2.2 and 5.2 s, respectively (11). In Japan, the 490 m facility of the Microgravity Center in Kami-Sunagwa, Hokkaido, has been built at an old abandoned mine and allows a 10 s duration of free fall (11). The Bremen drop tower, unique in Europe, was built in 1990 in the University of Bremen. The height of this facility is 146 m, and it can accommodate modules weighing 250 kg. In the drop mode the capsule is released from a height of 120 m giving 4.74 s of microgravity experiment time. Since 2007, the Bremen facility also offers a catapult mode in which the capsule is catapulted vertically to the top of the tower and then drops back down the deceleration chamber. Using this mode, the microgravity experiment time can be extended to 9.3 s. Unlike the drop mode, the capsule and its enclosed experiment experience an upward acceleration of up to 35 g before the experiment begins (17,18).

Evacuation of the drop tube has improved the weightlessness level to 10^{-6} g, which is currently the best Earth-bound microgravity condition (17,18). The drop tower is suited for fast physical and biological processes, such as studies of the electrophysiology of biological membranes and gravitaxis (18) (Table I). A typical experimental "campaign" involves 10 to 15 drops.

Parabolic Flights

Flying an aircraft in a ballistic trajectory of a parabola is another platform aimed to achieve free-fall conditions. The parabolic flight maneuvers reach an altitude of at least 3 km and provide microgravity for up to 25 s (11). The parabolic segments of the flight start from a steady horizontal flight level (horizontal phase), followed by flying upwards for 20 s till the nose of the airplane is around 47° inclination (pull-up phase) with accelerations between 1.8 and 2 g. All aircraft engines thrust is then strongly reduced for about 20 to 25 s compensating the effect of air drag (parabolic free fall, which is the microgravity phase). When the aircraft dives at 42° (pull-out phase), the engines are fully powered again and another phase of 1.8–2 g for 20 s terminates the parabola to come back to the steady horizontal flight (18). The range of microgravity level is limited to approximately 10^{-2} g by aerodynamic forces and turbulences. A level of 10^{-3} g can be achieved for free floating experiments. However, the gravity of ascent during maneuvers should also be considered**.** Most parabolic flights in Europe are performed by Novespace, a subsidiary of the space agency of France CNES (Centre National d'Etudes Spatiales), using an Airbus A300 Zero-G (18). This is the only opportunity for most scientists to experience weightlessness, through participating in the flights (Fig. 2). Experiments that have been carried out in parabolic flights include studies of signal transduction in human immune cells and osteoblasts, neuronal responses in experimental animals, and protein crystallization projects (Table I).

Sounding Rockets

Sounding rockets are rockets launched on a ballistic trajectory with a free-fall in vacuum at high altitude. A two-stage sounding rocket can achieve peak altitudes over 400 km and attain for 5 to 6 min a microgravity level of 10^{-4} g. The major disadvantage is the recovery of experimental modules from remote locations and the related costs. Sounding rockets have been used for microgravity studies by the USA, Germany, France, Japan and China (11). Examples of research performed using sounding rockets are analyses of membrane transport, gene expression, signal transduction pathways, cell physiology and morphology, and biotechnological experiments (18) (Table I). As a direct consequence of the development of small launchers, an increase of the availability

t1.1 **Table I** Available Microgravity Research Platforms

t1.2 Microgravity platform	Gravity force (g)	Duration	Applicability	Examples	Limitations
t1.3 Ground simulators	10^{-2}–10^{-3}	Hours	Preliminary μG screening studies Timescale of hours or longer	Microbial responses, mammalian cell behaviour in microgravity	Cannot properly simulate μg for relatively fast molecular and cellular processes
t1.4 Free fall towers	10^{-2}–10^{-6}	2–9 s	Fast processes	Electrophysiological studies, fast gravitropic reactions in fungi	Short study duration
t1.5 Parabolic flights	10^{-2}–10^{-3}	25 s	Fast processes	As for free fall towers + signal transduction, protein crystallization studies	μg phases interrupted by phases of hyper-g accelerations
t1.6 Sounding rockets	10^{-3}–10^{-4}	Minutes	Slower processes	Gene expression & signal transduction pathways, free-flow electrophoresis	Short study duration
t1.7 International space station	10^{-5}–10^{-6}	Months	All types	All of the above and slow processes, *e.g.*, crystallization of monoclonal antibodies, identifying new drug targets in models of aging & disease	Scarce flight opportunities
t1.8 Unmanned nano-satellites	10^{-5}–10^{-6}	2–3 years	All types	All of the above, *e.g.*, protein crystallization, organs on chip and 3D cell cultures, tissue engineering	Currently limited launch opportunities, expected to expend

Adapted from Thomas *et al.* (11)

of sub-orbital flights onboard sounding rockets is expected within the next few years (19).

The International Space Station

The International Space Station (ISS) is the largest scientific and technological international cooperative program worldwide. The ISS is based on a partnership between the USA (NASA), Canada (Canadian Space Agency, CSA), European countries (the European Space Agency, ESA), Russia (Roscosmos), and Japan (Japan Aerospace Exploration Agency, JAXA) (18). The 360-ton structure orbits at an altitude of approximately 250 miles (400 km) and has more than 820 cubic meters of pressurized space which accommodates a crew of six persons and a vast array of scientific facilities. Crew members aboard the ISS conduct experiments in diverse fields, including human physiology, biology, physics, and astronomy. For more than 18 years, over 230 people from 18 countries have lived and worked continuously onboard the ISS, conducting 2400 research projects Over 200 new experiments will be launched in 2019 (20). Examples of studies conducted onboard the ISS include growing and analyzing crystals of leucine-rich repeat kinase 2 implicated in Parkinson's disease in space in order to develop drugs that target the condition more effectively (21,22), examining the physiology of aging and age-related disease progression in mice (ISS expedition duration for both projects October 2018 to April 2019) (23), and evaluating the molecular interactions and efficacy of azonafide antibody-drug conjugates in cancer cells under conditions of microgravity (expedition duration April 2017–February 2018) (24). More than 1200 microgravity-related patents were granted between 1981 and 2017, indicating value creation and signifying economic potential (25).

