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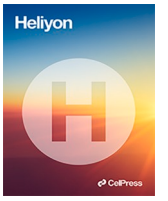

Research article

High-altitude balloon platform for studying the biological response of living organisms exposed to near-space environments

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ABSTRACT

The intangible desire to explore the mysteries of the universe has driven numerous advancements for humanity for centuries. Extraterrestrial journeys are becoming more realistic as a result of human curiosity and endeavors. Over the years, space biology research has played a significant role in understanding the hazardous effects of the space environment on human health during long-term space travel. The inevitable consequence of a space voyage is space ionizing radiation, which has deadly aftereffects on the human body. The paramount objective of this study is to provide a robust platform for performing biological experiments within the Earth's stratosphere by utilizing high-altitude balloons. This platform allows the use of a biological payload to simulate spaceflight missions within the unique properties of space that cannot be replicated in terrestrial facilities. This paper describes the feasibility and demonstration of a biological balloon mission suitable for students and scientists to perform space biology experiments within the boundary of the stratosphere. In this study, a high-altitude balloon was launched into the upper atmosphere (~29 km altitude), where living microorganisms were exposed to a hazardous combination of UV irradiation, ultralow pressure and cold shock. The balloon carried the budding yeast *Saccharomyces cerevisiae* to investigate microbial survival potential under extreme conditions. The results indicated a notable reduction in biosample mortality two orders of magnitude (2-log) after exposure to 164.9 kJ m^{-2} UV. Postflight experiments have shown strong evidence that the effect of UV irradiation on living organisms is stronger than that of other extreme conditions.

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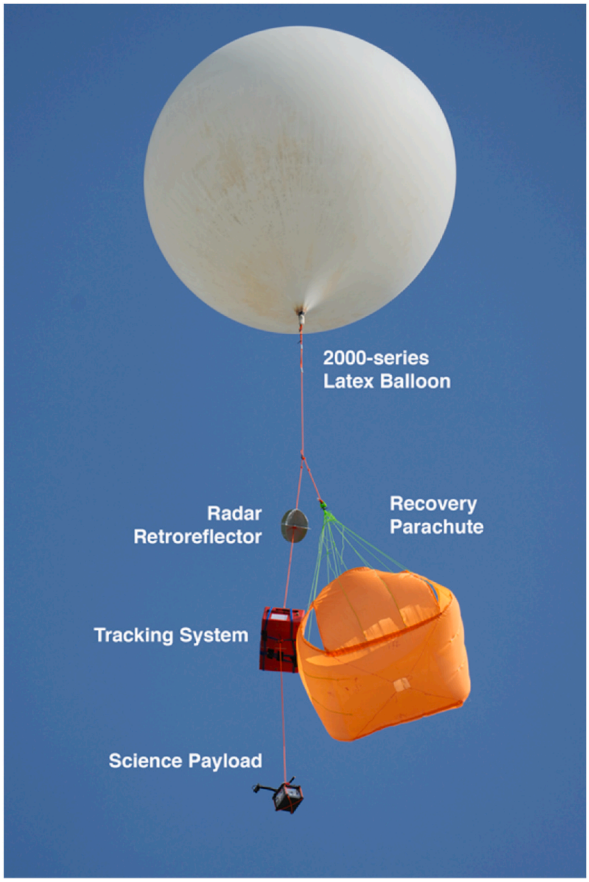

Fig. 1. The overall structure of the high-altitude balloon (HAB) system.

1. Introduction

For many decades, humans have traveled to space with the ultimate goal of exploring the universe. However, the outer space environment poses an enormous obstacle to humanity realizing one its longest-lasting ambitions. Without the protection of the Earth's atmosphere, astronauts will be exposed to two vital conditions, space radiation and microgravity. The hazardous nature of space can influence many health risk symptoms in most living organisms, such as chromosomal damage, oxidative stress induction, immune system dysfunction and bone loss [1,2]. Furthermore, space radiation poses the most crucial danger to the human body. Extraterrestrial radiation originates from various sources, including galactic cosmic rays (GCRs) and solar particle events (SPEs) [3]. HZE ions are high-energy protons and highly charged (Z) and energetic (E) galactic cosmic rays that notably have sufficient penetration power to cause irreversible damage to astronauts, especially through cellular mechanisms [4,5]. During Mars exploration, astronauts would receive a dosage of more than 1000 mSv within a few years of the space journey [6]. In comparison, the annual global average radiation dose is 2.4 mSv on Earth's surface [7]. The expected dose of the Mars venture would be as high as 622 mSv for only round-trip travel [8]. This radiation amount has strong potential to cause a vast number of health diseases, especially cancer [4,9]. In addition, the symptoms of these diseases could be made much more severe symptoms when combined with weightlessness. Understanding the health risks of these harmful factors could lead us to overcome the remaining hindrance in space exploration.

