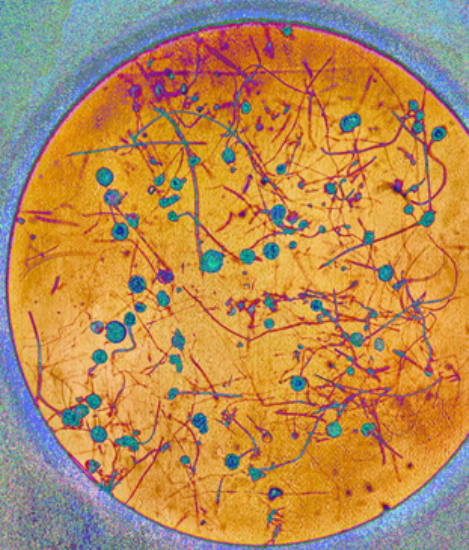
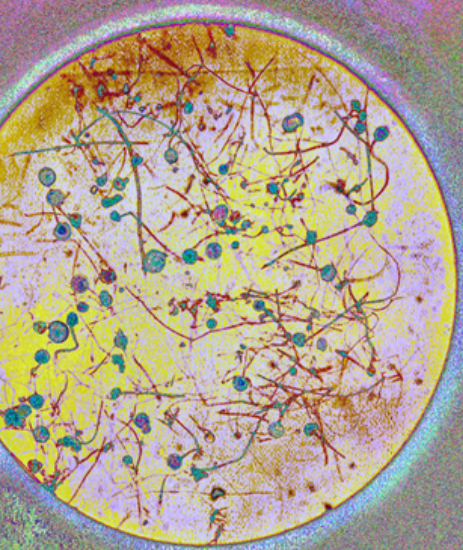


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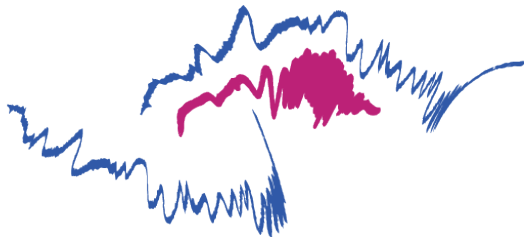


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LETTER FROM THE EDITOR

In my role as the graduate student editor, it brings me pride and joy to present the thirteenth volume of the *Florida Atlantic Undergraduate Research Journal (FAURJ)*. Since its launch in 2012, we have showcased the hard work and dedication of students involved in research at Florida Atlantic University.

We want to thank all the students who have submitted their manuscripts for this edition of *FAURJ*. It is their intelligence and sophisticated research efforts that render this edition noteworthy.

We would like to extend gratitude and thanks to the wonderful faculty at our university who have generously given their time and effort to peer-review each manuscript. Their dedication and scholarly guidance have been critical in ensuring this journal edition scholarly rigor and quality.

We invite you to immerse yourself in the intellectual richness found within the pages of this edition of *FAURJ*. We hope this collection of scholarly works captivates, inspires, and ignites a passion for exploration.

Sincerely,
ANA TYULMENKOVA

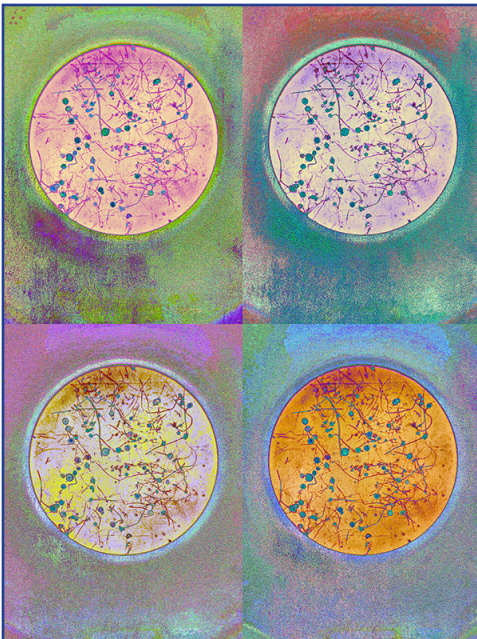
COVER DESIGN ARTIST STATEMENT

“RHIZOPUS REVELATION”

This image offers a close-up view of a *Rhizopus* sporangia, a pivotal fungal reproductive structure commonly found in diverse ecosystems. Captured in my biodiversity lab, the photograph showcases the vibrant colors that accentuate the sporangia’s intricate details, which often go unnoticed by the naked eye. To add a touch of artistry, a subtle grainy filter was applied, emphasizing the microscopic linework within the image. Additionally, enhanced saturation was used to create a sharper and more vivid representation. With four color variants, this image not only celebrates the captivating beauty of this microscopic world but also underscores the diversity inherent in the natural sciences.

— **Sakshi Kumari Pandit**

Undergraduate Student
Charles E. Schmidt College of Science



ATTEMPTING to BLOCK CANCER'S IMMUNE EVASION "DON'T EAT ME" SIGNAL IN A COURSE-BASED UNDERGRADUATE RESEARCH EXPERIENCE

David Harbaugh*, Marissa Shank*,
Jordan Merritt, Ph.D. (Faculty Advisor)

* Denotes Equal Contribution

ABSTRACT

In a semester-long Course-based Undergraduate Research Experience (CURE), 16 students attempted to kill breast cancer cells by blocking their immune evasion technique. The human breast cancer cell line MCF-7 upregulates cell surface CD47 to interact with signal-regulatory protein alpha (SIRP α) on THP-1 macrophages to evade phagocytosis, enabling cancer cell survival. Thrombospondin-1 (TSP1), a known high-affinity ligand of CD47, was used to outcompete SIRP α on THP-1 macrophages. By preventing the CD47-SIRP α interaction or "Don't eat me" signal, an immune response was expected from THP-1 macrophages. Interestingly, there did not appear to be phagocytosis of the MCF-7 cells by THP-1 macrophages but there was a detectable production of pro-inflammatory cytokine TNF α , compared to controls. With mixed results, further optimization and experimentation are needed to confirm the effects of TSP1 on phagocytosis. Further understanding of ways to manipulate the CD47-SIRP α interaction between cancer and immune cells could lead to novel combinational immunotherapies.

INTRODUCTION

A growing problem in advancing research is a lack of laboratory space for students, individuals, and start-ups (Patel, 2021). At universities, many research faculty can only support a handful of students in their laboratories and there is low turnover when student researchers are working towards a thesis or dissertation. Course-based Undergraduate Research Experiences (CURE) have emerged as a possible supplement to direct research with a faculty member (Lopatto, 2007).

CUREs are designed to conduct novel research in a large classroom and/or laboratory training environment. As a result, students who complete CUREs should have a working knowledge of marketable laboratory techniques with the possibility of presenting their results at a conference or submitting them for publication. If research opportunities for undergraduate students continue to be restricted by one-on-one interactions with faculty, the lack of laboratory training beyond standard classroom material can have a detrimental effect on the number of prepared principal investigators (PIs) joining the workforce to conduct research on disease treatments and cures, including cancer.

Original Hallmarks	
1. Avoiding immune destruction	6. Evasion of growth inhibitory signals
2. Reprogramming energy metabolism	7. Growth signal autonomy
3. Evasion of cell death	8. Unlimited replicative potential
4. Genome instability and mutation	9. Tumor-promoting inflammation
5. Angiogenesis	10. Invasion and metastasis

New Hallmarks	
1. Dedifferentiation and transdifferentiation	3. Altered microbiome
2. Epigenetic dysregulation	4. Altered neuronal signaling

Table 1. Original and novel hallmarks of cancer

Cancer remains a worldwide leading cause of death. As the population grows, so will the number of cancer cases and deaths (Torre et al., 2016). About 10 million deaths and 19.3 million new cases caused by cancer were reported in 2020 (Sung et al., 2021). In 2020, the most diagnosed type of cancer worldwide was breast cancer, with 2.3 million new cases (11.7%; Sung et al., 2021). In 2040, 28.4 million cases of cancer are expected (Sung et al., 2021). The burden of cancer is expected to remain and grow worldwide unless novel screenings and therapeutics are available to detect and eliminate it.

There are fourteen hallmarks of cancer that enable immortality, growth, and dissemination as shown in Table 1 (Senga & Grose, 2021). Regarding the fourteen hallmarks, avoiding immune destruction is a challenge for the body's cancer immune surveillance and therapeutics in mitigating breast cancer. The body's immune system typically uses innate and adaptive immune responses to observe, detect, and extinguish budding cancer cells. However, cancer cells have developed strategies to escape

immune clearance by using immunosuppressive factors or decreasing their immunogenicity (Bates et al., 2018). Without mechanisms to eliminate the immune-resistant cells, they will continue to proliferate and be unchecked for regulation.

Macrophages are prominent players within the immune system that can be a target for novel therapeutic approaches. Macrophages have been shown to target tumor sites, directly phagocytose tumors, initiate a tumor's microenvironment, and display noteworthy antigens (Sloas et al., 2021). A prominent checkpoint that acts to prevent phagocytosis by macrophages is cluster of differentiation 47 (CD47), which is a transmembrane protein expressed on all cells. When CD47 on a host cell interacts with a Signal Regulatory Protein Alpha (SIRP α) receptor on a macrophage, a self-cell signaling cascade informs the macrophage that the other cell is a healthy self-cell and to not mount an immune response (Chao et al., 2019). Unfortunately, cancer cells, including breast cancer cells, will overly express CD47 to mimic a healthy self-cell and continue to proliferate (Chao et al., 2019). Currently, anti-CD47 monoclonal antibodies have shown a significant response in diminishing tumor progression by enhancing macrophage activity (Takimoto et al., 2019). An example of a humanized anti-CD47 antibody showing such results in-vitro and in-vivo is CC-90002 (Narla et al., 2022). A concentration-dependent CC-90002 mediated phagocytosis was found in hematological cancer cell lines of acute myeloid leukemia (AML), acute lymphoblastic leukemia, and lenalidomide-resistant multiple myeloma (MM) (Narla et al., 2022). Moreover, immunodeficient mice with MM cell line-derived xenografts showed a similar dose-dependent antitumor activity of CC-90002 (Narla et al., 2022). The success of targeting and blocking the CD47-SIRP α interaction with monoclonal antibodies opens the door for exploring other known CD47 ligands as potential blocking agents. Testing for ligand interaction and the result on cancer cells involves techniques that are ever-evolving, while also not being too tedious for early researchers to master. This type of novel research is a natural fit to be conducted in a large classroom/laboratory CURE setting.