Today private companies offer payload services supporting experiments onboard the ISS. Examples are NanoRacks (US) which provides the NanoLab container, Space Tango (US) with its Tango Labs, Space Application Services (Belgium) with its ICE Cubes, and ISIS (The Netherlands) with the ISIS CubeSat platforms. These are three types of experimental plug-and-play modular box containers that differ in their sizes, payload cards, types of connectors and power supply, usually 1 U CubeSat research modules (10 cm × 10 cm ×10 cm) or modular combinations of that basic size that house science experiments to be run on the ISS. Within such containers, small experiments of a predefined geometry can be connected with a standardized interface to a shared power, telemetry, and a thermal management. Recently, SPACE-BD joined the list of payload services suppliers, facilitating the access of Japanese groups to the ISS.

Findings gained through studies on the ISS are expected to both provide data to support long-duration deep space missions, *e.g.*, to Mars, and benefit life on Earth. However, the ISS is expected to operate only until 2024, with the partners discussing a possible extension until 2028 (26). This, combined with limited flight opportunities available and the general trend of space commercialization, resulted in development of alternative microgravity platforms for conducting research in space. Microgravity experiment designers have been working on solving these issues by miniaturizing and automating their experiments (19).

EMERGING MICROGRAVITY PLATFORMS

While retrievable orbital payloads have simplified access to the ISS, an inherent difficulty common to those devices it is a need in the constraining manned operation by astronauts. In addition, only government space agencies have access to such research. Hence, microgravity research at the ISS is very expensive and is associated with a long waiting list from the design of the experiment until its execution. This has led yet new actors to think one step further and dissociate long-duration microgravity research from human spaceflight, by simply flying microgravity experiments on stand-alone automatic satellites**.** An illustrative example is the unmanned, autonomous, remote-controlled miniaturized microgravity lab platforms developed by our Swiss-Israeli company SpacePharma, which are described below.

Autonomous Microgravity Lab Platforms

SpacePharma's approach is to simplify the complicated process of sending experiments to space making it more accessible, affordable and valuable, by providing complimentary or alternative microgravity lab platforms that do not require human intervention. These integrated end-to-end miniaturized state-of-the-art microgravity laboratory systems operate independently through nanosatellites, on which experiments can be controlled from the ground by the scientists themselves. The platforms enable researchers to conduct reliable, repetitive, and calibrated experiments.

The first microgravity platform (SPmgLab) that was developed consists of three CubeSat units, one for the service module and two for the actual laboratory within a total dimensions of 30 × 10 × 10 cm (Fig. 3). The entire microgravity space lab is placed inside a pressurized atmospheric box (Fig. 3a). The main lab components are the plunger unit (cassette) which contains fluid reservoirs, a manifold which directs the fluid flow, an observation chamber, and a light source which is placed under it (Fig. 3b). The lab additionally contains a light microscope and a spectrometer which are placed above the observation chamber. The lab is divided into four sections (experiments), with two experiments on each cassette (Fig. 3c). Each experiment contains two reservoirs connected directly to the observation chamber, a main chamber and a third reservoir which is connected to the observation chamber through the main chamber. The observation chamber is shared for all experiments and is observed by the light microscope and the spectrometer. The fluids from the reservoirs are transported to the main chamber or to the observation chamber using a spring activated plunger. During activation of the plunger in reservoir C (containing for example a protein solution) the fluid is pushed and mixed with the fluid in the main chamber (containing an antisolvent), and together they flow to the observation chamber where the protein can crystallize. Since the observation chamber is shared, each experiment ends with a clean observation chamber because it is being flushed before the next experiment with fluid from reservoir A or B.

The SPmgLab is suitable for biochemical reactions, crystallization processes and studying colloidal systems. Once the satellite is in space, users can control their experiment using a proprietary software that can be installed on laptops and smartphones. Experimental results and data are transmitted to a ground station in Switzerland (Fig. 4) for further analysis and evaluation. The automated labs contain sensors and readers and can be used in various microgravity platforms, from ground simulators to parabolic flights, nanosatellites and the ISS (Fig. 5).

The first SPmgLab was launched to space on February 2017 from India through the Indian Space Research Organization (ISRO)'s PSLV-C37 rocket which carried the SpacePharma's DIDO-2 nanosatellite (Fig. 6). SpacePharma's DIDO nanosatellites are 3 U CubeSat satellites for micro-gravity research weighing approximately 5Kg and orbiting at an altitude of 500Km. The DIDO satellites are equipped with solar cells and batteries for power supply and communication system and contain the miniaturized and autonomous end-to-end SPLab microgravity platforms that can be remote controlled from anywhere. The DIDO-2