Over several decades, humanity has investigated Martian surface characteristics, seeking the possibility of future colonization. The extreme conditions on Mars indicate similar health risks as space voyages that could induce biological damage to astronauts. Unlike on Earth, wideband ultraviolet (UV) radiation can penetrate the thin atmosphere of Mars due to its lack of an ozone layer [10,11]. Moreover, all extraterrestrial radiation directly reaches the planetary surface due to the weak magnetosphere. The temperature and pressure on Mars are also significantly lower than those on Earth [12,13]. However, these hazardous conditions cannot be safely utilized to test the limits of living organisms. Existing terrestrial facilities cannot simulate all the possible lethal combinations together. Experiments on Earth are limited to only simulating a few conditions in the laboratory [14,15]. On the other hand, stratospheric conditions are comparable to Martian environments and include fully wideband UV irradiation, low temperature and ultralow pressure [16–19]. Thus, high-altitude balloons could be powerful tools for replicating Mars-like conditions in the stratosphere. Most of the UV radiation is typically absorbed by the ozone layer in the atmosphere. Balloon flight could simulate UV conditions similar to those of Mars's surface when floating above the ozone level [20,21].

In the early 19th century, the high-altitude balloon was first invented as a weather balloon to investigate atmospheric phenomena. Currently, one of its notable applications is for biological experiments of space's extreme conditions through the near-space (middle stratosphere layer) environment. Many scientific balloons have been constructed to study microbial survival under Mars-like conditions [22–25]. Additionally, multiple balloon flights have been used to collect aerosols from the atmosphere to identify the microbial cells that appear in the near-space environment [26–28]. In this study, our team recently developed a scientific balloon platform for conducting space biology experiments within the Earth's boundary. The balloon design consists of two primary components: 1) a biological payload box with a system for monitoring UV intensity, temperature and pressure and 2) a high-altitude balloon with an integrated tracking system. These platforms would allow students and scientists to study the biological responses caused by the dynamic nature of extraterrestrial hazards.

Ultimately, the scientific flight mission of a high-altitude balloon has shown the successful demonstration of carrying out the budding yeast *Saccharomyces cerevisiae* to the upper atmosphere (~29 km altitude). The objective of this study was to observe cell mortality after exposure to Mars-like conditions. This low-cost balloon platform would be an intriguing tool for students and scientists with limited resources. Additionally, the robust payload box gathered environmental information throughout the mission in order to later interpret those harmful parameters. Furthermore, postflight laboratory assays were performed to examine the impact of the microbial survival rate in response to acute exposure to space-extreme environments. The results revealed the deadly influence of outer space on living organisms, especially UV bombardment. In the end, we would like to introduce our design platform, which might be suitable for students to conduct experiments under conditions similar to those in space. This pilot study has indicated the promise of future space biology missions as a vital stepping stone that paves the way for exploring the mysteries of the universe.

2. Materials and methods

2.1. Launch vehicle platform

The overall structure of the high-altitude balloon (HAB) system is shown in Fig. 1, including a balloon at the top, a radar retroreflector, a tracking system, a recovery parachute, and a biological payload. The balloon was targeted to reach an apogee 29 km above sea level in the stratosphere. The balloon used in the mission was specified as a 2000-series latex balloon, initially inflated using 14 cubic meters of helium gas, providing the required lifting force for the ascent. The launching platform and tracker used were developed by Ngamdeevilaisak et al. [29].