The Immunology Tech Lab CURE was created to teach students marketable laboratory techniques and contribute knowledge to the field through their results. In this CURE, alongside control experiments using anti-CD47 monoclonal antibodies, thrombospondin-1 (TSP1), which is a

known angiogenesis inhibitor and CD47 ligand (Kaur et al., 2021), was used to determine if it can outcompete the CD47-SIRP α interaction. As shown in Figures 1 and 2, TSP1 is a protein encoded by *THBS1*, and its expression decreases in the tumor microenvironment when malignant melanoma and breast carcinoma cell lines develop (Kaur et al., 2021). As expected, an increased expression of TSP1 has been shown to suppress carcinogenesis in squamous cell carcinoma, melanoma, cervical carcinoma, glioblastoma, and prostate carcinoma (Kaur et al., 2021). With this information about TSP1, the CURE students incubated TSP1-treated MCF-7 breast cancer cells with THP-1 macrophage to determine the presence of phagocytosis, via confocal microscopy, and immune response through detection of pro-inflammatory cytokines IL-1 α and TNF α , via Enzyme-Linked Immunosorbent Assay (ELISA).

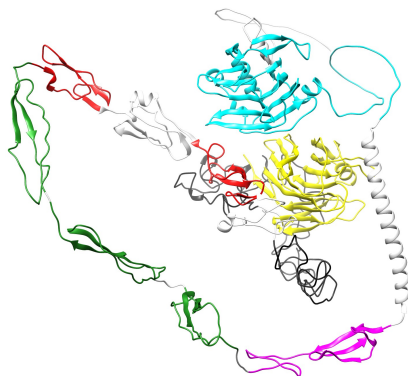
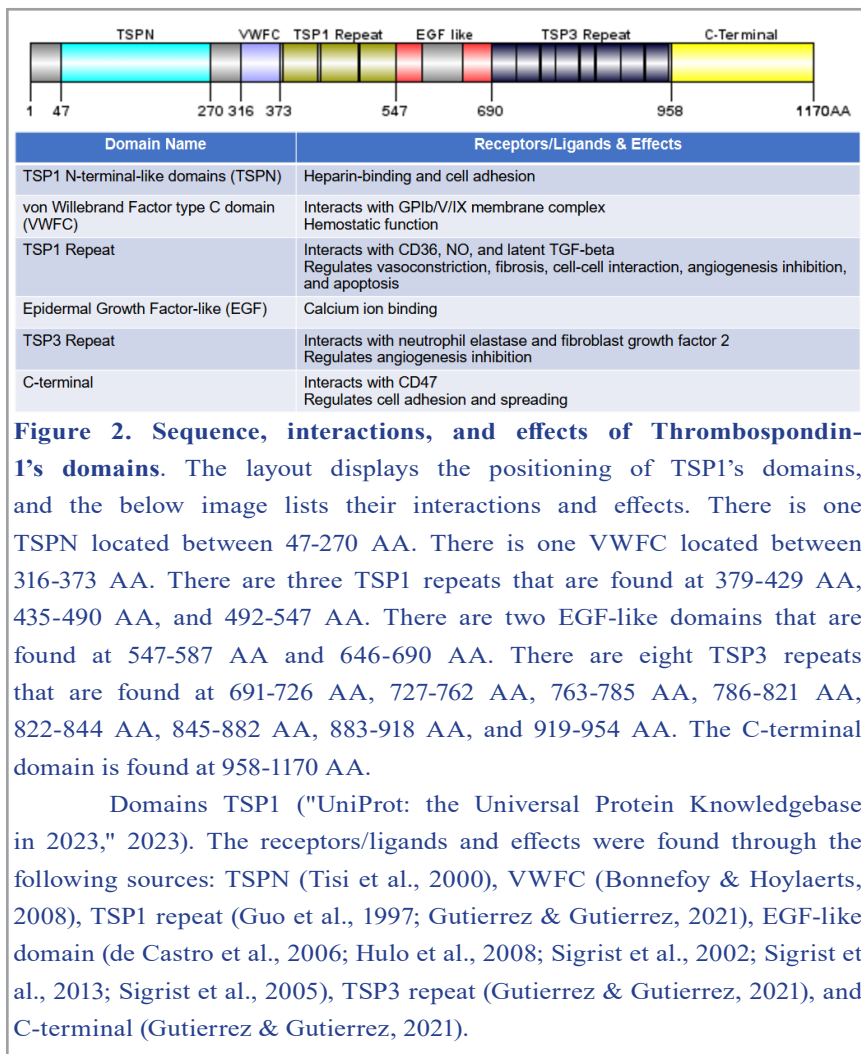


Figure 1. Crystal structure of Thrombospondin-1. The figure displays color-coded domains of the 2D domain sequence in Figure 2. Teal is TSPN, magenta is VWFC, green is TSP1 repeat, red is EGF-like, black is TSP3 repeat, and yellow is C-terminal. Crystal structure of TSP1 (Jumper et al., 2021; Varadi et al., 2022).



We hypothesized that TSP1 will bind to the CD47 receptors on breast cancer cells, thus blocking the interaction of CD47 with SIRP α on macrophage, leading to an increase in phagocytosis of breast cancer cells and the creation of proinflammatory cytokines IL-1 α and TNF α .

MATERIALS AND METHODS

Cell Lines:

THP-1 macrophage (#TIB-202) and MCF-7 breast cancer (#HTB-22) cells lines were obtained from American Type Culture Collection (ATCC).

The THP-1 cells were maintained in Roswell Park Memorial Institute (RPMI) 1640 Medium, the MCF-7 cells were maintained in Eagle's Minimum Essential Medium (EMEM), and both cell lines were supplemented with 10% fetal bovine serum (#16140071; ThermoFisher Scientific) at 37°C in a humidified atmosphere of 5% CO₂. The THP-1 cells were maintained at 1-6x10⁵ cells/mL of media. The MCF-7 cells were treated with Trypsin/EDTA (#25-053-CI; Corning) and split when 60-90% confluency was observed under a light microscope.

Labeling MCF-7 Cell Line:

CellTracker Deep Red was obtained from ThermoFisher Scientific (#C34565). The CellTracker Deep Red dry powder was reconstituted in a stock dilution of 1 mM with dimethyl sulfoxide (DMSO; #317275-55ML; Millipore Sigma). The stock dilution was then turned into a working dilution with a concentration of 1 μM. MCF-7 cells were grown in a 24-well plate to a minimum of 50% confluency. The complete-EMEM media was aspirated from the MCF-7 cells. The cells were washed with serum-free media 2 times and 500 μL of pre-warmed CellTracker Deep Red working solution was added to each well at a concentration of 1 μM. The MCF-7 cells were incubated at 37°C for 30 minutes. Following incubation, the CellTracker Deep Red working solution was removed and 2 mL of EMEM complete media was added to each well.

THP-1 Cell Differentiation:

Approximately 1x10⁵ THP-1 cells in 500 μL of RPMI 1640 complete media were suspended and added to a 24-well plate with round coverslips in each well. 500 μL of phorbol 12-myristate 13-acetate (PMA) solution (100ng/mL) in complete RPMI 1640 was added to the wells containing THP-1 cells while 500 μL of complete RPMI 1640 was added to control wells. The 24-well plate was placed in an incubator at 37°C in a humidified atmosphere of 5% CO₂ for 3 days to allow the THP-1 monocytes to differentiate to M0 resting phase macrophages and adhere to the cover slips. After 3 days, the cells were washed with serum-free media and maintained in complete RPMI 1640.

Co-Culture of THP-1 and MCF-7 Cells:

After culture, MCF-7 cells labeled with CellTracker Deep Red

were gently lifted using Trypsin/EDTA, counted and approximately 1×10^4 cells were added to 2 mL centrifuge tubes. A thrombospondin-1 (TSP1) solution was added to each tube containing CellTracker Deep Red labeled MCF-7 cells to a final working concentration of 5 $\mu\text{g}/\text{mL}$ of TSP1. The tubes were mixed well and then added to the 24-well plate with differentiated THP-1 macrophage growing on coverslips. The cell plate was tilted to mix the reagent and cells and then incubated for 24 hours at 37°C in 5% CO_2 . After 24 hours, the cell supernatants were collected and preserved at -20°C for analysis by Enzyme-Linked Immunosorbent Assay (ELISA).

Labeling of THP-1 Cells and Visualization of their Interaction with MCF-7 with Confocal Microscopy:

After the removal of co-culture supernatants, 1 mL of phosphate-buffered saline (PBS; #BP3991; Fisher Scientific) was added and mixed in each well. The PBS was removed and 500 μL of 4% formaldehyde was added to each well and incubated for 20 minutes at room temperature. The 4% formaldehyde was removed, and each well was washed twice with 500 μL PBS. Then, 500 μL of solution containing monoclonal mouse anti-human CD14-PE conjugated antibody (R&D Systems) was added to the cells at a concentration of 10 $\mu\text{L}/10^6$ cells. The cells were incubated in the dark for 1 hour at room temperature. The anti-CD14-PE solution was removed, and each well was washed twice with 250 μL PBS. The coverslips, with macrophage attached, from each well were carefully removed and placed face down on a microscope slide. The cells on the slides were visualized using a Nikon Eclipse 90i confocal microscope using a 10x eyepiece and Plan Apo VC 60x oil immersion lens.