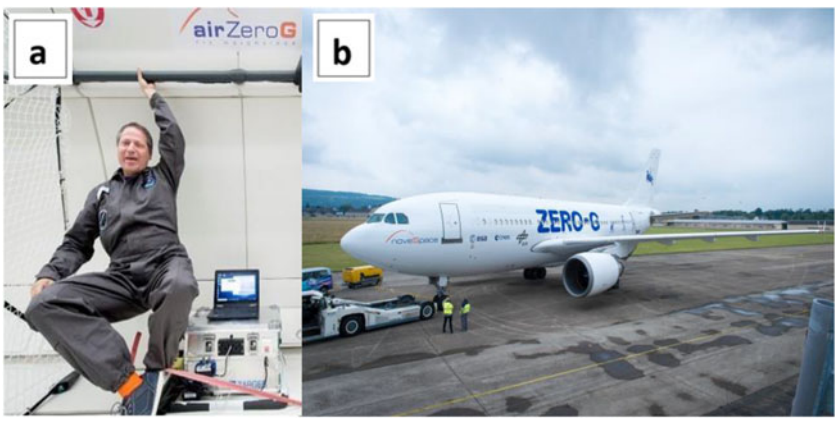

Fig. 2 SpacePharma's SPmgLab microgravity lab tested on board of an Airbus A300 NoveSpace/Zero G parabolic flight campaign above Switzerland during Swiss Parabolic Flight mission on June 2018. (**a**). A SpacePharma's engineer floating during a free fall phase of the flight. (**b**). The Airbus A300 on the ground.

nanosatellite was the first ever use of a free orbiting unmanned autonomous nanosatellite for microgravity research performing biochemical reactions and crystallization processes in space. The platform offered 380 min of satellite communication per week and 4 experiments completed with over 17,000 microscope captures and over 1000 spectrometer measurements. In this first mission, formation of crystals, kinetics of an enzymatic reaction and self-assembly of macromolecules were tested in orbit.

The company's Advanced Lab (SPAD) is a miniaturized, remote-controlled device for performing biological experiments in extreme conditions such as outer space at an altitude of above 100 km. The SPAD is a customizable, plug-and-play modular system designed to enable researchers to remotely conduct end-to-end autonomous experimentation in orbit, aboard the ISS or other extreme environments. Its modularity enables adaptation of the system to support wide range of experiments using tailor-made lab-on-chips which can include 3D cell culturing, organs-on-chip (OOC) for tissue engineering, disease modeling, tumor spheroids, bacterial growth, vaccine research, etc.

The first SPAD advanced lab for biological research was launched to the ISS in November 2018 onboard Northrop Grumman's unmanned resupply spacecraft Cygnus during its tenth flight to the ISS under the Commercial Resupply Services contract with NASA (Cygnus NG-10) (Fig. 7a). It returned to Earth in January 2019 on SpaceX's Dragon CRS-16 mission (Fig. 7b) after performing research on human muscle cells in orbit.

MICROGRAVITY RESEARCH IN LIFE SCIENCES

Microgravity improves protein crystals growth and contributes to optimization of nanofluidic systems for development of technologies in various fields, such as diagnostics and drug delivery. In addition, microgravity and spaceflight have been associated with physiological alterations in a variety of organisms, from viruses and bacteria to mammals, including humans. Changes induced by spaceflight may serve as models of ground-based conditions such as osteoporosis and aging of the immune system. The following section provides several examples of such initiatives for chemical, pharmaceutical and biological applications.

Applications for Chemistry

Protein Crystallization in Orbit

The applications of protein crystallization are wide, because most drug targets are proteins and because protein-based drugs, specifically monoclonal antibodies (MAB's) are the fastest growing segments in the pharmaceutical industry (27,28). Once the 3D structure of a protein is defined, it helps understand the protein's functions either as a drug target (*e.g.*, enzymes, transporters and receptors) or as the drug itself (1,2). The most important yet difficult stage in this process is generating an optimal crystal which will supply high resolution structures of the protein or a co-crystal of the protein and its ligands. Much effort has already been invested into optimizing the crystallization process, a work- and time-intensive task. In addition to usual crystallization variables (antisolvent precipitant, pH, temperature), the protein itself is a variable; the implicit assumption is that solubility and crystallization propensities vary across different constructs. Therefore, testing a reasonably large number of constructs of a target protein should increase the probability of success (29).

Microgravity substantially improves the growth of protein crystals. This is because, in the absence of buoyancy-induced convection, the movement of protein molecules in microgravity is driven only by random diffusion and is therefore much slower than on Earth (30). The crystals which are grown in space can be returned to Earth for protein mapping (31,32). Furthermore, when gravity as a masking factor is eliminated, other interactions can become prevalent. Consequently, other crystalline structures (polymorphs, see below) may arise, even though they are very rare on Earth. It might even be possible to crystallize materials which were not successfully crystallized in 1 g (33). For example, some of the proteins involved in neurodegenerative diseases crystallize on Earth but not with enough quality and uniformity to determine their structures (21). This approach has been applied to the hematopoietic prostaglandin D synthase, a protein expressed in certain muscle fibers of patients with muscular dystrophy. Crystallization of this protein in space resulted in the discovery of a new inhibitor, several hundred times more potent than the original drug (34,35).