2.2. Payload positioning and tracking

The custom-made payload positioning tracker attached to the high-altitude balloon (HAB) consists of two Global Navigation Satellite System (GNSS) receiver breakout modules: 1) the U-blox NEO-M9N and 2) the U-blox NEO-M6. These two modules are commonly used in HAB applications as the primary positioning module in the tracking system. The module's dynamic mode was set to support up to 80,000 m of absolute altitude and 250 m/s of horizontal velocity, which is the most suitable mode for HAB applications. The two-dimensional position of the tracking device can be slightly inaccurate when the altitude is more than 10,000 m above the mean sea level (MSL), which might introduce up to 50 m of lateral distance and altitude deviation. Each GNSS receiver was connected to a passive ceramic patch antenna with a circular polarization pattern (3 dBi gain) through an I-PEX MHF connector. The antenna's frequency responses are 1575.42 MHz (GPS L1 band) and 1561.00 MHz (BeiDou E1 band). Before launching the balloon, the precision of the GNSS receiver was tested on the ground by statically positioning the tracker at a single coordinate and setting up the same configuration as the flight operation for 20 min with 652 data points. The determination analysis indicated that the horizontal displacement exhibited a lateral standard deviation of 3.5 m and a range of 12.5 m, and the altitude had a standard deviation of only 1.5 m, which was 0.005% of the 29,000 m target altitude.

A position tracker with accurate and reliable long-range wireless communication was required because the scientific payload had to be recovered for postflight analysis. The use of LoRaWAN/LoRa technology is commonly recommended for high-altitude and long-range applications over other technologies, e.g., XBee, NB-IOT, and RFD900x, for these types of usage because it yields better performance for wireless communication over a long distance [30,31]. The LoRa device is used for communication between the tracking system and the ground control station (GCS) in peer-to-peer (P2P) mode. The LoRa RF module uses the spread spectrum modulation technique by varying the frequency at which the signal is transmitted continuously instead of via traditional serial binary formats.

For missions requiring a high data rate, the 915 MHz band provides a reasonable compromise between distance over the 433 MHz band and data rate over the 2.4 GHz band. The EBYTE E32-900T30D wireless RF module was chosen for this mission because it has a center frequency of 915 MHz and transmits in a burst at 1 W (30 dBm) of power. It also has a receiving sensitivity of −147 dBm. The SX1276 transceiver module and internal microcontroller were utilized for interfacing with the tracking system through the universal asynchronous receiver/transmitter (UART) protocol. The module was paired with a 4.3-dBi-gain omnidirectional monopole antenna on the transmitter end and a 12-dBi-gain directional Yagi Uda antenna on the receiver end at the GCS. Moreover, the module implements a forward error correction (FEC) channel coding algorithm, which has a strong error correction ability under a low signal-to-noise (SNR) ratio during long-distance communication; in this case the distance was over 50-km line of sight (LOS). The calculated communication range was obtained using the Egli model from Oluwole et al. which suited the mission's use cases for predicting path loss of peer-to-peer links [32]. A 47.5 km communication range was predicted for the setup described earlier.

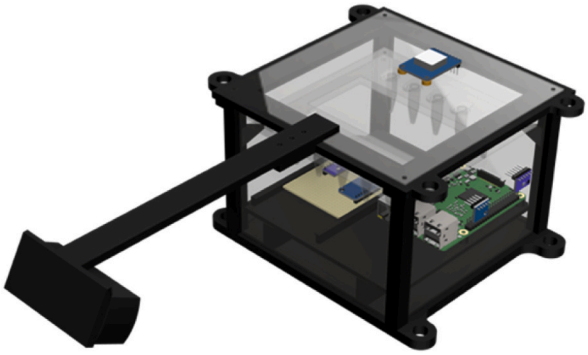

Fig. 2. A 3D model of the biological payload.

Fig. 3. A snapshot image of the biological payload at the apogee during the stratosphere flight mission.