Enzyme-Linked Immunosorbent Assay (ELISA) for Tumor Necrosis Factor alpha (TNF α) and Interleukin-1 alpha (IL-1 α):

Human TNF α (#KHC3011, Invitrogen) and Human IL-1 α (#ab100560; Abcam) ELISA kits were used to analyze the supernatant from the co-culture of thrombospondin-1/MCF-7/THP-1 cells according to the manufacturer instructions. Standards, samples, and controls were added to a capture antibody-coated 96-well plate and incubated for 2.5 hours at room temperature. The solution was aspirated, and the wells were washed 4 times. A biotin conjugated detection antibody was added to

each well and incubated for 1 hour at room temperature. The solution was aspirated, and the wells were washed 4 times. A streptavidin horseradish peroxidase (HRP) solution was added and incubated for 45 minutes at room temperature. The solution was aspirated, and the wells were washed 4 times. A color-producing substrate specific for each ELISA kit was added and incubated for 30 minutes at room temperature in the dark. Stop solution was added, and the cell plate was scanned on a ThermoScientific Multiskan Skyhigh plate reader for absorbance (450 nm). A standard absorbance curve was created to evaluate the concentration of TNF α and IL-1 α detected from samples.

RESULTS

Confocal light microscopy was used to attempt to analyze the phagocytosis of the MCF-7 breast cancer cells by THP-1 macrophage. To visualize the cells on a confocal microscope, MCF-7 cells were labeled with a whole cell dye (CellTracker Deep Red) that shows as a red color, while the THP-1 macrophage were labeled with a cell surface receptor conjugated antibody (anti-CD14-PE) that shows as a green color. Analysis of phagocytotic activity was determined by taking images of whole THP-1 macrophage and examining the cell borders to locate if MCF-7 breast cancer cells were found within or outside of the macrophages, as shown in prior published studies (Chao et al., 2011; Majeti et al., 2009). The modifications made to these techniques included adding Thrombospondin-1, investigating a breast cancer cell line, using CellTracker Deep Red and anti-CD14-PE to visualize the cells. When imaging the negative control samples containing

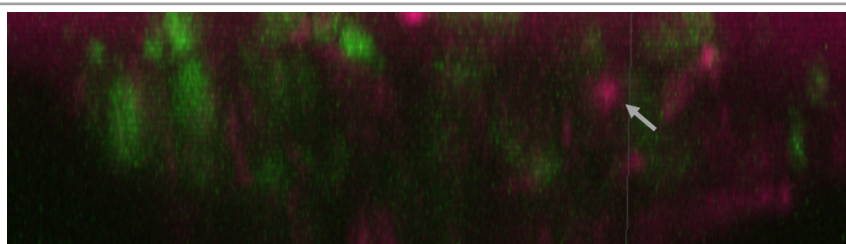


Figure 3. Negative control. The labeled MCF-7 breast cancer cells (red), indicated by the arrow, are outside of the THP-1 macrophage (green), indicating that no phagocytosis occurred. The cells were visualized using a Nikon Eclipse 90i confocal microscope at 600x magnification.

a co-culture of THP-1 and MCF-7 cells, it appears that there are MCF-7 cells found mostly outside of the labeled cell surface of the THP-1 macrophage based on the color variation (Figure 3). This is consistent with prior knowledge that the interaction of CD47 on the MCF-7 cells with SIRP α on the THP-1 cell will result in no phagocytosis (Takimoto et al., 2019). In the images of the positive control samples containing a co-culture of THP-1, MCF-7 cells, and anti-CD47, there appear to be MCF-7 cells found mostly inside of the labeled cell surface of the THP-1 macrophage (Figure 4). Again, this result is consistent with prior knowledge that anti-CD47 antibodies will block the interaction of CD47 on the MCF-7 cells with SIRP α on the THP-1 cell and result in phagocytosis of the MCF-7 cells.

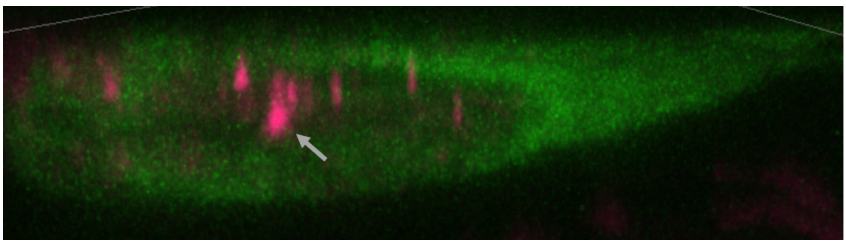


Figure 4. Positive control. Labeled THP-1 macrophage (green), MCF-7 breast cancer cells (red), and anti-CD47 were incubated together. The MCF-7 cells, indicated by the arrow, appear to be inside the THP-1 macrophage indicating phagocytosis. The cells were visualized using a Nikon Eclipse 90i confocal microscope at 600x magnification

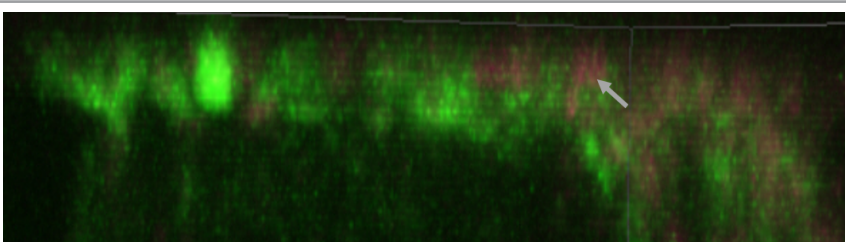


Figure 5. Experimental group. Labeled THP-1 macrophage (green), MCF-7 breast cancer cells (red), and TSP1 were incubated together. The MCF-7 cells indicated by the arrow appear to be outside of the THP-1 macrophage indicating that phagocytosis did not occur. The cells were visualized using a Nikon Eclipse 90i confocal microscope at 600x magnification.

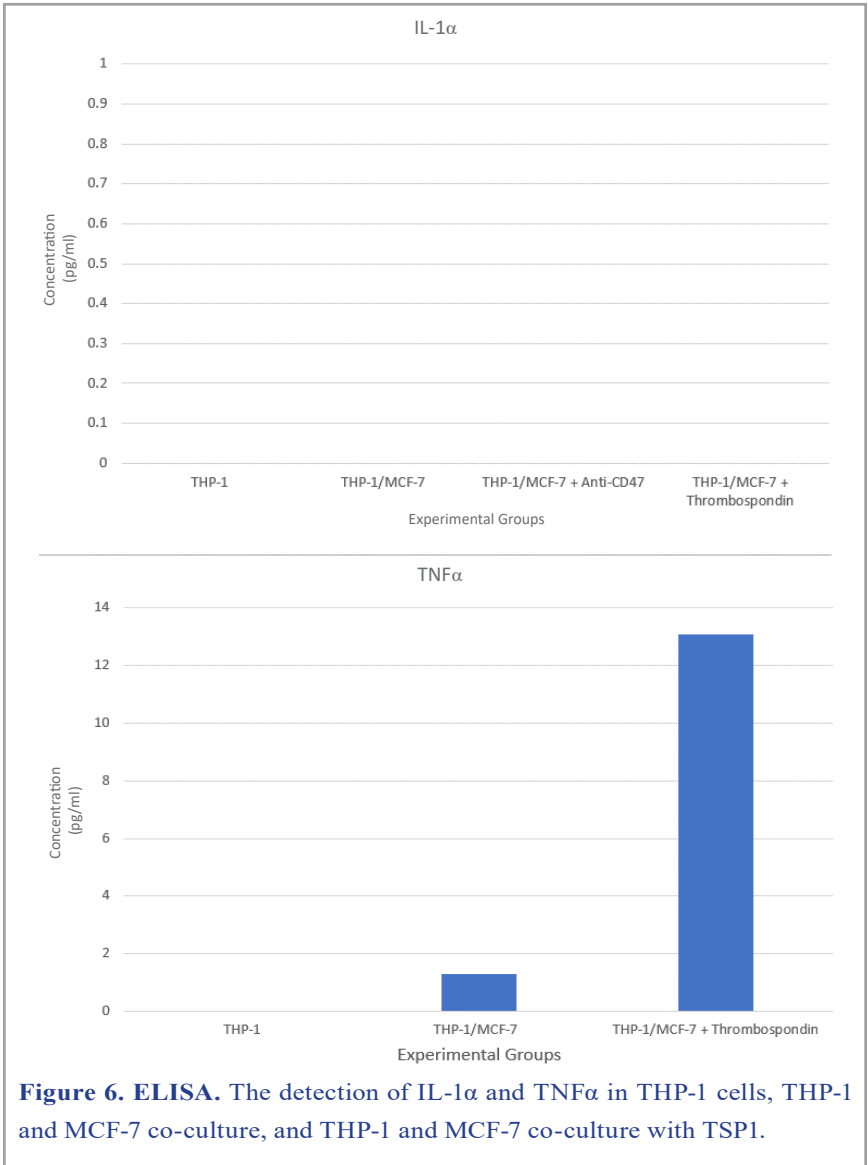
Interestingly, there appear to be MCF-7 cells found mostly outside of the labeled cell surface of the THP-1 macrophage in the images of the experimental samples containing a co-culture of THP-1, MCF-7 cells, and TSP1 (Figure 5). This may signify that immune evasion by the MCF-7 cells potentially took place, and the CD47-SIRP α interaction was not blocked.

To further determine whether immune evasion did take place, ELISA was used to test the concentration of secreted factors in the THP-1 cells, THP-1 and MCF-7 co-culture, and THP-1 and MCF-7 co-culture with TSP1 (Figure 6). There was no detectable IL-1 α in all three of the samples, which could signify that the concentration of IL-1 α was not detectable in our samples at the working volumes used. This is further supported by the positive control, THP-1/MCF-7 and Anti -CD47, not containing detectable levels of IL-1 α . Conversely, we did see detectable TNF α in the samples of THP-1/MCF-7 co-culture (1.3 pg/mL) and THP-1/MCF-7 co-culture with TSP1 (13.1 pg/mL). The difference in concentrations could signify that a baseline interaction is taking place in the THP-1/MCF-7 co-culture, while a larger immune interaction is possibly taking place in the THP-1/MCF-7 co-culture with TSP1 present.