Merck has been working with NASA and the Center for the Advancement of Science in Space (CASIS) growing crystals of monoclonal antibodies aboard the ISS for many years, thereby improving Merck's drug discovery, delivery and manufacturing processes as practical applications on Earth (36). Launched on SpaceX CRS-10 in February 2017, an experiment that involved growing crystalline suspensions of uniform crystals on the ISS aimed at improving the formulation and delivery of the company's cancer-fighting immunotherapy monoclonal antibody drug pembrolizumab (Keytruda) (37,38). Additionally, results from this investigation could lead to improved drug stability and storage of other monoclonal antibodies. SpacePharma has developed customized based lab-on-chips to perform batch and continuous crystallization experiments under microgravity where multiple crystallization parameters can be tested in one mission in order to find the optimal conditions for obtaining large crystals with improved quality. Using microfluidic droplet creation technology, the company is developing a microfluidics-based

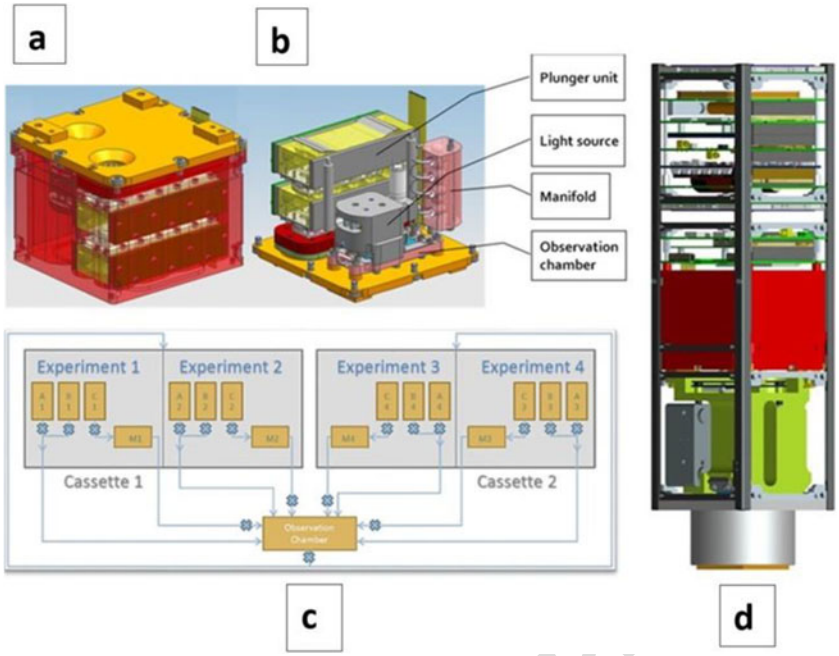

Fig. 3 Layout of SPmgLab. (**a**) Model of the outside view of the lab including the atmospheric box. (**b**) Model of the outside view of the lab (without the atmospheric box). (**c**) Cassettes and reaction chambers. (**d**) Nanosatellite with SPmgLab and accessories. The lab is divided into four sections (experiments), with two experiments on each cassette (**c**). Each experiment contains two reservoirs (A & B) connected directly to the observation chamber, a main chamber (M) and a third reservoir (C) which is connected to the observation chamber through the main chamber. The observation chamber is shared for all experiments and is observed by a light microscope and a spectrometer. A stirring bar is placed inside the observation chamber in order to stir its contents. The fluids in reservoirs A, B, C are transported using a spring activated plunger. During activation of the plunger in reservoir C the fluid is pushed from C and mixed with the fluid in the main chamber, together they flow to the observation chamber. During activation of the plungers in reservoirs A or B the fluid is pushed straight to the observation chamber. Since the observation chamber is shared, each experiment ends with a clean observation chamber, thus at least one of reservoir A or B is used for flushing the observation chamber before the next experiment.

crystallization lab that produces hundreds of microdroplets per minute and each droplet can have the same crystal growth conditions allowing many experiment repetitions as well as control and variation of experimental parameters. Successful crystallization experiments with improved, pure and large crystal compared to 1 g ground control were already performed in 2018 by SpacePharma using its miniaturized SPmgLab microgravity platform during a NoveSpace/Zero G parabolic flight mission (Fig. 8).

Small Drug Molecule Crystallization and Polymorphism

The most active pharmaceutical ingredients (APIs) of a drug can exist in several polymorphs (forms of crystal structures), pseudopolymorphs (solvates and hydrates), salts, and amorphous solids (39). Polymorphs of the same drug may vary in their physical properties, which translates to potential variability in manufacturing processes, bioavailability and efficacy of the active compound. For example, due to differences in

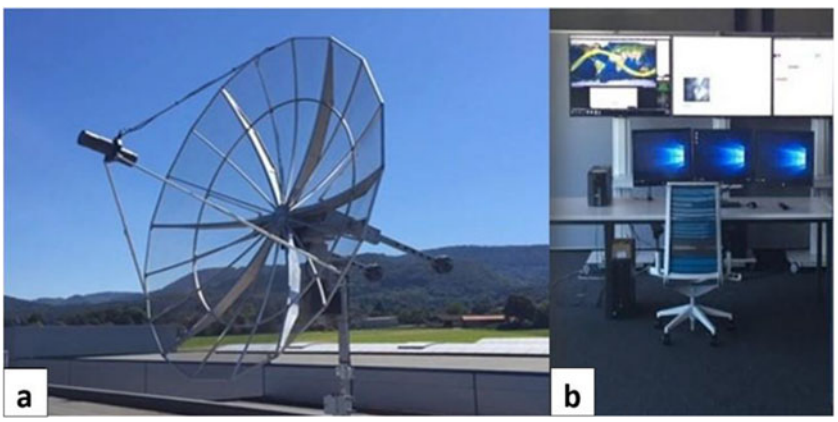

Fig. 4 SpacePharma's ground station at Courgenay, Switzerland. (**a**) Antenna serving satellite operators with real-time Telemetry Tracking and Control (TT&C) and payload data delivery and data processing services provided by RBC Signals. (**b**) Satellite control, monitoring and communication room.