2.3. Balloon biological payload

The biological payload of this mission was made of lightweight polylactic acid (PLA) 3D-printing filament, which has a mass of 700 g and dimensions of 150 mm × 150 mm × 100 mm, excluding a built-in mounting for integration onto the balloon. The payload was enveloped in thin transparent acrylic sheets to allow the biosamples to be exposed to UV radiation directly. The specimen tray was also built with thin acrylic sheets, which can carry up to 10 samples with the size of a 1.5 ml microcentrifuge tube. This platform design allows the payload to have a plug-and-play sample slot that can handle numerous organism species, such as bacteria, fungi, human cells, and mammalian cells. With this platform, the biological samples must be loaded into a 1.5 ml tube, even if they are cultured with a dissimilarity technique. The computer-aided design (CAD) model of the payload is shown in Fig. 2. The payload system comprises an onboard computer (Raspberry Pi 3 Model B) and various sensors for collecting UV intensity, atmospheric temperature, payload ambient temperature, atmospheric pressure, GPS receiver and webcam camera. A data logger was implemented onboard the computer with timestamps from the real-time clock included-GPS module.

2.4. Model organisms and sample preparation

The *S. cerevisiae* strain INVSc1 (Invitrogen, USA) is a diploid strain that is auxotrophic for histidine, leucine, tryptophan, and uracil selection markers. In this study, *S. cerevisiae* strains that carried the episomal plasmid pYES2 were cultured in yeast selective media lacking uracil (yeast nitrogen base without amino acids, dextrose, amino acid mixture without uracil) to avoid microbial contamination and were incubated at 30 °C with 180 rpm shaking for 12 h. Then, the yeast samples were diluted to an aliquot with an OD600 of 1.0 in 1 ml (approximately 2×10^7 cells/ml). The samples were divided into two groups: 1) 10 ground control samples and 2) 10 flight samples. The flight samples were attached to the balloon platform to be directly exposed to UV radiation, low ambient pressure and stratospheric temperature.

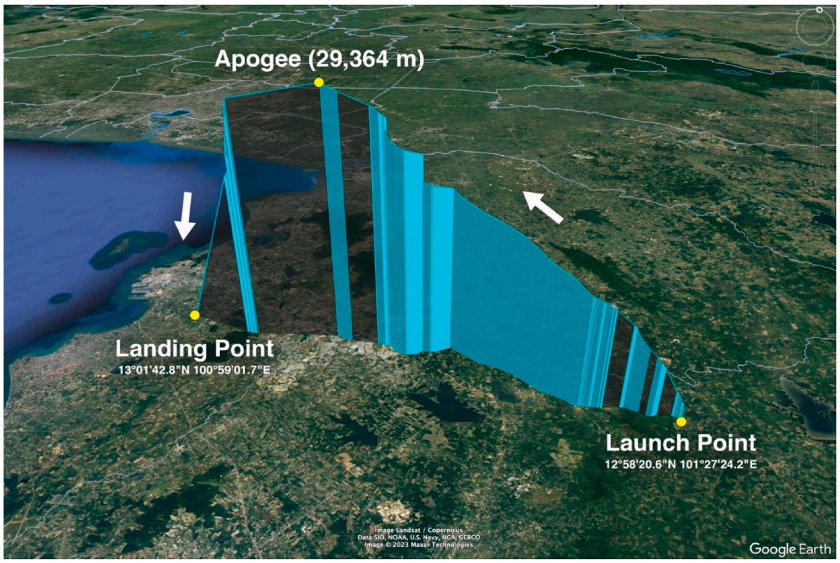

Fig. 4. Balloon flight profile pathway.

2.5. Survival assay

Postflight yeast samples were diluted with distilled water to an appropriate dilution. The cell suspensions were plated on YPD agar (1% yeast extract, 2% dextrose, 2% peptone, 2% agar) using the spread plate method and incubated at 30 °C for 48 h. The total number of plates was counted to determine the number of colony-forming units (CFUs). The survival rate was determined by calculating the ratio of the number of flight sample CFUs to the number of ground control CFUs that grew on the YPD agar plates. At least three biological replications ($n \geq 3$) of the CFU assay were performed.

3. Results

3.1. High-altitude balloon flight experiment

The high-altitude balloon carrying the biological payload was launched on May 4, 2023 at Wangchan Valley, Rayong, Thailand (12°58′20.6″N 101°27′24.2″E). It ascended for 2 h until reaching an absolute altitude of 29,364 m or approximately 29.4 km (Fig. 3). After the balloon popped at the apogee, the payload descended safely with its parachute, allowing the payload to slow to a targeted velocity of 15.0 m/s while entering the troposphere. It landed 52 km laterally northwest of the launch site at 13°01′42.8″N 100°59′01.7″E. The operation and geocoordinate plotting from "Launch Point" to "Landing Point" are illustrated in Fig. 4 using Google Earth Pro software.