CONCLUSIONS AND FUTURE DIRECTIONS

As diseases become more prevalent there is an increased need for capable scientific researchers to find better treatment options, vaccines, and cures. In the university setting, the research training model of one-on-one interaction with a faculty mentor is straining to bring novel research experiences to as many students as possible. Unfortunately, there is a small maximum that any faculty mentor can train at one time because of the needs associated with one-on-one training. Interestingly, many research projects can easily be adapted to Course-based Undergraduate Research Experiences (CURE) taught as a formal class in larger classroom-type laboratories. The CURE format provides a higher volume of students with training on marketable and valuable research techniques each semester. In this CURE, a class of 16 students in one semester learned about immune evasion techniques of cancer cells and attempted, as a group, to reverse one of those techniques (CD47-SIRP α interaction), leading to the elimination of cancer cells.

As breast cancer becomes more prevalent, the need for new treatment options grows. While it is already known that anti-CD47



antibodies can block the CD47-SIRP α interaction that allows cancer to evade the immune system, this study sought to find a new, potentially more effective way to block this interaction. In place of anti-CD47, a known high-affinity ligand for CD47, thrombospondin-1 (TSP1), was selected to test the ability of TSP1 to outcompete SIRP α on THP-1 macrophage for the CD47 on the MCF-7 breast cancer cells. Based on confocal analysis,

it appears that MCF-7 cells can be found outside of the THP-1 cells when TSP1 is present, which would signify that the interaction of CD47-SIRP α was still taking place and that TSP1 was not outcompeting SIRP α . Since the phagocytotic activity was dependent on qualitative observation, new methods to quantify the fluorescence intensity can be utilized in future studies to provide insight on the proximity of the MCF-7 cells to the THP-1 cells, such as the use of antibody-opsonized polystyrene beads (Babcock, 1999; Choy & Botelho, 2017). Moreover, the use of a surface receptor label for the THP-1 macrophage did not produce a uniform label detectable by confocal microscope. This made the location of MCF-7 cells in relation to the THP-1 cells hard to confirm. Therefore, the protocol can be improved for the next semester of CURE researchers by increasing the concentration of surface receptor label used, decreasing the working volume in the individual wells, or switching to a whole cell dye instead of a surface receptor targeting labeled antibody. Similarly, we expected to see varying levels of the pro-inflammatory cytokine IL-1 α by ELISA, like we did in our TNF α ELISA. A possible solution to help measure a detectable level of IL-1 α is to also decrease the working volume in the individual wells to help concentrate the cytokines present while also helping to bring the different cell types into a closer proximity to each other for immune interactions to take place. Once a protocol has been effectively established, future studies can employ statistical analysis by performing the assay in duplicates and triplicates and repeating the experiment at least two times for consistency. Ultimately, based on the results of confocal analysis and ELISA, it is not yet known if TSP1 is an effective inhibitor of the CD47-SIRP α interaction by outcompeting SIRP α . Additionally, activation of the THP-1 macrophages from an M0 to an M1 state and searching for the presence of more pro-inflammatory cytokines (IL-6, IL-8, and IL-12) in the supernatant could help improve future research examining the phagocytic ability of macrophages. Further experimentation must be conducted to optimize the methods and improvements detailed above.

Through this CURE, students were able to conduct novel research projects with hands-on training in a group setting, like students involved in one-on-one directed research with a single faculty mentor, while also learning to critically analyze results, determine experimental improvements to their research, and present their findings to various audiences in both written and oral forms.

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ANALYZING PLANT GROWTH AFTER A FLIGHT TO AN ALTITUDE OF 30,000 METERS VIA A CUBESAT SATELLITE CARRIED BY A HIGH-ALTITUDE BALLOON

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ABSTRACT

Knowledge of the effects of space travel on plants' growth and development has tremendous importance for the future of human exploration and colonization of the Moon and Mars, and in discovering methods for growing food in outer space. Environmental conditions at high altitudes are known to be hostile to living beings, including plants, due to low temperature, low pressure, and high levels of cosmic radiation. To explore the feasibility of growing plants during space flight, a miniature satellite known as CubeSat was developed by a team of undergraduate students. This prototype CubeSat contained an automatic sensors-equipped Environmental Control System (ECS) to house a plant growth chamber. The CubeSat carrying several alfalfa and tobacco seedlings flew to an altitude of 30,000 meters above sea-level by a helium-filled balloon. After a 27-minute flight, the CubeSat safely landed, and the plants were retrieved. The sensors worked as designed and the data during the flight were recorded and saved. The plants were recovered from the extreme conditions and grown normally in the lab. This experiment has demonstrated that the prototype CubeSat/ECS worked as expected and could serve as a cost-effective platform for future study of plant growth during high altitude or space travel. The results of this endeavor demonstrated a successful interdisciplinary collaboration and should be of significant interest to NASA, as well as other international space agencies to pursue a future of growing plant food in space.

INTRODUCTION

Understanding plant growth and development in out-of-Earth environments is vitally important not only for astronaut health, but for the success of future long-term off-Earth habitation (Wamelink et al., 2014). The increasing interest in space exploration, demonstrated by initiatives like NASA's Artemis and SpaceX's Starship, have raised crucial questions about cultivating food in space and on other astronomical objects. Particularly, little is known about the resilience of plants exposed to elevated levels of radiation in places other than Earth and the feasibility of plant growth in Lunar and Martian regolith (NASA Science Editorial Team, 2022). The risk of space radiation to both humans and plants is a great concern. To assess and understand the risks of cosmic radiation, NASA in collaboration with the U.S. Department of Energy has established the NASA Space Radiation Laboratory at Brookhaven National Laboratory (La Tessa et al., 2016). A high-power ion beam is used to simulate cosmic radiation and evaluate the effects on cells and different equipment. Even though experiments with the NASA Space Radiation Laboratory have been highly valuable to understand the radiation in plants (Zhang et al., 2022), experiments using actual space radiation for an extended period will be critical to examine the long-term effects on the growth, yield and development of plants. Space radiation is generated by various forms of high energy particles (e.g. protons, ions) emitted from the Sun or from outside the Solar System moving at a speed close to the speed of light. On Earth, the magnetic field and atmosphere provide natural protection from this radiation. Plants grown in the space environment would not have the protection that is naturally occurring on Earth.

The establishment of Lunar outposts, such as the Artemis Program's Gateway, underlies the urgency for low-cost investigations on the effects of chronic cosmic radiation and different soil media on various plant species (Connell, 2023). Despite the advances and numerous experiments of growth chambers on the International Space Station, there are still many critical gaps in knowledge for cost-effective, optimal plant production in space environments, including, among others, the effects of gravity, radiation, CO₂, pressure, airflow and pollination in space, as well as the development of water delivery/recovery systems, hardware/sensors, and automated processes for optimal yield. Understanding these effects is vital for the design and development of Lunar and Martian plant life,

contributing to the success of future space missions (Davies et al., 2003).

NASA's efforts to explore radiation effects involve platforms like the Materials International Space Station Experiment-3/4 (MISSE-3/4) and the Space Radiation Laboratory (Reckart, 2021; Brookhaven National Laboratory, n.d.-b). The MISSE program, initiated in 2001, utilized basil seeds to study effects of radiation on plant germination and growth in space, however, the results were not officially published (Reckart, 2021). In 2003, the Space Radiation Laboratory was established to emulate space radiation's effects on materials, organisms, and spacecraft (Brookhaven National Laboratory, n.d.-a). Nevertheless, challenges persist in replicating the continuous low-dose rates of space radiation found in chronic cycles on Earth (Simonsen et al., 2020).

Regarding regolith studies, NASA stationed the Veggie vegetable production system (VEG-01) and Advanced Plant Habitat (APH) on the International Space Station (ISS). The Veggie vegetable production system (VEG-01) is a small plant growth chamber to grow vegetables in space (Massa et al., 2017). VEG-01, initiated in 2014, relies on astronaut integration and lacks independent functionality. The APH, developed in 2017, is an autonomous system capable of having minimal astronaut interaction (Sempstrott, 2023). Despite its success in producing natural and nutritious crops, the APH is bulkier and requires a higher power output compared to Veggie (Costa, 2021), which would be cost prohibitive for growing plant food on the Moon or Mars.

As part of an undergraduate research project, Florida Atlantic University engineering students attempted to develop and optimize a self-contained robotic system to grow plants during space travel. This project addresses gaps in knowledge and the need for low-cost, high-volume data analysis via investigation of the potentiality of CubeSat for autonomous plant environments in space. Successful completion of this project required hands-on skills and problem-solving ability in the domains of engineering, such as systematic integration of various self-controlling and automatic data recording components for many parameters, and the technical procedures for launch of the CubeSat to space. It also required working knowledge and technical know-hows in the research areas of plant biology. Therefore, this project is a collaborative effect in the interdisciplinary investigation of astrobiology, a venture that bridges the realms between engineering and biology.

The CubeSat designed by us, featuring autonomous and integrative systems for CO₂-releasing, watering, lighting, atmospheric regulation, and sensor data collection, strives to serve as a research platform for beyond-Earth farming techniques. The CubeSat containing a plant growth chamber was launched on a high-altitude balloon to simulate an Earth orbit. It monitored plants during the flight, with automatic inputs and storage of environment parameters and ECS state data. Our CubeSat adhered to NASA's CubeSat structural, vibration, and thermal launch requirements, with the High-Altitude Balloon following FAA restrictions. Plants play a significant role in the field of biological sciences, both directly and in aiding space exploration endeavors. Plants offer invaluable insights into gravity, radiation, and other biological phenomena. They contribute significantly to advancing our understanding of how living things adapt to the unique environments encountered in space exploration (Paul et al., 2022).

