Research article

MINERVA: A CubeSat for demonstrating DNA damage mitigation against space radiation in *C. elegans* by using genetic modification

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A B S T R A C T

The ideas of deep-space human exploration, interplanetary travel, and space civilizations are becoming a reality. However, numerous hindrances remain standing in the way of accomplishing these feats, one of which is space ionizing radiation. Space ionizing radiation has become the most hazardous health risk for long-term human space exploration, as it can induce chromosomal damage and epigenetic changes. The Minerva mission aims to demonstrate cutting-edge technology to inhibit DNA damage against deep-space radiation exposure by using genetic modification. The concept of the experiment is to transform a creature with radiation intolerance into a transgenic organism that is radiation-tolerant. In this mission, *Caenorhabditis elegans* (*C. elegans*) will be genetically engineered with a protein-coding gene associated with DNA damage protection called damage suppressor (Dsup). Dsup is a nucleosome-binding protein from the tardigrade *Ramazzottius varieornatus* that has a unique ability to prevent DNA damage. This paper describes the feasibility of Minerva CubeSat, which will venture out to cis-lunar orbit with a biosensor payload capable of sustaining and culturing *C. elegans* under space environment conditions for 4 months. The mission will set in motion a paradigm shift corresponding to future space medicines and how they will be developed in the future, introducing a platform suitable for future experiments in the fields of space biology. Ultimately, the paramount objective of Minerva will be to test the limits of genetic engineering and incorporate it into the arduous journey of human perseverance to overcome the boundaries of space exploration—a vital step in making Mars colonization safe.

1. Introduction

Humans have wondered what lies beyond the sky and stars for as long as history. The concept of space and the vastness of the universe still perplexes us to this day, and space exploration poses the greatest challenges that even our sheer perseverance and tenacity struggle to overcome. The thought-provoking idea of an ever-expanding universe, with unlimited possibilities and the chance to find extraterrestrial life, has captivated us long before modern civilization. This idea leads humanity to the Mars mission, something of the near future, with only a few restrictions in the way. One of the most consequential, posing the most significant ramifications to human health, is space ionizing radiation. Within low earth orbit (LEO), a mere 87-day mission orbiting the earth in the international space station (ISS) resulted in a radiation dose of 178 milliSievert (mSv). On the other hand, a person receives approximately 2 mSv each year on earth. Furthermore, an estimated dose absorption has the potential to reach 1200 mSv during the mission to Mars, indicating the inevitable hazard potential from space radiation (Lloyd and Townsend).

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The shift to developing space medicines instead of constructing a higher quality shielding method came from the endeavor to prevent chronic long-term health damage induced by galactic cosmic rays (GCRs). High energy particles of GCRs present an annual dose of <20 cGy, which is not enough to cause acute health problems, but instead, instigates chronic health diseases from DNA damage (Townsend, 2005). High doses of ionizing radiation on biological matter are well understood and cause a variety of DNA lesions, and double strand breaks (DSB) are the main factor (Gulston et al., 2002). A comprehensive study on gamma irradiation biological effects found that ionizing radiation-induced lesions can include clustered DNA damage, not only isolated lesions (Friedberg et al., 2005). Ionizing radiation compromises the major DNA repair pathways that repair isolated lesions using base excision repair and strand break repair pathways (Dianov et al., 2001; Schärer and Jiricny, 2001). It has also been shown that an increase in the ionizing density of radiation decreases the repairability of DSBs in cells, which correlates to high-charge particles (HZEs) as they deposit a large amount of energy along their path, producing a considerable number of ions in biological matter (Blöcher, 1988; Bucker, 1974; Jenner et al., 1993). These issues lead to the idea of genetically modifying an organism, fit for future development and integration into medicine, as a new alternative to manage space ionizing radiation.

