Disrupting Herpes virus investigation in lunar orbit: A system for animal cells analysis

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(X) We apply for Student Prize.

( ) Please keep our idea confidential if we are not selected as finalist/semi-finalist.

Mission Objectives

The present project proposes sending a smallsat to the lunar orbit, in order to study the possible reactivation of Epstein–Barr virus (EBV) in animal cells into the smallsat. This investigation can open a whole new possibility to characterize how deep space environmental changes can lead to different situations in the cell development, further than the reactivation of the virus itself; changes such as slow the cancer cell proliferation, reduce the virus replication, induce gene mutations that can lead to a carcinogenesis process, and more important contributions to cancer research, alternative therapies and to think of precautions for the upcoming space flights, in order to seek for the astronauts health.

Epstein-Barr virus is a member of the herpes virus family, which infects more than 90% of the world's adult population, the individual can carry the virus through life, since long-term EBV coexist within most human host without serious consequences, but in some individuals the virus is implicated in the development of malignancy, since is a virus directly implicated in carcinogenesis [1]. According to this, the International Agency for Research on Cancer (IARC), classified it as a type I carcinogen, owing to the fact that infected cells would have the potential to develop lymphomas, nasopharyngeal carcinoma and gastric cancer [2].

A research done years ago [3], demonstrated an increased reactivation of latent herpes virus like Epstein-Barr (EBV), Varicella-Zoster virus (VZV) and Cytomegalovirus (CMV) in astronauts during a short duration space shuttle flight of 10-16 days. The radiation of latently infected cells alone is likely a major contributor to the reactivation of herpes viruses observed in 23 astronauts [3].

Also, it has been demonstrated the presence of EBV in cervical carcinomas [4], participating in the disease that caused around 12.7 million cancer cases and 7.6 million cancer deaths estimated worldwide in 2008 [5].

It's known that spaceflight induces a complicated immune dysfunction in astronauts, deep space can cause low and medium immune problems if it is a long-term journey or a mission respectively, and this could be a probable cause of the subclinical reactivation of latent herpes viruses [6].

Most of the abnormalities are believed to be strongly related to the microgravity environment in space. The important question is whether such abnormalities can be further modified by space radiation. Although the interactive effects of radiation and microgravity have been extensively examined since the early days of space experiment history, the results vary among experiments [7].

The reactivation of herpes viruses is an excellent biomarker to confirm clinically relevant *in-vivo* immune alterations in astronauts and the spaceflight-associated immune dysregulation may represent a model for patients on Earth that experience secondary immunodeficiency. Cell culture studies have hinted at a direct role where microgravity may inhibit T cells function, anyway this experiments and investigations to characterize the dysregulation of the immune system have been made onboard the International Space Station [8].

Apoptotic cell death under microgravity conditions has attracted cancer scientists to look for new cancer therapy since most cell lines have shown increased apoptosis under microgravity [9]. Also, microgravity provides an environment for cell culture that can induce changes and processes in cellular lines that can't be replicated with normal conditions, and this is another reason why the importance of microgravity in different research fields such as cancer, where this can help to understand and suppress tumor metastasis [10].

Concept of Operations

First, it is necessary to have the cell line with the Epstein-Barr virus, which it has been decided to obtain by the ATCC organization, the cell line is called HHV-4 (VR-1492) and it costs 450 USD [11], they must be integrated frozen (-16 °C degrees) in their corresponding equipment in the smallsat. In order to confirm the results, it is necessary to have a cell line control without the virus to evaluate if the changes are due to the reactivation of the virus and not due to the cellular environment, so it's proposed to buy another cell line in this case without any virus to study it together and observe its differences. This second cell line is called HeLa-NR1 and is priced at 249 [12].

SpaceX company was chosen as the smallsat launcher specifically with a Falcon-9, because they recover and reuse a large part of their rockets and thus have managed to lower the costs of putting satellites into orbit, where the approximate cost of lifting a 100 kg satellite in a GTO is around 8,500k USD [13]. From the moment of launch until its arrival in cislunar orbit, the duration time can be a maximum of 5 days, where the advantage of the cislunar orbit is to experience microgravity and radiation conditions that are different from Earth orbit. Once the satellite is in lunar orbit, the cells are thawed by means of the incubator system and the parameters necessary for the cells to live will be maintained, which is mentioned later. Activities include daily monitoring of the cell, photographs, and automated constant maintenance, to appreciate the morphology changes and detect the moment of reactivation of the virus as well as the effects of it in the cells. The following diagram and tables show the above mentioned activities according to the time set for the mission:

Table 1. Schedule of experiment activities.