As a first step towards understanding plant growth response in space, we chose to test alfalfa and tobacco plants. Alfalfa is a fast-growing, hardy, and nutritious legume plant, which may serve as a viable candidate for food supply for long-term space travel (The Editors of Encyclopaedia Britannica, 2023). Tobacco is a genetic model organism widely used in genetic and biotechnology studies. We decided to take advantage of the numerous genetic varieties created in our lab that were particularly suitable for studies of environmental stresses such as cold and radiation (Hill et al., 2016; Murashige & Skoog, 1962). Via a high-altitude balloon carrying the CubeSat with a self-controlled and self-contained Environment Control System (ECS) where a plant growth chamber was housed (Zabel et al., 2016), we attempted to record the growth of alfalfa and tobacco seedlings during the short trip to the Earth's stratosphere. Therefore, this endeavor allowed us to test the reliability of the CubeSat system and to observe plants' responses to the high-altitude environment. This project established the foundation for future improvement of launch vessels and greater investigation of biological processes of plants in non-Earth environments.

In this report, we present the efforts in the design, development and launch of the CubeSat research platform (illustrated in Figure 1) and the assessment of the growth of plants that had experienced an altitude of 30,000 meters above sea level for a brief period of time.

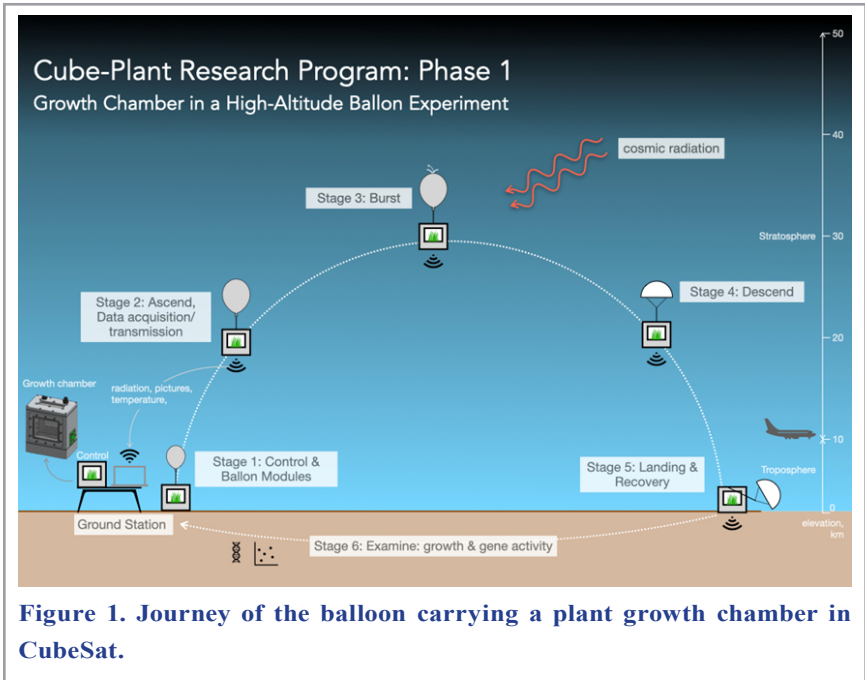


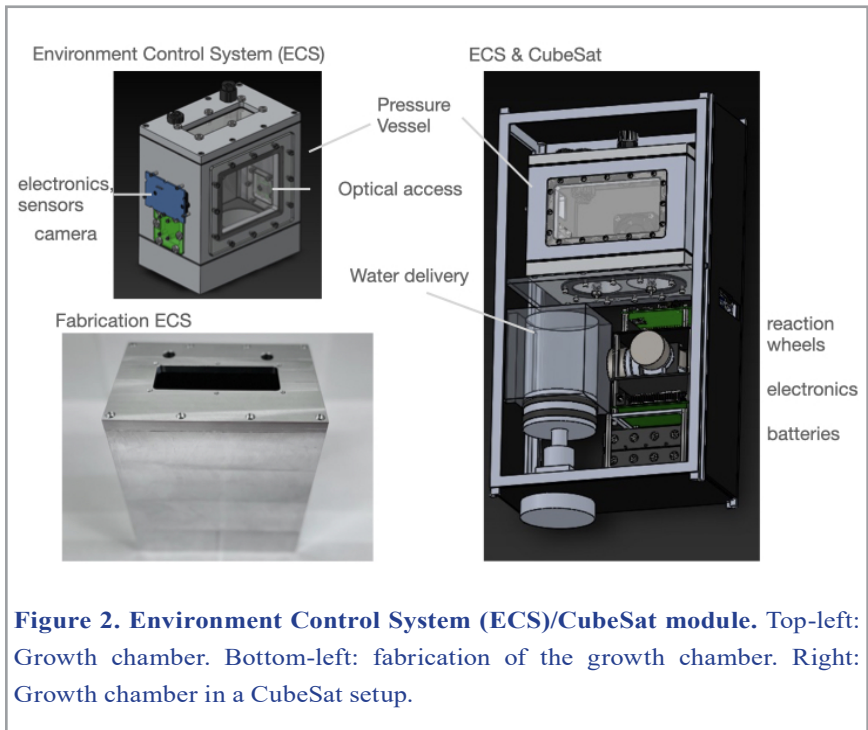
Figure 1. Journey of the balloon carrying a plant growth chamber in CubeSat.

MATERIALS AND METHODS

1. Design of Environment Control System for plant growth in a CubeSat

The Environment Control System (ECS) was designed to house a growth chamber that was able to hold multiple small plants in the 6-unit CubeSat, equipped with cameras and auto-controlled sensors. This CubeSat could monitor and record pressure, temperature, gas composition, humidity, and radiation. The CubeSat also contained a water delivery system for the plants. The ECS plays a vital role in maintaining optimal conditions for plants throughout the launch process. Its frame is built using A1-6061 T6, ensuring NASA material properties regarding launch are maintained, but also providing lightweight, soft metal for ease of assembly. The ECS components include the Grove SCD30, which monitors CO₂ levels, temperature, humidity, and a BarO2 for temperature and pressure backup data. To protect against extreme cold temperatures (~-65°C) experienced during the High-Altitude Balloon mission and while in orbit, the ECS is equipped with a multi-layer insulation (MLI), an industry-standard thermal control method. Concurrently, the 5V heating elements, coupled with the heat sinks on the GrowLED PCB, ensured a

consistent temperature of 25°C during the day and 20°C at night; creating a stable environment for the plants. The GrowLED PCB also supplied a diverse color spectrum for optimal plant growth. Other components of the ECS include RaspberryPi camera boards, Pocket Geiger radiation detector, ExHale CO₂ bag, two plant pillows, and a WetLink penetrator. Overall, the ECS components, including sensors, insulation, and heating elements, work together under the control of a custom-designed PCB motherboard to safeguard plant health during launch and the mission. The ECS/CubeSat is shown in Figure 2.



2. High-altitude balloon

A high-altitude balloon was purchased from Kaymont Consolidated. Before the launch, helium was pumped into the balloon to achieve an expected ascent rate of $5 \text{ m/s} \pm 2 \text{ m/s}$ to an altitude of $30 \text{ km} \pm 3 \text{ km}$, maintain altitude for 60-90 minutes, and descend at a rate of $3 \text{ m/s} \pm 2 \text{ m/s}$.

3. *Plants as payload for high-altitude balloon*

Three types of plants were used in this experiment: (1) alfalfa (*Medicago sativa*), (2) wild-type tobacco (*Nicotiana tabacum*), and (3) genetically modified tobacco named DsupR that expresses a protein called Damage Suppressor Protein (Dsup) from the tardigrade *Ramazzotius varieornatus* (Kirke et al., 2020). The Dsup gene produces a DNA-associating protein which lowers the rate of DNA breaking due to radiation, stabilizing the DNA. The transgenic plant allows for greater examination of possible cellular or genomic damages occurred during the up and down flight, exposed to high levels of radiation. In theory, the DsupR seedling should be more resistant to radiation exposure and other stresses than the wild-type plants.

The seeds were planted in the round cheese-cloth pats (also called pillows) (4-cm diameter and 3.5-cm height) filled with potting mix soil (Sta-Green) and grown in the growth room at 25°C under 8 hours of darkness and 16 hours of cool white light of $\sim 200 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$. The day before launch, two pats with a mixture of two-week-old alfalfa and tobacco seedlings were placed in the growth chamber in the CubeSat. The day after the CubeSat landed and returned to the lab, the plants were removed from the soil pats, transplanted in 6-foot soil pots and grown in the growth room to maturity.

RESULTS

1. *Launch of a high-altitude balloon*

On March 28, 2023, the high-altitude balloon was launched from Duette, Florida, carrying a 6-unit CubeSat with ECS where growing alfalfa and tobacco plants were securely placed, traveling to an altitude of 30,000 meters and 125 kilometers from west to east, and safely landed in South Florida (Figure 3). The entire flight lasted approximately 27 minutes.

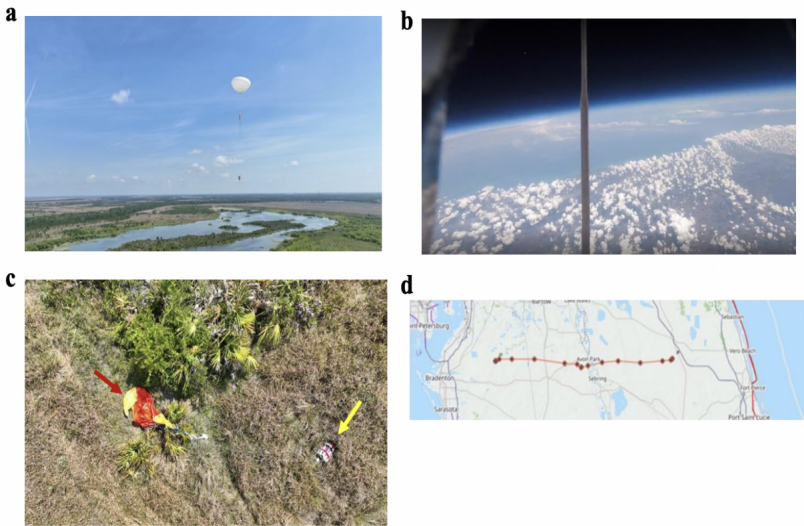


Figure 3. A short journey to “space” by the plants living in CubeSat.