The genome-editing technique against space radiation will utilize a synthetic recombinant protein from the tardigrade genomic DNA template to mitigate the radiation-related biological damage. The tardigrade *Ramazzottius varieornatus* or the water bear, one of the most tolerant extremophile species, is able to withstand extreme amounts of radiation doses, up to 4000 Gy with alpha particles, comparable to that of radiation received in space (Horikawa et al., 2008). This tardigrade possesses a unique protein-coding gene associated with DNA damage protection called a damage suppressor (Dsup) (Minguez-Toral et al., 2020). The resemblance of sequences between Dsup genes and the human nucleosome binding domain enables the Dsup protein to protect against DNA damage from radiation, preventing DNA fragmentation due to reactive oxygen species (ROS) production. The HMGN-like sequence in Dsup protein allows it to bind specifically to nucleosomes, which is functionally significant for its ability to protect chromatin from hydroxyl radicals (Chavez et al., 2019). Dsup plasmids have been transfected into the HEK293 human kidney cell line with successfully enhanced radiotolerance, alleviating DNA damage induced by X-ray exposure by 40%. This experiment provides strong evidence that Dsup proteins could play a key role against GCRs during long-term space exploration (Hashimoto et al., 2016). There also have the hypothesis that Dsup could behave similarly to HMGN1 and create a more open chromatin landscape in human cell lines (Westover et al., 2022).
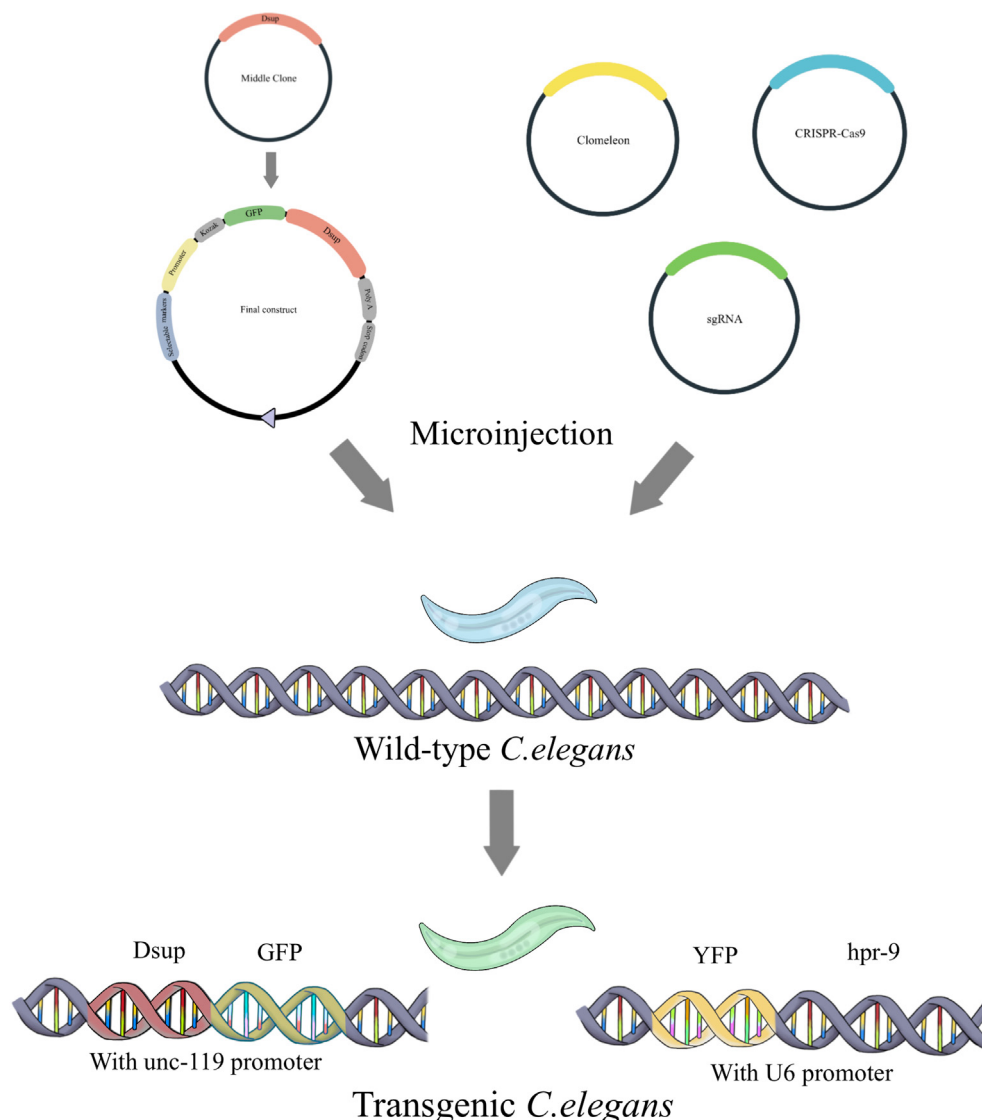

Figure 1. Gene editing process of transferring Dsup proteins into *C. elegans*.

The objective of the Minerva mission is to observe the radioresistance capability of transgenic *C. elegans*, which express Dsup proteins. The onboard experiment will serve as a proof of concept that the genetically modified *C. elegans* can tolerate more radiation than a controlled *C. elegans*. Since LEO or ISS cannot replicate the nature of deep space radiation within Earth magnetosphere protection, the experiment must be carried out in deep space as it best represents GCRs that astronauts may receive during long-term space exploration. Our steppingstone mission has intended to provide astronauts with possible space medicine technology capable of countering the DNA damage effects on human health in extreme conditions of the deep-space environment. The experiment on Minerva is unique among the CubeSat missions performed to date, as it can experiment with living Animalia species rather than microorganisms, especially for over four months. This paper describes the preliminary design for Minerva CubeSat, focusing on the platform design and feasibility of the Minerva mission. Minerva payload will consist of two scientific components: 1) an AIBO biosensor, which comes up with real-time DNA damage monitoring on *C. elegans* and 2) radiation dosimeter. These two payloads allow CubeSat to study the relationship between the biological response and the dynamic nature of the space environment. The Minerva mission aspires the CubeSat platform suitable for future space life science missions that will conduct the experiments under space conditions in a nanosatellite.

2. Material and methods

2.1. Caenorhabditis elegans

The model organism, the nematode *Caenorhabditis elegans* (*C. elegans*), is a free-living, transparent, small-size (approximately 0.25–1 mm), short lifespan organism with a complete genome sequence and cell lineage (Brenner, 1974). Its genome contains up to 83% of human homologous genes, which considers *C. elegans* an appropriate model organism for further research on space medicine development (Lai et al., 2000). The life cycle of *C. elegans* is approximately three days, including the embryonic stage, four larval stages (L1-L4) and the adult stage (Corsi et al., 2015). The maximum lifespan of *C. elegans* is 18–20 days, which is not sufficient to conduct an onboard experiment (Zhang et al., 2020). Intriguingly, *C. elegans* can form dauer through controlled starvation. If the eggs of *C. elegans* hatch in the absence of food, they will enter the Dauer state to prolong the survival of starvation for up to 4 months (Wang et al., 2009). The Dauer state halts the postembryonic development of *C. elegans* to conserve energy, ensuring that *C. elegans* will not grow without adequate nutrition. It is advantageous for our experiment, as Minerva is able to perform an onboard experiment with a living organism for over four months. Moreover, dauer larvae can be stimulated to continue growing by flooding it with nutrients. After it leaves Dauer state, Minerva will be able to observe the growth rate and movement of *C. elegans* via the AIBO platform.