Mission Activity	1 week	2 week	3 week	4 week	5 week	6 week
1. Freeze the cell line						
2. Unfreeze the cell line						
3. Monitor CO2 levels						
4. Monitor O2 level						
5. Monitor temperature						
6. Pictures of cell morphology						
7. Transmit data						
8. PBS and change culture medium						
9. End of the mission						

Figure 1. Operations Diagram related to activities in Table 1.

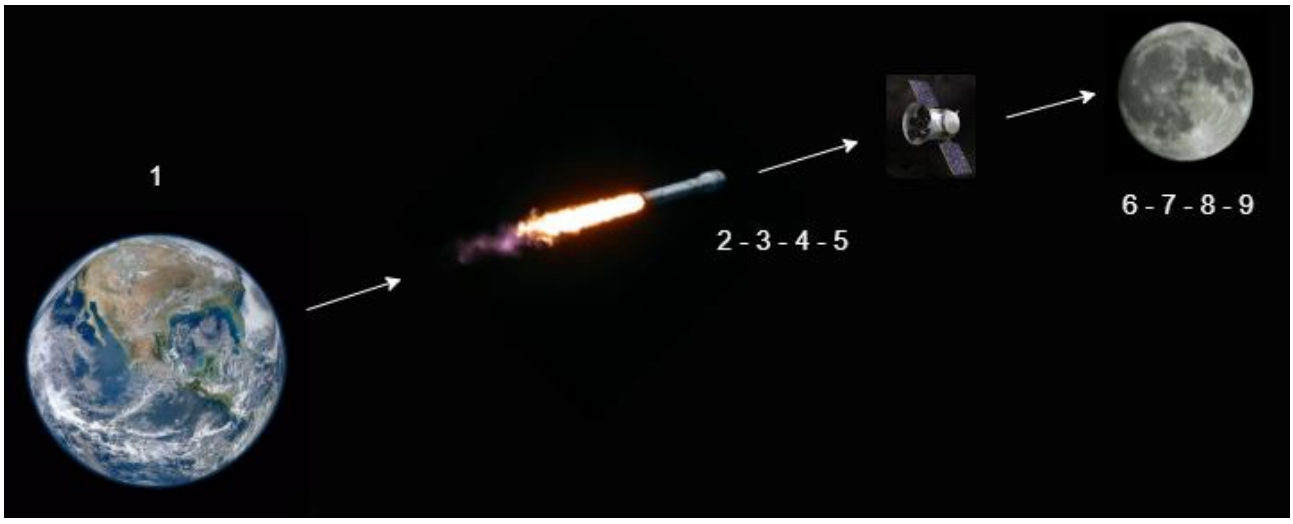


Table 2. Schedule of the general mission.

Activity	2021	1st 2022	2nd 2022	1st 2023	2nd 2023
Mission analysis					
Preliminary design review					
Closure design review					
Assembly and testing					
Launch the smallsat and end of the mission					
Funding search					

Key Performance Parameters

The cells will be surrounded by an environment that provides the optimal conditions for them to remain alive, which would be: Temperature of 33-37 °C degrees, 93%-96% of O2 and 4%-7% of CO2, using an incubator created by us, which will have the possibility of modifying remotely by means of software its temperature from -16 °C to 37 °C degrees, necessary for the cell to thaw correctly, similar in previous experiments in the ISS [14]. In this case, the tool proposed by this group with the name of "Deep Cell Container", would be in charge of maintain the cells in optimal conditions, and would look like the example in Figure 2:

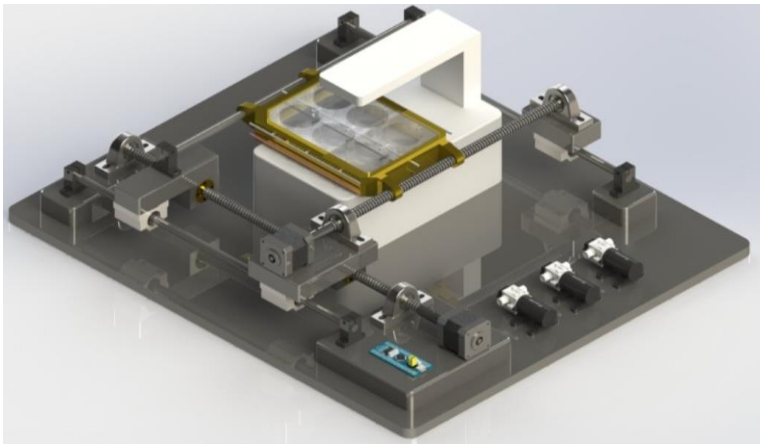
Figure 2. Deep Cell Container.