- a.** The balloon carrying the CubeSat in ascendance.
- b.** The moments before the balloon ruptured at 30, 000 meters.
- c.** The CubeSat payload (yellow arrow) and parachute (red arrow) post-landing.
- d.** HAB launch trajectory

2. Plant growth-related environmental conditions during the balloon flight

During the balloon flight, atmospheric pressure outside the CubeSat dropped as the balloon ascended, to -15 Hpa at the altitude of 30,000 meters (green line in Figure 4a). After the balloon ruptured and the CubeSat descended, the outside atmospheric pressure rapidly rose (grey line in Figure 4a). In contrast, the pressure inside the ECS remained steady at around 1,000 Hpa (red line in Figure 4a), which is close to the pressure at the sea level on the ground, suggesting the ECS maintained a suitable condition of pressure for plant growth.

Likewise, as shown in Figure 4b, the outside temperature decreased as the altitude increased, dropping to as low as -55°C at an altitude of 30,000 meters, which would instantly kill plants. Yet, inside the ECS maintained a plant-friendly temperature of 22 to 26°C throughout the flight (Figure 4b).

Plants need a constant supply of carbon dioxide (CO_2) to survive (Wheeler et al., 2001). Inside the ECS, CO_2 was released from two CO_2 -emitting bags (ExHale CO_2) installed on the walls of the ECS, so that the chamber maintained a CO_2 level of around 780 ppm (Figure 4c) to support plant growth.

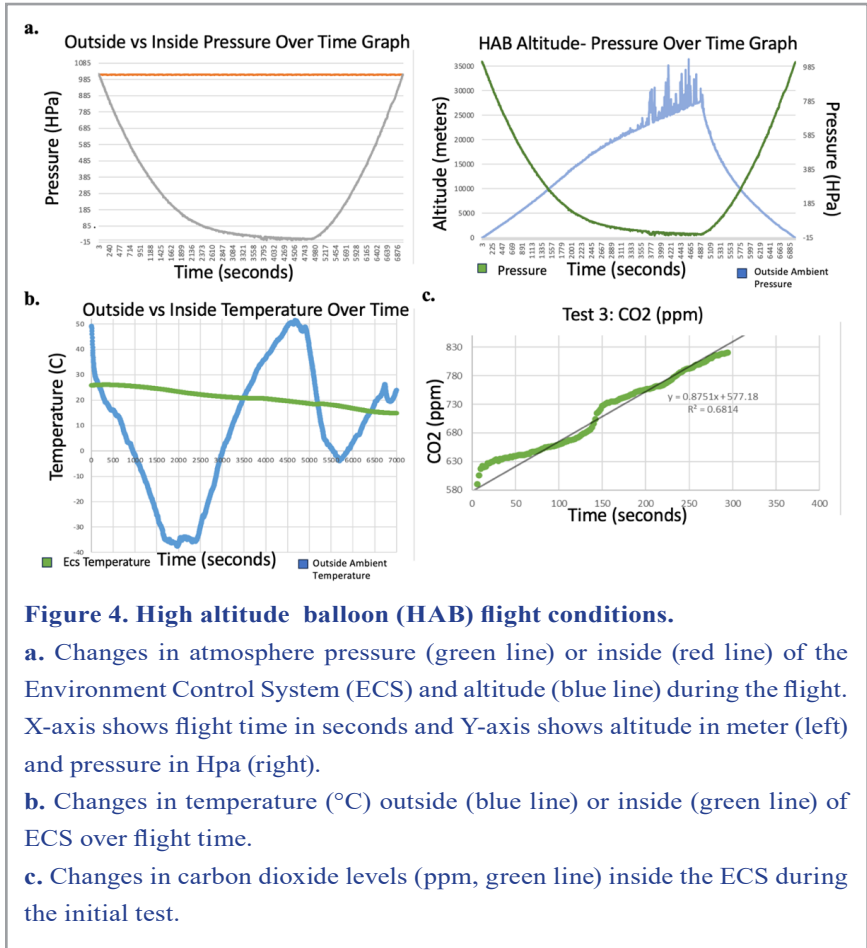


Figure 4. High altitude balloon (HAB) flight conditions.

a. Changes in atmosphere pressure (green line) or inside (red line) of the Environment Control System (ECS) and altitude (blue line) during the flight. X-axis shows flight time in seconds and Y-axis shows altitude in meter (left) and pressure in Hpa (right).

b. Changes in temperature (°C) outside (blue line) or inside (green line) of ECS over flight time.

c. Changes in carbon dioxide levels (ppm, green line) inside the ECS during the initial test.

The plants experienced a total ionizing dose of 0.243 $\mu\text{Sieverts}$. The CubeSat was exposed to an average of 0.6 $\mu\text{Sieverts}/\text{hour}$ for 27 minutes, which was an equivalent value of 12.7 $\mu\text{Sieverts}/\text{day}$. This radiation level is approximately 2.7 times higher than the average daily dose humans are exposed to on Earth.

The gravity at the altitude of 30,000 meters is about 99.1% of that at sea level. Therefore, gravity changes during the balloon flight should have negligible effect on plant growth.

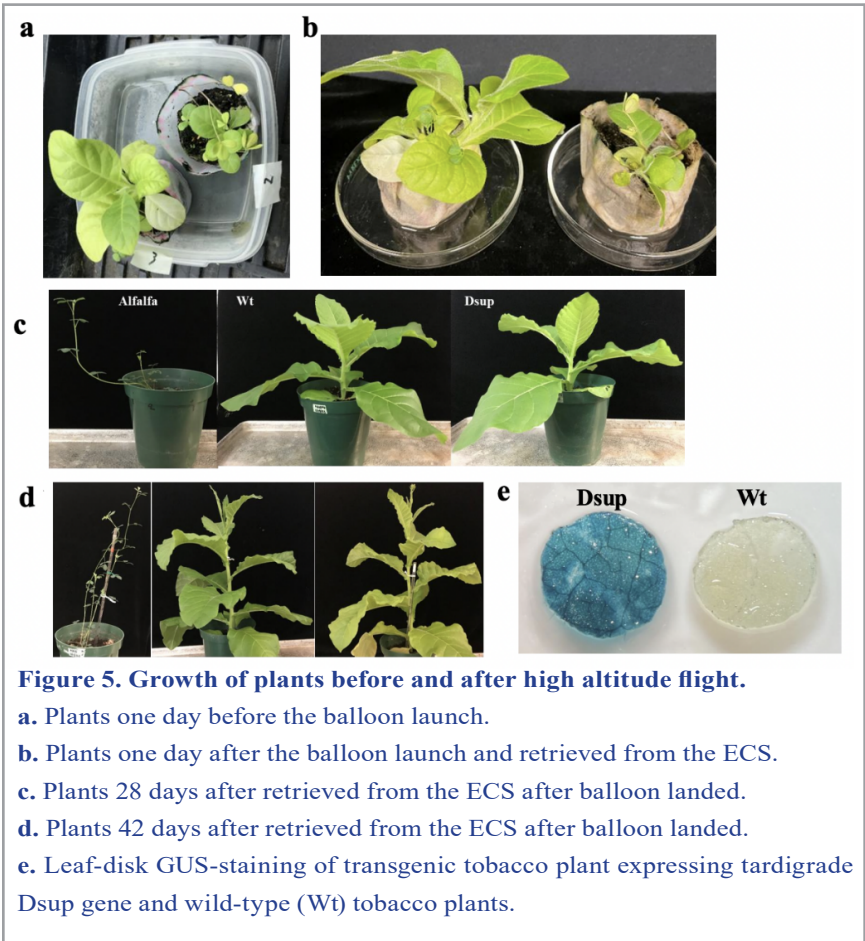
3. *Plant growth after the flight*

After traveling to ~30,000 meters above sea level, the CubeSat landed by a parachute and was transported to the FAU Boca campus. The plants were retrieved from the ECS (Figure 5a and 5b), immediately transported to garden soil-filled pots, and grown in the growth chamber. Those plants grew normally, and no obvious phenotypic abnormality was observed as compared to non-flown plants. Figure 5c and Figure 5d show, respectively, the representative plants of alfalfa and tobacco 28 days and 42 days after the CubeSat landed. To verify the genotypes of the tobacco plants, leaf disks from these plants were stained for beta-glucuronidase activity (GUS staining) (Bottino, 2020). Upon completion of the GUS staining test, plants with a positive result displayed a blue color whereas negative results remained green. The DsupR plants that possess the GUS gene showed blue color, whereas the wild type plants showed green (Figure 5e).

DISCUSSION

This research demonstrates the successful design of the Environmental Control System (ECS) and CubeSat, to withstand launch and return via a high-altitude balloon. The automated sensors integrated within the system have efficiently recorded and saved data on pressure, temperature, and carbon dioxide. More importantly, our research has demonstrated that plants can remain alive inside the ECS even when conditions outside become rapidly harsh and non-survivable for plants. The plants landed with no obvious damage and grew normally in the lab to maturity. We have not yet attempted analysis of any possible genetic mutations for these plants.

For future investigations, several considerations should be noted. Due to short flight time, we were not able to collect sufficient data of radiation levels at high altitudes, which is important to analyze cellular and genomic changes in plants' response to radiation. A longer stay at the high altitude, e.g. hours instead of minutes, would provide more informative data. Additionally, the high-altitude balloon did not reach the



necessary altitude to experience zero gravity, preventing analysis of its effects on plants. The preliminary test served to assess the feasibility of the system. For future missions, we plan to extend the duration of the plants at higher altitudes as the ECS has displayed successful results in those conditions thus far. Our long-term target is to launch this CubeSat into low-Earth orbit for extended and realistic testing. This research holds significant promise in furthering advancements in development of crops for future space expeditions, and eventual establishment of low-cost greenhouses for plant growth in Lunar and Martian soil (Wheeler et al., 2001). The findings reported here would aid in future development of innovative technologies supporting human exploration and potential habitation on other space bodies.