2.2. Transgenic Caenorhabditis elegans

In this mission, genome modification will be utilized to engineer Dsup proteins into wild-type *C. elegans* strain. The procedure of protein transfer is described as it is the most significant task to accomplish the Minerva mission. During somatic gene editing, the promoter unc-119 will be used to initiate transcription, which is known to express the protein at the neurons of *C. elegans* (Maduro and Pilgrim, 1995). For the gene of interest, the Dsup sequence will be inserted along with the multiple cloning site of the worm vector backbone named pCFJ601 (Frøkjær-Jensen et al., 2012). The coexpression of green fluorescent protein (GFP) or the purification tag is another essential element that indicates the existence of Dsup proteins in the animal model. To terminate the transcription process, a polyadenylation signal will be added after the Dsup sequence. The Kozak sequence and the stop codon will be utilized as the translation initiator and terminator, respectively, during protein translation. These

are the main components required for our transgene. The final construct of assembling all plasmid materials, composing a 5′ entry clone, a middle entry clone, and a 3′ entry clone, will be built by utilizing the multisite gateway cloning technique. Each component includes a promoter, gene of interest, and UTR unit. Each vector also contains homologous attB sites as its adjacent clone. The transgene will be developed by amplifying the Dsup gene sequence through the polymerase chain reaction (PCR) cloning method and delivering it to the gateway donor vector via BP reaction, applying 5′entry and 3′ entry clones for the construction process. Then, the LR reaction is used to recombine all the materials into a single plasmid (Merritt and Seydoux, 2010). Ultimately, the final construct is ready to be duplicated through the amplification processes in DH5alpha *Escherichia coli* (*E. coli*) and be chosen from the bacteria with selectable markers named NeoR - KanR and AmpR, kanamycin and ampicillin resistance. After the aforementioned processes, the plasmid will be ready for the transfer procedure, which will nevertheless utilize the microinjection method (see Figure 1).

According to the initiation of the microinjection procedure, DNA preparation will be performed by applying the purification method as the guide of the QIAGEN-tip 20 miniprep kit. Subsequently, to begin the injection process, the temperature will be dropped from 20 °C to 15 °C to lower the movement of the L4 stage nematodes. The injection will be at the distal gonad syncytium to inject the complete plasmid. The transgenic hermaphrodite progeny that inherit the genetic materials are identified and selected according to the observation of fluorophores (Rieckher and Tavernarakis, 2017). With the property of integrating the target gene at a specific locus, the transgene will be introduced to the *C. elegans* genome using the Mos1-mediated single-copy transgene insertions (MosSCI) technique. Mos1 from floxed NeoR containing plasmid is responsible for the induction of double-strand break at the ttTi5605 site of *C. elegans* genome. The plasmid will mediate the transposition of a gene into the previously created flanking region between the landing site. The methodology will follow the instruction of Frøkjær-Jensen et al. (2008).

Apart from using GFP as an indicator to ensure the existence of Dsup, 724 bp optimized Clomeleon fluorochrome or yellow fluorescent protein (YFP) will be inserted in the *C. elegans* gene associated with the ATM/ATR pathway to report DNA damage severity. Clustered regularly interspaced short palindromic repeats (CRISPR) and Cas9 protein, will be utilized along with homologous directed repair (HDR) to transfect fluorescent protein at the target site. CRISPR-Cas 9 and the PCR screening process will follow a protocol of Kim et al. (Kim and Colaiacovo, 2019). Single-guided RNA (sgRNA) with binding site are chosen specificity to a hpr-9 protein, where hpr-9 is responsible for responding to DNA damage and cell cycle checkpoint signaling (Stergiou and Hengartner, 2004). The GG dinucleotide protospacer adjacent motif (PAM) locates in front of the gene in the range of less than 10 bp at the genomic position III:10621541–10621542 and serves as a binding site of Cas9 protein. Short-range HDR CRISPR is applied in this experiment as our double strand breaking site, which is close to a PAM and has the desired specificity (Paix et al., 2017). The vectors containing Dsup-GFP, Mos1, CRISPR-Cas9, sgRNA, and chameleon with homology arms are inserted in unison via microinjection. These methods were chosen to ensure the presence of both Dsup and DNA damage will be observed through a fluorescent stereomicroscope. Both light emissions of GFP and YFP can be excited with the same frequency range and will emit a different color of light back to the instrument.