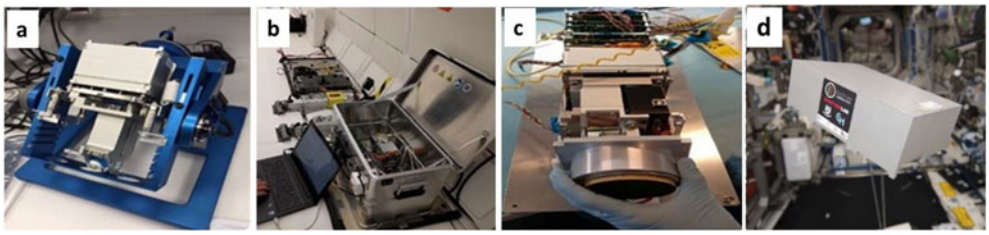

Fig. 5 Supported microgravity platforms. (**a**) SPmgLab mounted on an RPM ground simulator; (**b**) SPmgLab aboard a parabolic flight; (**c**) SPmgLab mounted on a nanosatellite; (**d**) SPmgLab at the ISS (image courtesy of NASA).

solubility, one polymorph may be more active than another. In addition, co-crystals (crystalline complexes of two- or more neutral molecules) of pharmaceutical materials can improve properties such as dissolution rate and stability (40). Co-crystals can also be employed for chiral resolution and might play a major part in the future of API formulation. A company may choose to patent a specific crystallized state or polymorph of a drug, thereby extending its period of market exclusivity after the original drug has been patented. Thus, discovering in advance all existing drug polymorphs of a new API and their properties is crucial (41,42).

New processes for preparation of novel API crystalline polymorphs using microgravity environment can be developed with potential applications for new intellectual property and patent extension of generic drugs. The results of the microgravity crystallization experiments can be used to solve new crystalline structures. Polymorph screening can support exploring new or rare polymorphs and obtaining the optimal conditions for crystallizing the same molecules on Earth or as new polymorphs with improved physicochemical properties. Using the SPmgLab microgravity platform, SpacePharma has conducted in 2018 successful experiments aboard the ISS on the crystallization of a small molecule with superior crystal morphology outcome compared to Earth product made with the best technologies available.

Fig. 6 SpacePharma's DIDO-2 nanosatellite on orbit following launch to space on February 2017 from India aboard the Indian Space Research Organization (ISRO) PSLV-C37 rocket. Shown is onboard camera view of satellite deployment. The arrowhead indicates the DIDO-2 satellite.

Self-Assembly of Biomolecules in Microgravity

Peptides are highly promising in nanotechnology because they are biocompatible, versatile, and may be decorated with additional molecular entities. Hence, they can be utilized as building blocks for studying self-assembly of molecules to generate complex architectures (43). Natural convection affects many self-assembly processes since they are usually delicate. When the masking of gravity is removed, chemical and physical interactions become more prominent. As a result, studies of self-assembly processes in microgravity allow observing and measuring the forces affecting the assembly processes (44).

Examples of proteins that undergo self-assembly are cytoskeletal microtubules. These are hollow, cylindrical cytoskeletal polymers built of αβ-tubulin protein heterodimers. In eukaryotes, microtubules play key roles in cellular structure, transport and division. Solution conditions, including ionic strength and the presence of microtubule-associated proteins can strongly affect microtubular polymerization. In addition, several neurodegenerative diseases involve impaired interactions of microtubules with their associated proteins (45), and some widely-used anticancer drugs, such as paclitaxel, function by interfering with microtubule dynamics (46,47).

Tabony *et al.* used sounding rocket experiments to demonstrate that microgravity impairs the assembly of microtubules structures, likely due to density fluctuations during self-assembly (48,49). Thus, the microgravity environment of space facilitates new studies for shading light on the mechanisms by which microtubule-associated-proteins and microtubule-targeted drugs act.

Applications for Pharmaceutical Sciences

Behavior of Colloidal Systems in Microgravity

Many pharmaceutical formulations are based on colloidal system (CS)-like suspensions, emulsions, liposomes and microparticles, which may destabilize over time, resulting in reduced product quality (50) -. Improving stability may also reduce the need for stabilizers, thus increasing API concentrations while reducing packaging, storage, and conveying costs.

The absence of sedimentation and buoyancy in microgravity allows studying phase separation and aggregation without mass convection caused by density differences. Without

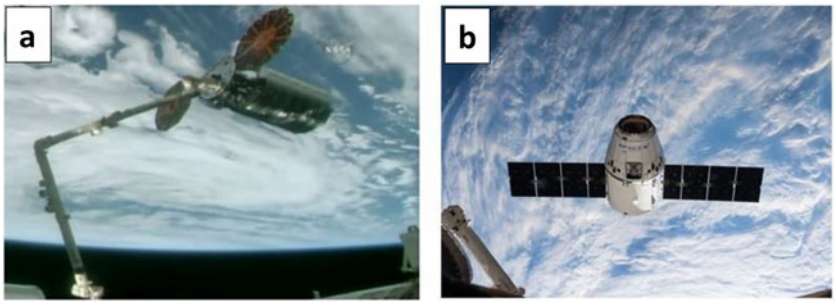

Fig. 7 Transportation of SPAD to the ISS and back. (**a**) Docking of Northrop Grumman's Cygnus carrying the SPAD to the ISS during Cygnus NG-10 (November 2018); (**b**) Return to Earth of SPAD on board of SpaceX's Dragon (CRS-16 mission; January 2019) (Photos courtesy of NASA).

gravity as a masking factor, the contribution of other parameters, such as composition and polydispersity, becomes more prominent (51–53). Recent Space Shuttle (54) and ISS (55,56) experiments with colloidal formulations provided outcomes such as a) partial phase diagrams of mixtures, since sedimentation does not interfere with observing the microstructure evolution over long periods of time (months) (54); b) quantitative measurements of the parameters affecting destabilization (56); c) internal structures of aggregates and the kinetics of aggregation to predict product quality degradation due to aggregation (55).