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LAS FUNCIONES DE LA IRONÍA EN “LA CAMISA DE MARGARITA” DE RICARDO PALMA

**Amparo Godoy Pastore,
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RESUMEN

En este ensayo se propone un análisis de la ironía como herramienta de crítica social en “La camisa de Margarita” de Ricardo Palma utilizando como marco teórico la obra “Las complejas funciones de la ironía” (1992) de Linda Hutcheon. La autora identifica y clasifica las funciones de la ironía en una escala de “carga afectiva”, que oscila desde la ironía decorativa y humorística hasta la elitista, desafiante o crítica. En este artículo se señalan las formas irónicas de Hutcheon presentes en “La camisa de Margarita”, incluyendo la “enfática”, “subversiva” y “autoprotectora”. Luego, se las analiza como factor clave en la denuncia palmista de las instituciones sociopolíticas y religiosas que concentraban el poder en la sociedad peruana del siglo XIX. Se concluye, por tanto, que el uso de la ironía en el cuento construye una crítica social y defiende la ideología liberal de Palma eficazmente.

INTRODUCCIÓN

El escritor peruano Ricardo Palma (1833-1919) incursionó tanto en el ámbito político como en el literario a lo largo de su vida. Fiel a su ideología liberal, participó en el intento golpista de 1860 liderado por José Gálvez, y secundó la sublevación de Manuel Ignacio de Vivanco contra el gobierno de Ramón Castilla. Su obra literaria, a pesar de ser muy vasta y diversa, se caracteriza por la fusión de su preocupación nacionalista y su amor por la historia y las costumbres peruanas. De esta combinación surge la «tradición», un subgénero narrativo de tipo histórico-ficticio, en el cual el marco costumbrista y el tono humorístico dan pie al comentario social. Núñez, autor de “El impacto de Ricardo Palma en América Latina” (1999), escribió lo siguiente sobre los elementos fundamentales que definen la “tradición” de Palma:

La "tradición", tal como la definió el propio Ricardo Palma, es una mezcla de historia y ficción. Pero habrá que agregar que, aparte de esos elementos fundamentales, por añadidura se encuentran en ella

otros ingredientes: los giros de lenguaje local o antañón, la copla popular, los decires y refranes del pueblo, el cuadro costumbrista, los vocablos de significación especial, el efecto escénico (de procedencia dramática) (párr.6).

“La camisa de Margarita” fue publicado originalmente en Barcelona por Montaner y Simón como parte del tercer tomo de *Tradiciones peruanas* (1893), un conjunto de relatos histórico-ficticios breves, que indagan en diferentes costumbres y etapas de la Historia de Perú. En el relato costumbrista, el autor peruano exhibe la crítica humorística de las instituciones políticas y religiosas del país en el siglo XIX, propia de la tradición palmista. A través del discurso irónico, Palma construye su crítica social, política y eclesiástica, incluyendo temáticas como la disparidad socioeconómica, el clasismo, las élites del poder y la familia como institución social.

Linda Hutcheon, autora de “Las complejas funciones de la ironía” (1992), entiende la ironía como una relación dinámica, un proceso comunicativo mucho más complejo que un simple juego de significaciones (p. 220). Utilizando como marco teórico las funciones de lo irónico descritas por Hutcheon, particularmente la “enfática”, la “subversiva” y la “autoprotectora,” propongo un análisis de las diferentes formas en las que se manifiestan la ironía y la sátira en el cuento de Palma. Asimismo, examinaré el papel fundamental de lo irónico como mecanismo de crítica de las instituciones sociales y religiosas que dominaban la sociedad peruana del siglo XIX, y como defensa de la ideología liberal del autor peruano.

LA COMPLEJIDAD DE LA IRONÍA SEGÚN HUTCHEON (1992)

Hutcheon refuta la simple definición tradicional de la ironía como una oposición semántica en la que el significado irónico se sustituye por otro literal (p. 219). La autora propone una definición más amplia y dinámica de la ironía como una relación dinámica en la cual lo dicho y lo no dicho compiten en un espacio con cierta “carga afectiva” y un “margen crítico” (p. 220). Además, según Hutcheon, la ironía tiene múltiples “motivaciones operativas inferidas” o funciones, que no necesariamente dependen de la intención del ironista. La autora identifica diferentes tipos de ironía y los clasifica en una escala según su carga afectiva, que oscila desde la ironía decorativa y humorística hasta la elitista, desafiante o crítica (pp. 220-221).

En la escala de Hutcheon, la función más básica de la ironía es la de “énfasis”, que permite mayor precisión en la comunicación, aunque también puede resultar meramente decorativa, o incluso un obstáculo en la transmisión del mensaje (p. 222). Es posible también interpretar la ironía como una “complejidad” típica del arte, una forma de ambigüedad controlada; la contracara de esta función es, por supuesto, que se presta para malentendidos (p. 222). Subiendo en la escala de margen crítico, la ironía tiene una función “lúdica” que, dependiendo del lector, será admitida con humor o como una trivialización del arte, superficial y vacía (p. 222). La autora describe luego su uso como “mecanismo de distanciamiento”, a menudo asociado negativamente con la indiferencia, pero que es también un signo de una nueva perspectiva y del rechazo de la tiranía que significa el juicio explícito en un momento en que dichos juicios podrían no ser apropiados o deseables (p. 223).

Un uso muy criticado de la ironía según Hutcheon es el “evasivo”, asociado con la hipocresía, el engaño y la duplicidad, pero la autora explica que esto se deriva de una visión general de la ironía como la actitud de quien, frente a la elección entre dos cosas mutuamente excluyentes, elige ambas, lo que equivale a decir que no elige ninguna (p. 224). A esto añade que el doble significado actúa para contrarrestar la tendencia a asumir una posición dogmática de la “Verdad” a través del reconocimiento de la provisionalidad (pp. 224-225). Otra función muy debatida es la ironía “autocrítica”. Por un lado, es una forma de señalar el propio auto posicionamiento (como marginal o marginado) o el rechazo de asumir la superioridad, pero también es una forma de jactancia indirecta, de modo que el autodesprecio y la autoprotección son las dos caras de la misma moneda (p. 225). De hecho, la función “autoprotectora” puede aislar tanto como resguarda, camuflando personalidades perturbadas que dependen de ella para preservar la cordura (p. 225).

Por otro lado, la ironía “defensiva” se justifica como medio de comunicación cauteloso, mientras que la ironía “subversiva” se utiliza como herramienta pasivo-agresiva de represión política (p. 226). El carácter “transideológico” de la ironía la hace maleable a una gran variedad de contextos políticos y, cuando se la emplea en la sátira, sirve como herramienta correctiva (p. 226). Inevitablemente, será interpretada por algunos como “ofensiva” o un ataque a la autoridad, pero en su mejor versión actúa como contra discurso para cuestionar patrones de

pensamiento y de expresión (p. 228). A pesar de su potencial de exclusión, en ocasiones sirve para unir a las personas, creando un ámbito colaborador y comunitario (p. 230). Por último, la función “liminal” de la ironía consiste en crear configuraciones novedosas de ideas y significados, proporcionando un espacio paradójico para el pensamiento creativo (p. 231).

Más allá de estas categorías, Hutcheon afirma que la ironía es una herramienta poderosa para la crítica y el análisis social y político, en tanto que permite a los hablantes y escritores expresar ideas complejas y controvertidas de manera indirecta y sutil. Reconoce, a su vez, que puede ser un arma de doble filo por su propensión a la ambigüedad y lo sencillo que es malinterpretarla.

La intención de Hutcheon al analizar las funciones de la ironía es entender cómo “diferentes actitudes generan diferentes razones para interpretar la ironía o usarla”, ya que “la falta de distinción entre estas diferentes funciones es una de las causas de confusión y desacuerdo sobre la pertinencia y el valor del tropo” (p. 220). Si bien la autora no tiene en cuenta la obra de Palma en su análisis, la complejidad de la ironía en “La camisa de Margarita” y su papel en la crítica social se prestan a ser entendidas de mejor manera al aplicar la clasificación de Hutcheon.

LA IRONÍA COMO HERRAMIENTA LITERARIA DE CRÍTICA SOCIAL EN “LA CAMISA DE MARGARITA”

A lo largo de su vida, el activismo político de Palma lo llevó al exilio en varias ocasiones. A partir de 1872, el escritor peruano fue dejando la política activa para dedicarse más exclusivamente a la literatura. En este contexto, Palma utiliza la ironía en sus obras como lo que Hutcheon (1992) declaró un “rechazo de la tiranía de los juicios explícitos en un momento en que tales juicios no eran permisibles” (p. 223). Esto le permitió mantenerse involucrado en la vida política y expresar su ideología liberal sin la amenaza inminente del exilio, la censura o la persecución. Pérez Garay (2010) escribe en su tesis “Liberalismo criollo: Ricardo Palma, ideología y política: 1848-1919” que “las Tradiciones Peruanas de Ricardo Palma expresan y reflejan en sus numerosos relatos satírico-humorísticos de historia y ficción, gran parte de las ideas liberales de su autor, así como también su intensa preocupación e interés por el mundo de la política” (p. 247).

En cierta forma, la ironía es una característica intrínseca de la complejidad literaria que desafía las expectativas del lector y permite múltiples significados. “La camisa de Margarita” no es una excepción. En su nivel más superficial, la historia del escritor peruano sobre la odisea que atraviesan la joven limeña, Margarita, y el español, Alcázar, para poder casarse pese a la oposición de don Raimundo y don Honorato, busca explicar el origen del dicho peruano “más caro que la camisa de Margarita Pareja”. A pesar de la aparente simplicidad narrativa del cuento, éste tiene un intrincado plano secundario de significados irónicos que se expresan a través de sus personajes y que le permiten a Palma construir una crítica social discreta y humorística.