2.3. Spacecraft onboard experiment

During the experiment in cis-lunar orbit, three types of *C. elegans* will be involved: 1) transgenic *C. elegans* as the primary model organism of the Minerva mission. 2) Space-controlled *C. elegans* with the expression of GFP and YFP. 3) ground-controlled *C. elegans*, which remains on Earth. The onboard experiment will operate through the Autonomous Intelligent Biological Operating system (AIBO), which is the scientific payload of Minerva. In AIBO, the *C. elegans* will be separated into 16 chambers
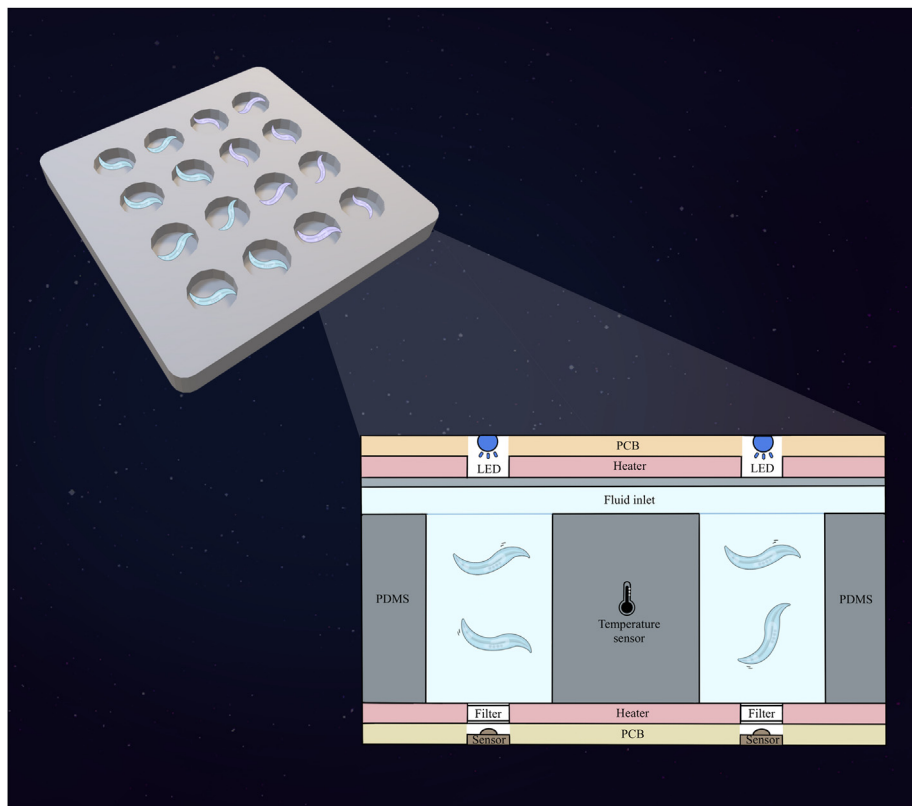
Figure 2. A cross-section of AIBO microfluidic chips integrated with blue and near-infrared LEDs to track DNA damage in *C. elegans* through photodetector.

with ten *C. elegans* each. Control factors such as nutrients and temperature will be automatically adjusted and controlled by AIBO. *C. elegans* will be loaded into the microfluidic system of AIBO while in the Dauer state.

The AIBO payload platform will be separated into eight sets of biosensors. Each set of biosensors will contain two models of *C. elegans*: transgenic type and space-controlled type. Biosensor numbers 1 to 8 will be conducted for 16 weeks in total, with a two-week gap between each experiment. Each experiment aims to compare the differences in radiation response between the two *C. elegans* models in space and ground control *C. elegans*. The results will be quantified and examined to determine whether transgenic *C. elegans* can withstand space ionizing radiation and continue growing as it would on Earth.

2.4. Experiment analysis method

Chronic DNA damage in neurons of *C. elegans* after absorbing space radiation will be observed by utilizing the amount of YFP appearance in neurons and comparing it with control groups to determine the enhancement of radioresistance in transgenic *C. elegans*. YFP will be expressed along with hrp-9, a biomarker for the DNA damage response pathway in *C. elegans*. An increased amount of YFP expression indicates a higher level of DNA damage in neurons, which respond to space ionizing radiation. Coexpression GFP will be added along with the Dsup protein sequence to quantify the expression of Dsup proteins in genome-engineered model organisms, as GFP should be expressed in accordance with Dsup proteins. Since the mentioned technique utilizes the quantitative comparison of both GFP and YFP expression, these fluorochromes will be added into both control groups to perform this method. In addition to measuring DNA damage, this method can also be applied to gather the quantity of remaining *C. elegans*, as some might not survive during the long-term spaceflight mission. The *in vivo* emission spectrum

of GFP and YFP in live *C. elegans* allows AIBO to monitor the change in GFP and YFP expression via a multidimensional (multicolor) imaging technique. Minerva is able to provide real-time insights into the neuronal DNA damage of *C. elegans* through AIBO, a fully automated biosensor platform that is capable of collecting the biological response data every hour. In addition, the growth rate of *C. elegans* can be tracked via AIBO after provoking it out of the Dauer state.

2.5. Radiation dosimeter

A Timepix-based linear energy transfer radiation spectrometer (LETS) will be applied for deep-space dosimetry in the Minerva mission as one of the primary payloads. It will provide radiation dose measurements during traveling in a near-rectilinear halo orbit, including GCRs and SPEs (Stoffle et al., 2015). The received total ionizing dose (TID) will be computed and collected into onboard storage of the payload before transmission back to the Earth. It is essential to analyze the radiation resistance ability, biological damage, and cis-lunar orbit radiation level of subjects.