In Figure 2, are presented 4 types of gas transport pipes of different colors depending on the tasks they perform. The red tubes (both sides below) are to add Phosphate Buffered Saline (PBS) to the cells for its washing, and RPMI medium, which contains the necessary nutrients for its optimal growth, the medium has to be changed every two or three days, using PBS to remove any cell waste substances. The black tube (in the center below) is used to remove the previous culture medium and remove liquids deposited by the cells (using PBS), in order to achieve the wash of the cells and subsequently add fresh medium with the red tubes, the white tube (in the center above) is to transmit the O_2 and finally the gray ones (both sides above) will transmit CO_2. Each one is connected to its respective storage tank for said resources. After fulfilling the necessary conditions for the cells to survive, a digital microscope will photograph the cells and send the data for their respective analysis on the ground.

To keep the range of temperatures that the cell needs, electrical heaters are used to warm the Deep Cell Container case made of a conductive material. The Deep Cell Container also has a digital inverted microscope as mentioned before, that will be utilized for the monitoring of each cell culture with an electromechanical system to move the Deep Cell Container and see each cell well. When the analysis is complete, the data will be sent from the platform towards a ground station for an in-depth review of the results, using in that case the Deep Space Network [15]. Finally, it is necessary to monitor with sensors for each of the variables that are going to directly affect the cells, these variables are temperature, radiation, gravity, and levels of the gases of the cell. In the experiment it's proposed to use hardware redundancy to avoid a fracture-critical system. For the microcontroller STM32 ARM Cortex M7 its going to use a watchdog timer to automatically detect software anomalies and reset the processor if any occur. The redundancy for the PT1000 temperature sensor, NDIR CM1106H-NS CO2 sensor, InPro6800G O2 sensor, and finally a sensor to measure radiation it's a triple modular redundancy so in case the one sensor fails there are still two sensors

measuring. The final system will look like the image in Figure 3:

Figure 3. Mechanical system model



Space Segment Description

First, it is necessary to clarify that a BCP-100 has been chosen as a main structure and that it's a pre-tested platform which has favorable aspects for each individual area that makes up the mission [16]. These aspects are visible in Table 3. These characteristics make it possible for the scientific and technological instruments to be brought along the mission without problems, showing the possible capacity provided by the platform and the actual parameters used in the project like a final mass of 15kg, 50x50x50cm of volume and more factors, verifying that the established requirements are met:

Table 3. Experiment parameters.

Parameters	Platform possibilities	Real used in Payload
Payload Mass	70 kg	15 kg
Payload Orbit Average Power	200 W best case orbit and 100W worst case	60 W
Payload Volume	0.14 m3	0.125 m3
Bus voltage	28 V ± 6 V dc	12,5,3.3 V dc
Interface Temperature	-20 °C to 50 °C	32-37 °C

Table 4. Other parameters.

Variable	Definition
Orbit Altitude	400 to 850 km
Launch Vehicle Compatibility	Delta IV ESPA, Atlas V ESPA, Minotaur I, Minotaur IV, Pegasus, Falcon 9, Falcon Heavy
Orbit Inclination	0° to 98.8°
Stabilization Method	3-axis
Pointing Modes	Nadir, ground target tracking, inertial point, payload sun point, safe
Attitude Knowledge	0.03° 3σ
Attitude Control	0.03° to 0.10° 3σ depending on mode
Communication Frequency	L-Band uplink, S-Band downlink (encrypted SGLS), NASA STDN, and commercial options
Command/Telemetry Rate	2 Kbps uplink/32 Kbps downlink
Mission Data Rate	2 Mbps downlink; 5 Mbps downlink option

Additional considerations

In the additional considerations, the mechanical equipment made by our team is mentioned, both the Deep Cell Container and the incubator that can be implemented even in cell study laboratories due to its automation. Other things to consider are the possible errors that have been

cataloged before, during and after the launch of the experiment itself in the smallsat. One of them is the great limit of the period of time in which the experiment can be carried out.

With respect to the virus, it depends entirely on the life of the cell line, so if the cultures perish, the viral content will also do [17], for that, there is no risk at biological level since the cell and the virus are inert. As the measures taken on redundancy of the technical system were clarified above, these solutions are also presented in the scientific part of the project to obtain statistical data from many cells with the EBV and others without the EBV implanted.

Finally, the importance of performing this experiment on the Moon and not on the ISS is emphasized, since recent studies have stressed the possibility that simultaneous exposure of human fibroblasts to simulated microgravity and radiation, results in more chromosomal aberrations than in cells exposed to radiation alone, although this is still an unknown criteria [18]. Therefore, a better understanding of radiation, microgravity, a different Earth's atmospheric pressure, more oxygen solubility, and large amounts of helium in the Lunar environment can influence the reactivation of herpesviruses, that should provide important knowledge for spaceflight as well as for terrestrial medicine.

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