The mission's apogee altitude was targeted at 29,000 m above mean sea level, while the actual apogee altitude measured from the primary GNSS receiver was approximately 29,364 m. The balloon flight profile was determined from the 2525 data points recorded in the external flash memory, which shows an average payload velocity of 5.0 m/s during ascent and 17.0 m/s during descent. During the onboard mission, the payload tracking system was able to acquire only an estimated 60% of the high-quality GNSS information caused by the occurrence of payload free fall after the balloon popped, which led to unexpected hardware shutdown during the descending phase. However, the missing data gap was filled in through the inverse distance weighting interpolation method to complete the imperfect flight pathway information.

3.2. Stratosphere onboard flight observation during the balloon mission

The mission objective of this scientific balloon was to investigate yeast survival after exposure to numerous extreme conditions during balloon flight. Various sensing techniques were used during this mission to observe the hazardous environment of the stratosphere that might affect microbial mortality. The biological payload data collected during the flight mission, which included UVA/UVB intensity (280–400 nm), atmospheric temperature, payload temperature and atmospheric pressure, were described. These parameters are crucial for interpreting biological samples exposed to UV irradiation, cold shock, and low ambient pressure in a near-space environment. The data were collected in a data logging system implemented on an onboard computer at a 1 s sampling rate. In addition, the high-altitude balloon system provided a video snapshot of the mission through its webcam, which is available at "https://youtube/dqT6ywTkrgU".

UV dosage measurement is necessary in any biological space mission, and especially in this study. Exposure to UV rays increases the

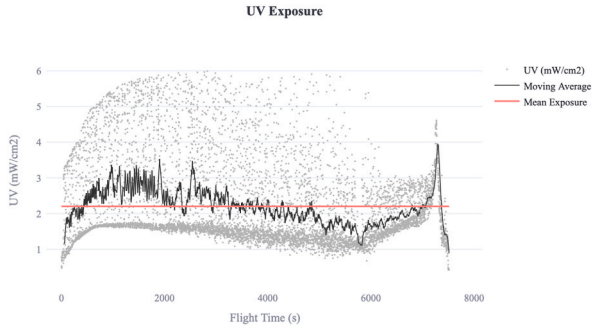

Fig. 5. UVA + UVB exposure during high-altitude balloon missions.