2.6. Autonomous intelligent biological operating system (AIBO)

A fully automated in-situ biosensor that integrates microfluidic and optical sensors into the system, provides DNA damage observations in neuron cells of *C. elegans* with exposure to space radiation (see Figure 2). The AIBO payload consists of eight biosensors; each comprises both transgenic and space-controlled *C. elegans* within its microfluidic system. It is also capable of gathering information via the use of biosensors, subsequently storing the data onto the onboard storage. This information will be sent back to Earth together with TID data of the dosimeter. With this payload, the biological response of *C. elegans* will be tracked every
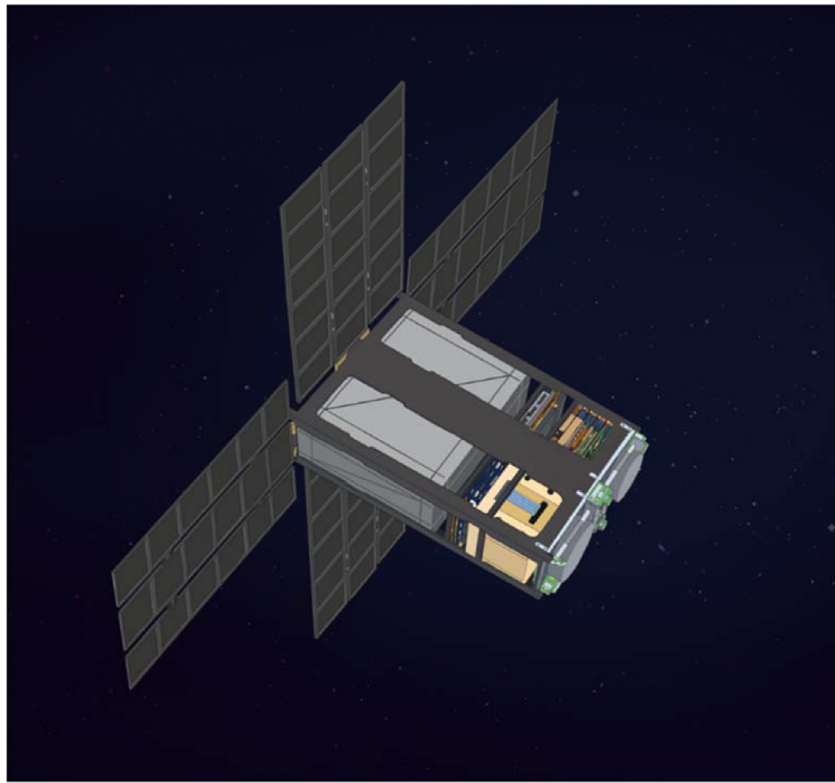
Figure 3. Minerva 6U nanosatellite.

hour or up to request. Details of the AIBO component are described below.

2.6.1. Microfluidic chips

The AIBO microfluidic system consists of sixteen 100 µL cylinder-shaped culturing wells 4 mm in diameter x 7 mm deep, connected microchannels and two 0.8 mL syringe pumps, which contain the *C. elegans* nutrient media. AIBO microfluidic chips will be fabricated by utilizing polydimethylsiloxane (PDMS) within an estimated size of 50 mm × 50 mm x 10 mm, except for syringe pumps. Each AIBO

microfluidic will separate into two clusters of 8 wells, comprising transgenic and space-controlled *C. elegans*. The frangible membrane will adhere between the syringe orifice and microfluidic nutrient inlet microchannel, which will be utilized for controlling Dauer state of *C. elegans*, as it should receive nutrient and arise from the Dauer state at a specific time.

2.6.2. Optical imaging system

The automated monitoring system of AIBO will perform by using the fluorescence detection technique, which requires a CMOS-based optical
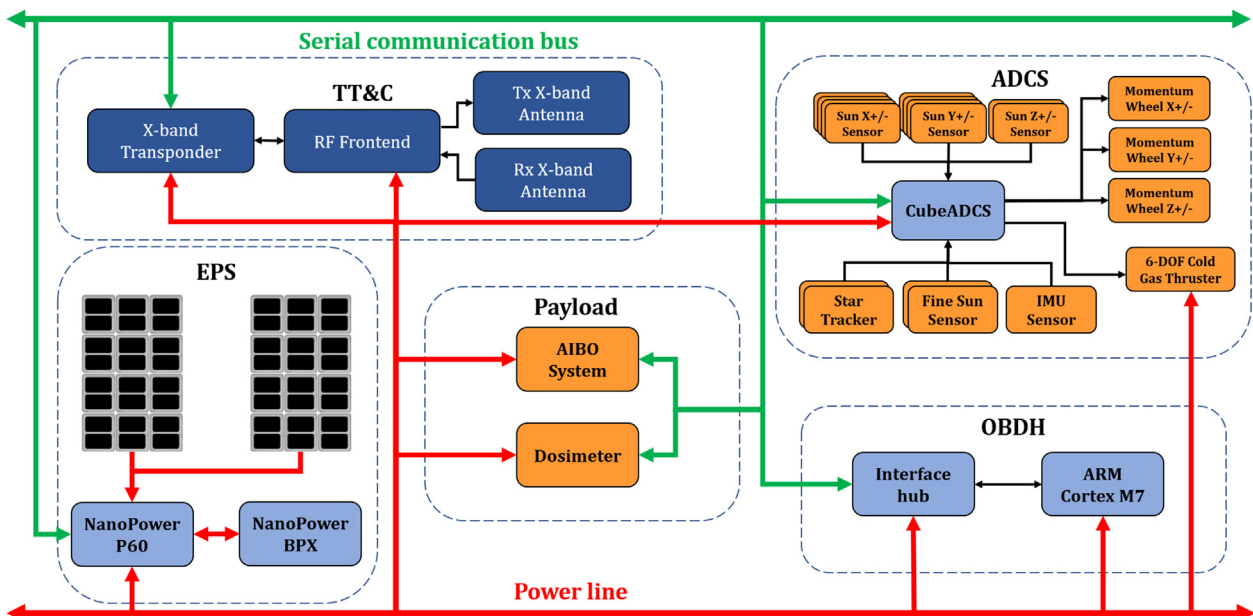

Figure 4. The system diagram of Minerva 6U nanosatellite.