One example of the use of novel microgravity platforms is the study of emulsions (57). Investigating the physical nature of emulsion-based systems is of great technological importance since it is required for the design of new and improved products, while maintaining high efficiency (58–61). The additives added in emulsions can hardly be studied in 1 g because gravity modifies the physical properties both at the microscopic and macroscopic scales. At the microscopic scale, gravity induces fluid fluxes and modifies interface thinness, such that the surfactant transfer and adsorption effects are masked. At the macroscopic scale, Microgravity conditions prevent drainage (creaming and sedimentation) and allow monitoring the complete interaction cycle of surfactants from adsorption until phase separation and destabilization (60). Interface elasticity is only driven by surfactant concentration; adsorption and diffusion of surfactants could be studied with a greater accuracy.

Given those advantages, microgravity has been suggested as an accelerated model for investigating the rules that govern the structure and dynamics of colloidal gels and emulsions in order to increase the shelf-life of products as described in the ESA's report on the fundamental and applied studies on emulsion stability (60). Today both academic groups and leading pharmaceutical companies conduct microgravity experiments in order to enhance their knowledge, and thus increase their product quality and stability on Earth.

Employing SPmgLab, such insights can be obtained by characterizing the microstructure evolution of colloidal systems for long periods of time under microgravity, *e.g.*, by using optical imaging, spectrometry, or other applicable techniques. Several experiments on emulsion stability under microgravity conditions were conducted using this platform by mixing water, an oil, and a surfactant from different reservoirs and at several ratios into the reaction chamber and following the emulsion droplets formation using a dye.

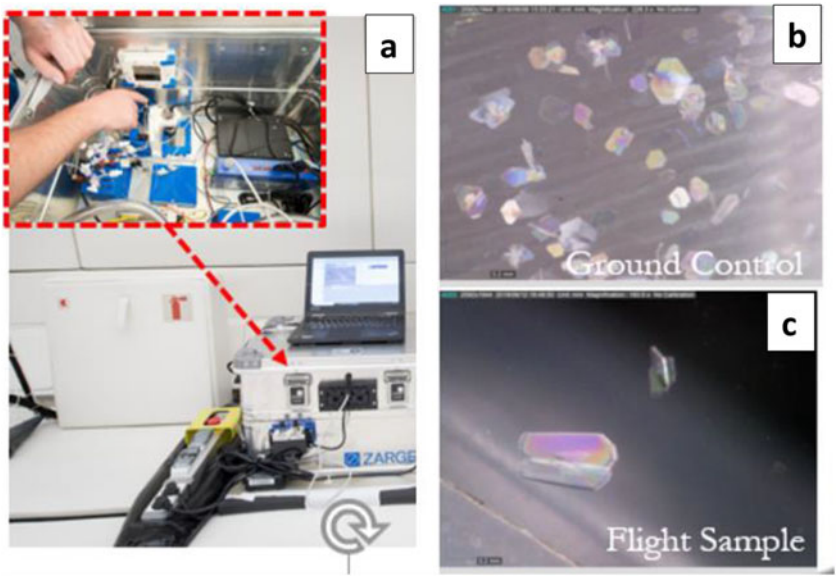

Fig. 8 Peptide crystals prepared using SPmgLab under microgravity conditions during NoveSpace/Zero G parabolic flight mission on June 2018. (**a**) SPmgLab aboard parabolic flight; (**b**) Peptide crystals grown on 1 g ground control; (**c**) Large peptide crystals grown on parabolic flight under microgravity conditions.

Microencapsulation and Drug Delivery Systems

Microencapsulation for improved drug delivery has been derived from microgravity research (10). Microencapsulation experiments on the ISS resulted in the development of the Microencapsulation Electrostatic Processing System-II (MEPS-II). Due to surface tension forces, the MEPS in space combined two liquids that do not mix on Earth (80% water and 20% oil) to spontaneously form liquid-filled microcapsules as spherical liquid-filled bubbles coated by a semipermeable membrane (62). Processes such as particle coalescence, flocculation, creaming, phase separation and sedimentation decreased in the microgravity environment resulting in better particle stability and improved shelf-life. The higher stability of the microencapsulated systems obtained in space may change when returned to Earth, but the aim of studying particle formation in microgravity is to better understand their physicochemical properties such as drug loading, and particle size and distribution. Thus, by evaluating particle formation in the space environment, pharmaceutical companies hope to identify opportunities to optimize the nanoparticle manufacturing to develop improved drug delivery formulations. For example, in June 2019 AstraZeneca launched a research project to the ISS National Lab seeking to advance a nanoparticle drug delivery system for therapeutic cancer vaccines (63). The drug delivery experiments conducted onboard the ISS included DNA encapsulation and microencapsulation of anti-cancer drugs (62). So far, research on ISS resulted in 13 licensed microcapsule-related patents. Microgravity studies for optimizing drug loading, size distributions, and various processing methods for specific drugs and therapeutic agents could also be conducted in orbit using SPmgLab or SPAD microgravity platforms.

Applications for Biology

Microgravity as an Accelerated Model of Aging and Disease

With an increasing aging population, there is a need in understanding how and why various functions of the human body decline with age and in finding means to slow or prevent these processes. Preventing age-related diseases could have significant economic impact on society and provide public health benefits for increased longevity (64). This task is complicated by the long time periods required for such studies. Even standard rodent models require 3 years to follow the changes over the lifespan, and studies in primates can last 15–30 years (65).