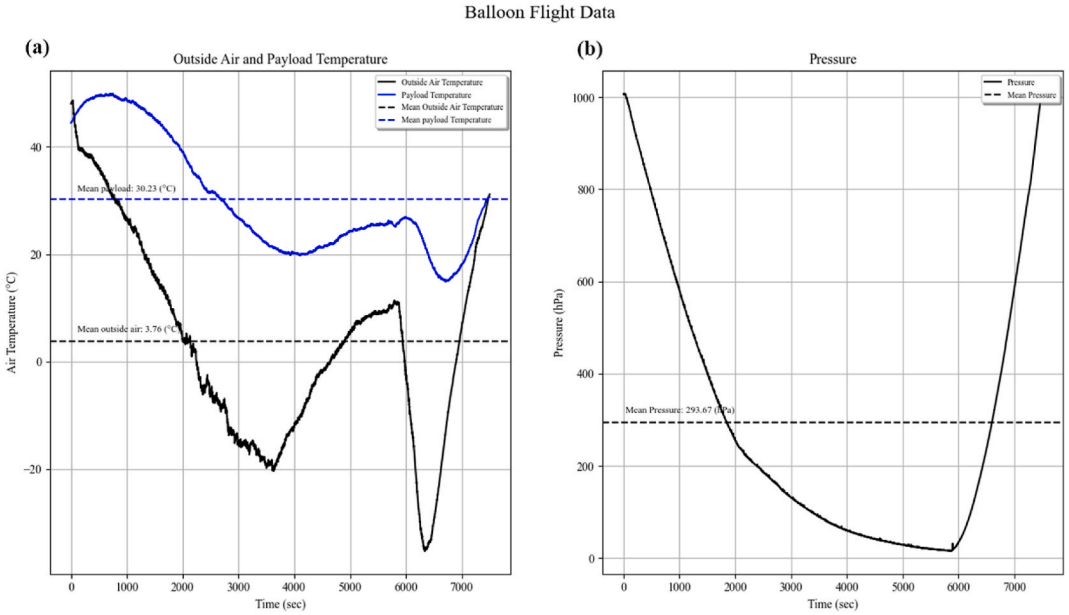

Fig. 6. Temperature (a) and pressure (b) conditions during high-altitude balloon missions.

risk of chromosomal damage and other harmful cellular responses in living organisms, especially microbial cells [33,34]. The total dose exposure of UV irradiation was computed from UVA/UVB intensity values that were gathered during a balloon flight mission. Fig. 5 shows the UV-ray data obtained from the UVA + UVB sensor throughout the flight mission. The data were similar to those of Caro and colleagues, who utilized the same measuring technique [16]. However, the UV intensity during flight was significantly lower than that during flight for Caro and the other teams. The flight time might be a crucial factor, resulting in the low intensity of UV light since the balloon was launched in the early morning. The mean exposure value of the UVA/UVB raw data was approximately 2.2 mW cm^{-2}, which was calculated from 7500 data samples with 1-s intervals between samples. The total dose estimation can be examined using an integration method between the mean exposure and flight duration, resulting in an estimated dose of 164.9 kJ m^{-2} of UVA/UVB exposure. The typical daily dose was only 3.4 kJ m^{-2} per day in Rayong Province, which could not be compared to the dose rate during balloon flight [35]. UV irradiation during this high-altitude balloon experiment was successful compared to the lethal UV radiation dose of 50.76 kJ m^{-2}, which killed one of the most radiation-resistant organisms, *Deinococcus radiodurans* [36].

On the other hand, temperature and pressure profiles during a flight mission cannot be used to determine their substantial role in causing cell death. The ambient temperature of the stratosphere dramatically decreased during flight but was not significantly lower inside the payload box, as shown in Fig. 6a. The calculated temperature from the International Standard Atmosphere (ISA) standard was −21.6 °C at the standard tropopause altitude (11,000 m), whereas the actual temperature was 3.7 °C at the same altitude [37]. Thus, the flight operation temperature was lower than the ISA standard of 25.3 °C (ISA+25.3 condition). The notable difference between these two temperatures might be caused by the tropical climate of Thailand, which is naturally hot and humid throughout the year. This information indicated the interference of solar temperature on cold shock exposure during this mission. Moreover, the payload box indicated that there was no significant change in temperature during flight. The mean temperature was approximately 30 °C, and the minimum temperature was 15 °C, which remained at almost the same level throughout the flight. The optimal

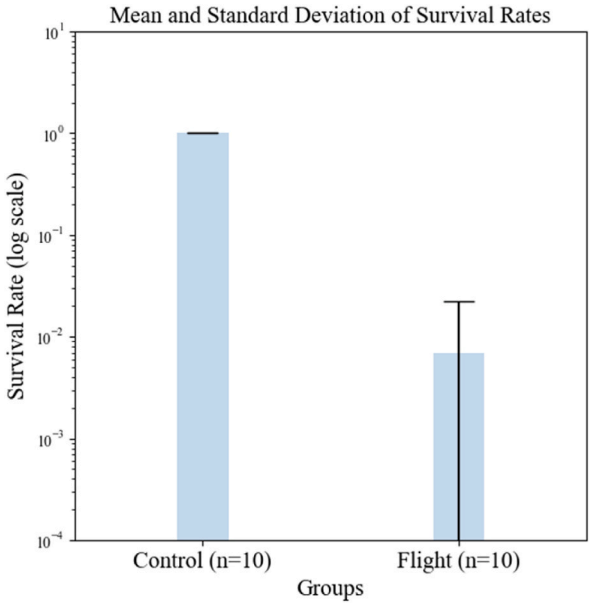

Fig. 7. The mean and standard deviation of the survival rate of yeast after exposure to stratospheric environments.

temperature for budding yeast cultivation is 30 °C, the optimal temperature for cell growth [38]. A harmful response occurs only when the temperature reaches a near freezing point (10 °C or below) [39]. Hence, the payload temperature did not reach a severe cold shock level to cause yeast mortality during the balloon mission. This unexpected result made the temperature effect negligible in our mission.