Table 1. Minerva specification.

Category	Technical restrictions and constraints	
System	Lifetime	Operated in orbit ≥3 months On-ground storage >1 year
	Envelope in launching state	100 mm × 226.3 mm x 366 mm
	Mass	≤12 kg
	Power	>80 W
Payload	Mass	≤5 kg
	Power	≤20 W (Peak)
	Biosensors	CMOS-based optical sensor Light emitting diode Wavelength: 470 nm and 485 nm
	Dosimeter	Timepix-based linear energy transfer radiation spectrometer
Structure	Mass	≤1 kg
	Transverse frequency	’25 Hz
	Longitudinal frequency	’50 Hz
Thermal control subsystem (TCS)	Power	≤15W (Constant)
	Control Method	Passive control with active as a complement
Onboard data handling subsystem (OBDH)	Process capacity	2.14 DMIPS/MHz, I2C data bus
	Process storage	RAM >2 M, Flash>256 K
Electrical power subsystem (EPS)	Mass	≤2 kg
	Un-regulated power bus	Up to 32 V
	Battery capacity	2.6 A h at 30% DOD
Attitude determination and control subsystem (ADCS)	Control mode	3-axis stabilized based on three momentum wheels
	Attitude determination accuracy	≤0.1° (3σ)
	Pointing accuracy	≤0.2° (3σ)
	Stabilization	≤0.1°/s (3σ)
Telemetry, tracking and command subsystem (TT&C)	X-Band transmitter	Downlink data rate: ≤6.25 Mbps
	X-Band receiver	Uplink data rate: ≤4 kbps

Table 2. CR3BP patch point for the NRHO North family case.

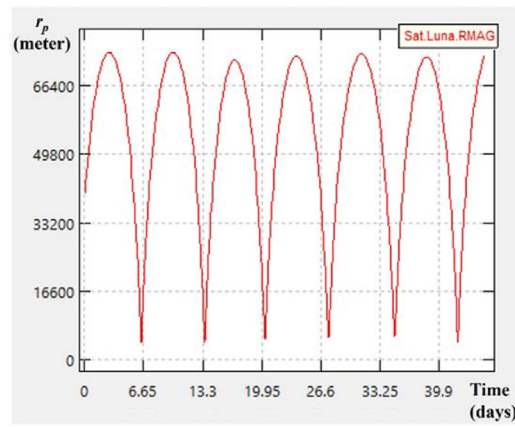| Parameter	Initial condition at 1/8 Rev	Perigee (m)	Apogee (m)
r_x	6060.483	-247.122	16023.07
r_y	19452.284	0	0
r_z	34982.968	-4493.21	71816.65
v_x	0.08267	0	0
v_y	0.00682	1.44467	-0.121.97
v_z	0.368434	0	0

3. Results

Minerva is a custom 6U nanosatellite with a biological space mission to investigate the capability of genome manipulation against radiation exposure (see Figure 3). It will have two deployable solar cells (MMA eHaWK) embedded over 2 sides of satellite 2U surfaces and wings that can pivot on a single axis of rotation using a modification gimbal to span across the 1 U × 2 U face. It allows the power bus to generate up to 84 W. To maintain the orbit, a 3-axis attitude determination and control subsystem (ADCS) and cold gas thruster are used to maneuver the satellite back to its orbit due to the quasi-static nature of NRHO. The navigation tracking method will utilize radio transmissions from deep space network (DSN) to obtain the satellite position via telemetry, tracking and command subsystem (TT&C). There are 4 modes of operation: detumbling mode to control the satellite to zero angular velocity, earth-pointing mode to transmit and receive the signal from the earth, orbit maintenance for adjusting the orbit every 7 days, and sun-pointing mode to maximize the generated power.

Minerva will carry two primary scientific payloads: AIBO biosensors and radiation dosimeters. It will orbit in near-rectilinear halo orbit (NRHO) within cis-lunar space, which is paramount for future space exploration. The overview, requirements and analysis of Minerva subsystems and payloads are described in this section (see Figure 4). As the result of a feasibility study, the main specifications of Minerva are shown in Table 1.
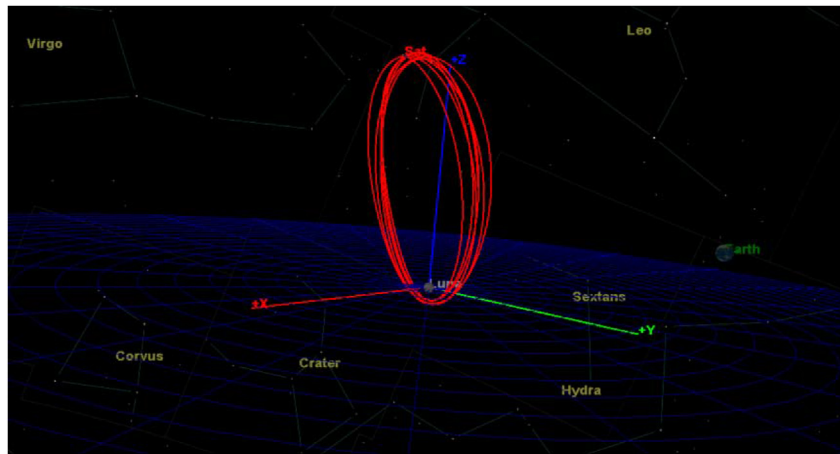
3.1. Orbit trajectory analysis