In microgravity, aging accelerates by up to 10 times, with a scale of days and weeks (66). Thus, the microgravity environment of space is fast becoming a novel model for accelerated experimental aging that is otherwise unavailable (7). This topic has been described in excellent reviews by di Giulio (5), Vernikos and Schneider (6), and Biolo *et al.* (4). Briefly, the many physiological changes that occur in space, such as bone and muscle loss (67,68), immune dysfunction (69–71), inflammatory response, and cardiovascular deconditioning resemble those observed during aging (72). Hormonal changes common to aging and to microgravity include mild hypothyroidism (73), increased levels of stress hormones (74,75), gonadal dysfunction (76–78), and insulin resistance (79,80). Interestingly, in the recently published NASA Twins Study (72), the length of telomeres increased during a year-long space flight and decreased upon return to the ground. Telomere elongation in space seems contrary to the acceleration of aging-related processes in space, because telomere length shortens with cell division and thus has been associated with human aging and age-related diseases. The underlying mechanism of the transient telomere elongation has not been identified, but could be related to healthy life style of astronauts, weight loss, or a shift toward cell populations with longer telomeres (72).

Therapeutic treatments for preventing microgravity-induced degeneration may be adapted to diminish the burden of age-dependent diseases, which is the goal of any pharmacologist developing new anti-aging treatments. An example of this approach is the collaboration of the biopharmaceutical company Amgen with NASA to develop a rodent-based experiment that could benefit astronauts and earthbound humans. Amgen's first space experiment (STS-108) in 2001 focused on understanding the role of an engineered version of the protein osteoprotegerin in bone loss. This study led to an FDA approval in 2010 of Amgen's new drug, denosumab, which is marketed under the brand name Prolia (81). During the Phase 3 trials, patients treated with this drug showed a 20–68% reduction in fractures, depending on the type of bone studied, and significantly higher bone density (82).

A recent retrospective, longitudinal analyses on diffusion magnetic resonance imaging data collected from 15 astronauts demonstrated significant changes in the white matter of the brain, that were only partially related to fluid shifts. The rate of changes was approximately 2-fold the reported changes during the same period with healthy aging (83). Studying the factors that contribute to the accelerated changes in microstructures of the brain in microgravity can enhance our understanding of brain aging. In addition, the enhanced molecular self-assembly in microgravity as described above can be utilized for characterization of amyloid formation under microgravity environment. Findings from studies can become a big step toward understanding the mechanisms of neurodegenerative diseases (84), including Alzheimer's, Parkinson's, Huntington's and prion diseases.

Certain immune cells tend to have altered activity with age, which results in higher vulnerability to illness (85). Because similar changes in the activity of those cells occur during

spaceflight, microgravity is an attractive model for researchers in this field. NASA and the NIH's National Institute on Aging have collaborated to support research aboard the ISS, with T cell activation in aging being one of the first studies in space (85).

Organ-on-Chip (OOC) and 3D Tissue Engineering

An OOC is a microfluidic device containing continuously perfused chambers in which living cells recapitulate the architecture, interfaces, and microenvironment of tissue and organ functionality, unlike conventional two-dimensional (2D) or 3D culture systems (86). The OOC technology enables the customization of the platform for specific diseases. Cross-species differences in preclinical studies make the platform more valuable. Additionally, it can be used for drug screening in parallel to *in vitro* assays and animal model studies (87). Thus, OOC platforms can improve hit-to-lid screening and the predictability of efficacy, toxicity and pharmacokinetics in humans (88). Moreover, OOC technologies can promote stratified medicine, the development of treatment in rare diseases, and nanomedicine.

The use of OOC models in space supports the studies of changes that could take years on Earth enables mimicking the effects of drugs on these changes and supports animal replacement for toxicity studies. In 2017 NIH/National Center for Advancing Translational Sciences (NCATS), together with CASIS, funded five projects whose focus is the development of tissue chips to improve human health on Earth through the Chips-in-Space program. The initial projects are a part of a four-year program aimed to use OOC platforms onboard the ISS for translational research (89). The project's goals are to evaluate the ability of microfluidic devices to reflect physiological principles while being delivered to orbit and to provide access to modular components that can be interconnected to understand the integrated behavior of complex human responses.

Differential Gene Expression in Microgravity

The space environment (microgravity and radiation) can alter gene expression and reveal new targets for gene therapy, as has been recently demonstrated in NASA's Twins Study (72). Gene expression studies are important for gaining better understanding of the genetic basis and molecular mechanisms of cellular response to the space environment, thus improving risk management, monitoring and countermeasures (90). For example, culturing human mesenchymal stem cells for 20 days on an RPM resulted in significantly altered expression of 144 genes (91). The expression of 30 of these genes increased, whereas that of the other 114 genes decreased. The majority of these belonged to 11 principal groups according to their biological roles in the cell. Corydon *et al.* used a RPM to show that simulated microgravity induces significant alterations in the cytoskeleton-related proteins of human adult retinal epithelium cells, in addition to changes in cell growth behavior and gene expression patterns involved in cell structure, growth, shape, migration, adhesion and angiogenesis (92).