Furthermore, the ambient pressure profile notably decreased throughout the balloon flight (Fig. 6b). The pressure at the apogee was as low as 15.9 hPa, approximately fifty times lower than the surface pressure. However, Diaz and colleagues reported 73% microbial viability when the cells were cultured in sea salt water during low-pressure treatment [40]. There was an insignificant change in cell death after ten days of ultralow-pressure exposure. The budding yeast in this study was stored in liquid medium throughout the flight, similar to the conditions used for Diaz's experiment. Additionally, the flight duration was only approximately 2 h, which is too short to cause considerable cell death in the absence of atmospheric pressure. It is possible that all extreme conditions during balloon flight can contribute to yeast mortality during this mission. However, insufficient cold shock treatment and temporary exposure to ultralow pressure might not be able to cause any remarkable damage to budding yeast under these conditions.

3.3. Yeast survival after stratospheric environment exposure

The survival rate of the budding yeast after exposure to atmospheric conditions was assessed by utilizing the number of colony-forming units on YPD plates and comparing the results with those of the control groups to determine the cell viability of the yeast. The colony-forming units of ten samples ($n = 10$) from both the balloon flight mission and ground control were used to calculate the mean and standard deviation of yeast mortality from the high-altitude balloon mission, as shown in Fig. 7. Most of the YPD plates from flight conditions revealed no surviving yeast colonies after 48 h of incubation. The biosamples showed a crucial reduction in cell survival of more than two orders of magnitude ($p < 0.05$), which illustrated a percentage viability of approximately 0.66% compared to that of the laboratory control group. This result indicated the deadly potential of UV irradiation in the stratosphere to significantly induce cell death in yeast during 2 h of high-altitude balloon flight.

4. Conclusion

During the Mars mission, hazardous effects from extraterrestrial environments that brutally strike an astronaut's body remain inevitable. The use of numerous simulated facilities could play a role in estimating the consequences of Mars's extreme conditions. However, this approach still cannot represent the true dynamic nature of outer space that humanity aims to encounter in the future. On the other hand, the middle stratosphere has shown great potential for simulating Mars-like conditions that can be accessed safely within the protection boundary of Earth's atmosphere. Hence, high-altitude balloons might be an alternative solution for replicating extreme combinations of space environments. Biological experiments can be conducted through scientific balloon missions to investigate the health risks of space travel. In this article, the platform design of the high-altitude balloon for space biology missions was described in detail. The payload box represents a breakthrough of low-cost components that perfectly measure all desired parameters from the atmosphere during flight missions. The results of the survival assay provided remarkable evidence of harmful UV bombardment in outer space, which almost completely eliminated the entire yeast sample on the balloon mission. Ultimately, we aspire to promote our space biology experimentation platform through the high-altitude balloon, especially for students or scientists

with a strong passion for space biology. The demonstration of the biological onboard experiment endorses the accomplishment of our payload platform, which successfully performed the flight task. Thus, this platform could play an essential role in exploring the mysteries of the universe, aiding mankind to overcome one of the greatest obstacles in human history.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Sumeth Klomchitcharoen: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Pongsakorn Wechakarn:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Tanchanok Tangwattanasirikun:** Writing – original draft, Methodology, Investigation, Formal analysis. **Noparin Smerwong:** Visualization, Validation, Software, Formal analysis, Data curation. **Phubase Netrapathompornkij:** Resources, Methodology. **Thanapat Chatmeeboon:** Resources, Methodology, Investigation. **Norawit Nangsue:** Software, Resources, Methodology. **Vivatsathorn Thitasirivit:** Software, Resources. **Krin Kaweewongsunthorn:** Software, Resources. **Suvijak Piyanopharoj:** Software, Resources. **Phachara Phumiprathet:** Writing – original draft, Visualization, Software, Resources. **Yodchanan Wongsawat:** Writing – review & editing, Validation, Supervision, Project administration, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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