As no terrestrial facilities can replicate the unique properties of the space environment, not even LEO orbit, the Minerva mission design concerns the amount of radiation dose *C. elegans* would receive; since a limitation time of *C. elegans* hibernation exists. Therefore, the near-rectilinear halo orbit (NRHO) is chosen as our pathway since this kind of path meets our requirements: 1) distance apart from Earth eliminates the shielding capability of the magnetic field, which enhances the strength of radiation exposure. 2) Its time frame according to this orbit could reduce the number of eclipses in which Minerva will be shrouded by the Earth or Moon shadow. 3) The satellite temperature will never be too high or too low (in contrast to L2 Halo orbit). From the literature, the NRHO has a distance between the satellite and the lunar surface of 3,000 km to 70,000 km. This orbit has an elliptical shape trajectory, which takes approximately 7 days for a period. Minerva will be launched to LEO orbit 250 km before maneuvering to Trans-Lunar Insertion (TLI). After arrival near the Moon, it will propagate through an outbound lunar flyby to insert into NRHO.

In this paper, an L2 North family NRHO was simulated in the circular restricted three-body problem (CR3BP) in General Mission Analysis Tool (GMAT) by applying the solution method from Williams et al. (2017) (Williams et al., 2017). The ballistic trajectory of Minerva was performed in the ephemeris model utilizing CR3BP patch points, including solar perturbations. The patch point with r_p of 4500 km for the NRHO North family case are shown in Table 2, presenting within a Moon-centered Earth-Moon rotating frame, where r_p represents periapsis radius with respect to the Moon. As a result, L2 North NRHO is shown in Figure 5. In

sensor. Each culturing well will comprise one photodetector with a narrow bandwidth optical filter and one 470–485 nm light-emitting diode (LEDs), which is used to observe the amount of light illuminated from GFP and YFP expression in *C. elegans* via a fluorescence detection system (Kitts et al., 2007).

2.7. Payload environment control

Temperature is one of the essential factors that must be controlled in AIBO payload as *C. elegans* is very vulnerable to the temperature gradient. Since the ideal temperature for *C. elegans* is approximately 15–25 °C, the payload inside will maintain the ambient temperature within an optimal range at 20 °C to sustain the lifespan of *C. elegans* during the entire mission. Heaters and thermal sensors will be added along with biosensors, which automatically provide appropriate and steady temperatures for the experiment. Airloy X114 has a thermal conductivity of 32 mW/m-K functioned as a thermal insulator, minimizing the thermal fluctuation. In addition, the payload bay will be sealed with aluminium to retain the internal pressure of AIBO throughout the Minerva mission. AIBO pressurized tanks will keep the pressure and oxygen concentration in the payload optimal and similar to that of the atmosphere on earth.

(a)

(b)

(c)

Figure 5. Minerva trajectory simulation in L2 North families NRHO: (a) Position of Minerva CubeSat in NRHO orbit with respect to the Moon. (b) Moon-centered Earth-Moon rotating frame. (c) Earth-centered Sun-Earth rotating frame.

addition, Minerva must nevertheless undertake periodic orbit mainte-nance maneuvers to remain in NRHO for the long term.

3.2. Power budget analysis

Four deployable solar arrays of MMA design will be embedded on the CubeSat structure to provide power generation for the Minerva bus, which consists of double-sided 2U surface solar arrays (eHaWK 27A-42R) and gimbal wings solar arrays (HaWK 17AS42). The electrical power subsystem (EPS) can generate up to 84 W BOL with these four deployable solar panels. The total average and peak power consumption of Minerva is approximately 31 W with a 63.1% margin and 79 W with a 6.4% margin, respectively, which satisfies the power balance of Minerva (Table 3).

Table 3. Power budget.

Category	Component	Average Power (W)	Peak Power (W)
TT&C	X-band transponder	12.6	35
	Tx/Rx X-band antenna	-	-
Structure	6U structure	-	-
EPS	Battery with heater	6	6
	Power module	0.6	0.6
	Solar panels	-	-
ADCS	3-axis ADCS	0.571	2.295
	Fine sun sensor	0.1	0.2
	Star sensor (x2)	0.284	0.528
	Propulsion	-	2
OBDH	Onboard computer	0.783	11.9
TCS	Aerogels insulator	-	-
Payload	Biosensor	10	20
	Dosimeter	0.05	0.07
Total		**30.998**	**78.593**

Table 4. Mass budget and volume budget.

Category	Component	Mass (g)	Volume (cm^3)
TT&C	X-band transponder	1200	500
	Tx/Rx X-band antenna	40	-
Structure	6U structure	1000	-
EPS	Battery with heater	500	450
	Power module	191	300
	Solar panels	600	-
ADCS	3-axis ADCS	554	750
	Fine sun sensor	0.03	-
	Star sensor (x2)	111	-
	Propulsion	682	1100
OBDH	Onboard computer	130	250
TCS	Aerogels insulator	480	-
Payload	Biosensor	7000	3950
	Dosimeter	100	200
Total		12428.03	7500
Threshold		15000	8282.58
Margin		2571.97	782.58

3.3. Mass budget and volume budget analysis

Minerva 6U nanosatellite will be made of an aluminum-based structure of EnduroSat, which has dimensions of 100 mm × 226.3 mm x 366 mm with a mass of less than 1 kg. The entire weight of Minerva is approximately 13 kg with a 17.1% margin, including two payloads and subsystem components. Inside the EnduroSat structure, the volume usage of Minerva is 7750 cm^3 with a 9.4% margin, except for solar panels and antennas that attach outside (Table 4).