To cross-validate findings obtained in independent research platforms, the dynamics of changes in gene expression during a parabolic flight and a suborbital ballistic rocket mission were investigated in human Jurkat T lymphocytic cells by Oliver Ullrich's lab from Zurich University (93). Gene expression was analyzed using an Affymetrix Array consisting of 44,699 protein coding genes and 22,829 non-protein coding genes. Within 20 s (parabolic flight) and 5 min (rocket) of microgravity, three gravity-regulated genes were identified: a vacuolar V-ATPase that mediates acidification during bone resorption (ATP6V1A/D), diversity genes of immunoglobulin heavy-chains (IGHD3-3/IGHD3-10), and an intergenic non-protein coding RNA (LINC00837). These rapid changes in gene expression led the authors to conclude that human cells are capable of efficient adaptation to changes in gravitational conditions (93).

Using human renal cortical cells in microgravity culture, Hammond *et al.* studied differential gene expression in steady-state cell culture on STS-90 flight and found altered expression of 1632 out of more than 10,000 genes that were evaluated (94). In Jurkat T cells that were flown onboard a space shuttle, Lewis *et al.* found upregulation of 11 cytoskeletal genes and downregulation of gelsolin precursor compared with ground controls (95).

Effects of Microgravity on Stem Cell Differentiation and Proliferation

Microgravity research can contribute to the field of stem cell therapy by providing the conditions for accelerated models of cell proliferation and cell differentiation. For example, the use of gelatin scaffolds and a RWV enabled generating spheroids of undifferentiated human mesenchymal stem cells with subsequent rapid osteogenic differentiation (96).

Long periods of microgravity lead to hematological disorders, including anemia, thrombocytopenia, and altered structure of red blood cells (97). Space shuttle missions STS-63 (*Discovery*) and STS-69 (*Endeavour*) contributed to understanding the effects of spaceflight on the hematopoietic system (98). $CD34^{+}$ bone marrow progenitor cells were maintained at microgravity (flight) or on the ground. Over a study period of 11–13-days, the cell number increased 41-66-fold on the ground but only 10–18-fold in space (a 57–84% decrease). Myeloid progenitor cells expanded to a greater extent compared to ground controls, but expansion of erythroid progenitor cells declined. In addition, the cultures maintained in space maturated/differentiated faster toward the macrophage cell lineage. These findings demonstrated that spaceflight

affects the proliferation and differentiation of hematopoietic progenitor cells *in vitro* and that the effect of gravity is lineage-selective.

Several studies demonstrated that simulated microgravity (SMG) may support expansion of stem cell cultures *in vitro* in the absence of supplements which may impair stem cells transplantations. Constantini *et al.* used the Rotary Cell Culture System (Synthecon) to evaluate the effects of SMG on human hepatic cell line (HepG2) and human biliary tree stem/progenitor cells (hBTSCs) (99). The generation of 3D cultures of both cell types and the maintenance of stemness contrasting cell differentiation were favored in SMG, in association with stimulation of glycolytic metabolism. Hence, SMG can advance the development of the biliary tree stem/progenitor cell-derived liver devices. Yuge *et al.* reported that culturing human mesenchymal stem cells in SMG using a 3D-clinostat significantly increases their proliferation compared with cells cultured under normal gravity conditions (13-fold *versus* 4-fold in a week) (100).

Only few studies utilized real-time imaging for analysis of stem cell proliferation and differentiation in space. Among them is the study by Lei *et al.* who utilized live cell imaging techniques on the TZ-1 cargo spacecraft to study these characteristics in mouse embryonic stem cells in space (101). The findings of this study reinforced the role of space microgravity in supporting 3D growth of embryonic stem cells, with a negative effect on terminal differentiation.

The studies summarized here and others show that microgravity offers a unique environment to study and control stem cells in order to improve their quality for therapies. In addition, since microgravity leads to cells aggregation into large and organized 3D structures, growing cells in simulated or true microgravity might be a highly promising new technique to produce tissue constructs in the absence of a scaffold.

Microgravity and Infectious Diseases

The space environment leads to major changes in microbial features that directly relate to infectious diseases, including altered growth rates of bacteria, invasion of host tissue, biofilm formation, and sensitivity to antibiotics. For example, the virulence of Salmonella typhimurium (8) has been shown to increase onboard space shuttle flights. In addition, host susceptibility (vulnerability) to infection increases in space due to the above mentioned altered immune function (102). Hence, microgravity enables studying virulence processes with a great potential to discover new factors involved in pathogenicity, which can advance the development of new antibiotic drugs and vaccines (8,102–110). Research on vaccine development using colloidal lipid-based delivery systems (liposomes, nanoemulsions, micelles) under microgravity conditions will also contribute to better understanding of antigen-adjuvant particle interactions in order to improve efficiency and shelf-life.

CONCLUSIONS

The microgravity in space affects all levels of biological organization, including cells, tissues, organs, and organisms, often in unique ways. Thus, microgravity and space research enable new understanding of living systems and novel directions of pharmaceutical research. Studies in microgravity conditions can promote elucidation of protein 3D structures and identification of novel pathways that regulate gene expression and new targets for developing drugs and vaccines. Additionally, aging and prolonged microgravity exposure during spaceflight share some notable detrimental effects on human physiology making the microgravity environment a unique and attractive accelerated, non-invasive tool for developing new anti-aging therapeutic treatments. Indeed, Microgravity R&D for life sciences has recently been gaining traction, with the aim of translating findings in space to address current clinical research and drug development. Traditional and new emerging platforms are available to perform pharmaceutical research under microgravity conditions, from clinostats to various systems in orbit. Unique among them is SpacePharma's sophisticated, miniaturized, autonomous, unmanned and remote-controlled lab systems containing sensors and readers that can work in different microgravity platforms, from ground simulators to the ISS. Such advances are expected to greatly contribute to new advances with applications both in space and on Earth.

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