3.4. Link budget analysis

The Minerva TT&C subsystem will transmit payload information and communicate with ground stations via Iris V2.1 CubeSat Deep Space Transponder. It utilizes the X-band uplink frequency range of 7.145–7.190 GHz and the X-band downlink frequency range from 8.400-8.450 GHz. Moreover, Minerva will also use uplink transmissions from deep space network (DSN) to navigate the nanosatellite trajectory through the iris transponder. Downlink budget and uplink budget of Minerva with utilizing DSN as ground station are shown in Table 5 and Table 6, respectively.

Table 5. Downlink budget.

Parameter	Value	Unit
Spacecraft transmitter power	3.8	W
Spacecraft transmitter power	5.798	dBW
Transmitter line loss	0.5	dB
Spacecraft antenna gain	11	dB
Spacecraft antenna pointing losses	0.0003	dBi
Spacecraft EIRP	16.298	dBW
Free space loss (max. distance)	170.927	dB
Atmosphere loss	0.083	dB
Ground station antenna gain	68.2	dBi
Ground station antenna pointing loss	0.075	dB
Ground station figure of merit (G/T)	54.2	dB/K
Ground station receiver feeder loss	14	dB
System noise temperature (max.)	358	K
Data rate	6.25×10^6	bps
Bit error rate	1×10^{-6}	-
E_b/N_0	34.519	dB
E_b/N_0 threshold	10.5	dB
Link margin	**24.019**	**dB**

Table 6. Uplink budget.

Parameter	Value	Unit
Ground station transmitter power	20000	W
Ground station transmitter power	43.01	dBW
Ground station transmitter feeder loss	7.635	dB
Ground station antenna gain	68.2	dBi
Spacecraft antenna pointing losses	0.075	dBi
Ground station figure of merit (G/T)	54.2	dB/K
Ground station EIRP (min)	89.5	dBW
Free space loss (max. distance)	169.497	dB
Atmosphere loss	0.083	dB
Spacecraft antenna gain	11	dBi
Spacecraft antenna pointing loss	0.0003	dB
Spacecraft receive noise figure	3.5	dB
System noise temperature	308	K
Data rate	4×10^3	bps
Bit error rate	1×10^{-6}	-
E_b/N_0	95.118	dB
E_b/N_0 threshold	10.5	dB
Link margin	**84.618**	**dB**

4. Discussion

Space is possibly the most hazardous and precarious environment mankind has explored. During deep-space exploration in the near future, crucial health impacts from space ionizing radiation posing on human physiology are inevitable. Since Dsup proteins could play a role in alleviating GCRs consequences, Minerva mission serves as an opportune time to integrate the use of space medicine, a growing field in the space industry, as a high potential alternative solution to space ionizing radiation. The Minerva mission also indicates the feasibility of space biology missions utilizing a living organism within a nanosatellite platform, paving the way for a better understanding of extreme space environmental impacts on terrestrial life. As a result of the 7th Mission Idea Contest (MIC7), Minerva mission idea has won the second prize of MIC7 by the UNISEC global review committees that have shown remarkable evidence to prove the feasibility of Minerva mission in being a notable space exploration mission (UNISEC Global, 2021).

These findings are ambiguous and should be considered as certain limitations still remain. Therefore, it is essential to conduct further studies on the more sophisticated transgenic animal, which could help expand upon the characteristics of chromosomal damage response from space radiation in living organisms. Preliminary research must be done in the near future to ensure that the Dauer state of *C. elegans* is able to illustrate the effectiveness of Dsup on DNA damage properly. The lack of preliminary results raises concerns about *C. elegans*, which indicate the success of the space mission. With limitations on the animal experiment, we could not perform the experiment on *C. elegans* at this time due to ethical issues of animal use. Therefore, we plan to conduct further research in the near future after ethical approval for the experiment. We have started to perform some terrestrial experiments on *Saccharomyces cerevisiae*, which will be utilized as a preliminary study for the ethical approval paper of the *C. elegans* experiment. The limitation of animal ethics has influenced many preliminary results, which should be indicated to ensure the mission possibility. With this limitation, this paper could not perform a preliminary study on AIBO sensor capability for detecting fluorescent proteins, the difference between radiosensitivity and Dsup expression in Dauer state and L4 larvae, the consequence of Dsup in both Dauer state and post-embryonic development, or any result that is based on the grounds of animal experiments. However, the analysis technique for observing DNA damage has been designed properly, which might be enough to identify DNA damage even if the accuracy is not equivalent to terrestrial laboratory methods.

In the end, we aspire to send a ripple through the space community that will get researchers inclined to do studies in this field as it shows incredible promise in being the next breakthrough in both deep space exploration and genetic engineering.

Declarations

Author contribution statement

Sumeth Klomchitcharoen; Noparin Smerwong; Tanchanok Tangwattanasirikun; Peetimon Arunwiriyakit; Pisitchai Tachavises: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Sean Gallup: Conceived and designed the experiments; Wrote the paper.

Jin Tangkijngamwong; Benjamard Jirapanyalerd: Analyzed and interpreted the data; Wrote the paper.

Pichamon Phatthanaanukun: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Siripak Chattanupakorn; Visarut Rungpongvanich: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Norawit Nangsue: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Krai Meemon; Suradej Hongeng Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Patompon Wongtrakoonkate; Yodchanan Wongsawat: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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