La ironía se presenta desde el comienzo del cuento con la descripción de la protagonista. El narrador afirma que "la muchacha era una de esas limeñitas que, por su belleza, cautivan al mismo diablo y lo hacen persignarse y tirar piedras" (Palma, 2000, p. 106). En primer lugar, esta cita es una alusión directa a la Biblia, el catolicismo y la fe cristiana. El acto de persignarse consiste en reivindicar la fe propia y “tirar piedras” es una referencia al pasaje bíblico “[e]l que de vosotros esté sin pecado sea el primero en arrojar la piedra contra ella” (*Reina-Valera Revisada* 1960, 1960, Juan 8:7). El hecho de que la belleza de Margarita pueda convertir al diablo en un católico o santo, es una obvia exageración. La ironía aquí radica no solo en la pomposa belleza de Margarita y la contradicción que resulta de la imagen de un diablo piadoso, sino también en la subversión de la concepción tradicional de la belleza y sensualidad femenina como algo que incita al pecado. Aquí, en cambio, la belleza tradicional de Margarita santifica. Siguiendo la clasificación de Hutcheon, en este caso particular se observa un tipo de ironía “subversiva” que, utilizada en la sátira, sirve como herramienta correctiva. Este método es efectivo en tanto Palma define a Margarita como un estereotípico personaje femenino, cuya única característica es su belleza sin fin y su única aspiración, conseguir un pretendiente. Por consiguiente, cada vez que el comportamiento de Margarita se desvía de la tradición que la rige, surge el elemento irónico.

Los enamorados, Alcázar y Margarita, se conocieron en la procesión de Santa Rosa de Lima, una de las más grandes de Latinoamérica, en la que se celebra a Isabel Flores de Olivar. Santa Rosa, al igual que Margarita, poseía una belleza admirable que atraía las miradas de los hombres. La renombrada patrona del Perú realizó votos de castidad y

dedicó su vida a Dios, los débiles, y los desprotegidos. Margarita, lejos de meterse a monja voluntariamente, lo utiliza como amenaza para que su padre le permita casarse con Alcázar: “Margarita perdía colores y carnes, se desmejoraba a vista de ojos, hablaba de meterse monja y no hacía nada en concierto” (Palma, 2000, p. 107). Es aún más cómica la alusión a Santa Rosa, que dedicó su vida a los más desfavorecidos (particularmente los negros e indios), en comparación con Margarita, cuya fortuna y belleza favorecerían al menos desafortunado: un español de ascendencia aristócrata que emigró a Latinoamérica para heredar la fortuna de su familia. Sobre todo, la ironía tiene aquí una función “enfática” que marca la diferencia entre lo que la tradición pretende ser y lo que es realmente. Por un lado, la imagen impecable y celebrada de la monja Santa Rosa y de la institución religiosa que representa; por el otro, Margarita que utiliza la religión como herramienta coercitiva. Es así como Palma hunde “la pluma en el pasado para luego blandirla en alto y reírse de él” (Haya de la Torre, 1927, p. 118).

El único personaje femenino en la obra es Margarita y, a pesar de que la historia gira alrededor suyo y su camisa, tiene mínima participación, lo cual es indicativo del rol de la mujer peruana en el siglo XIX y de su ausencia en la toma de decisiones que la conciernen. En el cuento, “Margarita que se anticipaba a su siglo pues era nerviosa como una damisela de hoy, gimoteó, y se arrancó el pelo, y tuvo pataleta, y si no amenazó con envenenarse fue porque todavía no se habían inventado los fósforos” (Palma, 2000, p. 107). Para aquel entonces la mujer estaba limitada en su participación en la sociedad y en la toma de decisiones importantes. Aunque Margarita es el personaje central de la obra, su papel es mínimo en comparación con el de los personajes masculinos, reflejando la posición de la mujer peruana en el siglo XIX, que a menudo era marginada y vista como una figura secundaria en la vida pública y privada: “las mujeres estaban orientadas al esposo y a los hijos, debían cumplir a cabalidad su papel natural que era la maternidad y desplegarlo en el ámbito doméstico, mientras que para los hombres estaba reservado el espacio público, donde la política, la guerra y la economía eran sus ámbitos de acción por ser seres racionales” (Rosas Lauro, 2021, párr. 2). Es irónico que, a pesar de ser el eje central de la trama, sus acciones y decisiones son en su mayoría reacciones a las situaciones que se le presentan, y su papel se limita a ser víctima de las circunstancias y de las

acciones de los personajes masculinos. Su carácter, "nerviosa como una damisela de hoy", sugiere otra subversión irónica de la expectativa tradicional de la mujer como una persona obediente, callada y compuesta. A su vez, la frase "y si no amenazó con envenenarse fue porque todavía no se habían inventado los fósforos" es otro uso de la ambigüedad de la ironía "lúdica" para demostrar la escasez de opciones que tenía la mujer contemporánea para expresarse y tomar control de su vida. Las medidas dramáticas y ridículas que toma Margarita para conseguir lo que quiere son en sí una forma de rebelión, una idea escandalosa dada la tradición que los personajes palmistas representan.

Margarita no es el único personaje que, mediante la sátira, transmite el liberalismo del autor peruano. Todos sus personajes son "irreverentes ataques y críticas –en tono burlón– hacia personajes e instituciones del período colonial y el período republicano" (Pérez Garay, 2010, p. 247). Cada uno presenta alguna de las cualidades que Palma critica a estos establecimientos sociopolíticos y religiosos, como el orgullo, la codicia y la ignorancia. Particularmente, el autor denuncia los vicios de la aristocracia, cuyo monopolio de poder persistió incluso tras la Guerra de la Independencia de Perú en 1821. Según Gustavo Montoya (2002):

[L]a proclamación de la independencia el 28 de julio de 1821, no fue ni más ni menos una formalidad, un pacto de clase, una transacción temporal entre realistas, ejército libertador y la clase dirigente peruana, ésta demostrado por la continuidad del ordenamiento social, y la mínima alteración del orden político (párr. 12).

Para mantener su unidad social, este grupo utilizó varias estrategias. En primer lugar, mantuvieron vínculos familiares a través de matrimonios entre personas de la misma clase social para preservar su estatus. Este es el conflicto central del cuento: don Raimundo no quiere que su hija Margarita se case con Luis porque pondría en peligro su estatus social, o como cuenta el narrador, "don Raimundo no quería ser suegro de un pobretón", sino que quería para su hija—y su orgullo—"un hombre que varee la plata" (Palma, 2000, p. 107). En segundo lugar, estos grupos adinerados se enfocaron en la protección de la familia, considerada como una institución vital que debía mantenerse en armonía constante. Hay varios ejemplos en el relato. Uno de ellos es la reacción de don Honorato ante el rechazo de su sobrino: "—¡Cómo se entiende! ¡Desairar a mi

sobrino! Muchos se darían con un canto en el pecho por emparentar con el muchacho, que no le hay más gallardo en todo Lima” (Palma, 2000, p. 107).

El propósito de la sátira en la obra de Palma es socavar desde adentro, subvertir políticas e ideologías, pero tras el escudo del humor y el pretexto de la tradición. En la cita, “Como los amantes olvidan que existe la aritmética, creyó don Luis que para el logro de sus amores no sería obstáculo su presente pobreza”, Palma nos presenta la estereotípica pareja de enamorados cuya pasión paraliza todo tipo de juicio racional (Palma, 2000, p. 106). Los principales interesados en consagrar las nupcias no tienen autoridad alguna en la decisión; no son más que peones, activos financieros de transacción para don Raimundo Pareja y don Honorato. Ahora bien, para que de esta “burla” satírica surja lo que Hutcheon llama la función lúdica de la ironía, es importante que exista cierta complicidad por parte del lector, que éste se confabule con el autor en su postura crítica. La ingenuidad de don Luis, el ataque nervioso de Margarita y la ridícula disputa entre don Raimundo y don Honorato, ambos insultados por la sugerencia de una reducción en su fortuna, son todas cualidades y reacciones hiperbólicas que enfatizan lo absurdo de los insignificantes conflictos de las familias pudientes.

El humor de la obra solo se manifiesta si el lector reconoce este absurdo irónico. Aquellos que lo identifican, son incluidos dentro de un pacto colectivo o “red colusoria” que opera en contra de los excluidos (Goffman, 1986). Don Luis Alcázar, por ejemplo, es un joven arrogante que ha venido a Lima desde Madrid y que vive “tan pelado como una rata y pasando la pena negra” (Palma, 2000, p. 106). El mayor deseo de Alcázar es casarse con su enamorada Margarita, quién le flechó el corazón instantáneamente, pero don Raimundo, no soporta la idea de ser suegro de un pobretón. Lo curioso es que a pesar de que todos los personajes del cuento tratan a Luis como si no tuviera un centavo, el joven español tiene asegurada la fortuna de su tío, un aragonés acaudalado. La ironía de que un joven de familia aristócrata, a quien le corresponde la enorme fortuna de su tío, sea tratado como pobre a lo largo del cuento denuncia dos cosas: el derecho autoproclamado del noble a recibir una enorme fortuna sin mover un dedo y las dimensiones del clasismo en Perú. En definitiva, si este tipo de discriminación social y adquisitiva está presente entre los grupos de la élite peruana, solo resta imaginar su efecto en los sectores más humildes.

Otro ejemplo es el de don Luis diciendo que “no es de cristianos que matemos a quien no tiene la culpa” cuando es bien sabido que la Iglesia ha cometido atrocidades y abusado su poder repetidamente a lo largo de la historia (Palma, 2000, p. 107). No es sorprendente que las “Tradiciones” de Palma hayan sido el horror del clero católico (Pérez Garay, 2010). Evidentemente, la ironía solo le resulta cómica a quien no sea partícipe del sistema que se denuncia o a quien sea consciente de sus hipocresías. Aun así, la obra de Palma no es un ataque directo, sino más bien una modesta pero efectiva defensa de su ideología liberal. En ella, la ironía se despliega con el fin “autoprotector” que Hutcheon (1992) explica sirve para atenuar el efecto del orden o norma social (p. 225). Esta táctica fue extremadamente efectiva, al punto que “por una curiosa paradoja, Palma se vio rodeado, adulado y desvirtuado por una troupe de gente distinguida, intelectuales, católicos, niños bien y admiradores de apellidos sonoros” (Haya de la Torre, 1927, p. 118).

CONCLUSIÓN

Fiel a su perdurable interés en la tradición, Ricardo Palma desentierra con agudeza los vestigios de las costumbres peruanas en "La camisa de Margarita". El autor peruano hace de esta obra un instrumento para criticar la situación sociopolítica y religiosa de la época en que vivió, en un Perú en el que el poder estaba concentrado en manos de una oligarquía que hizo lo posible por mantener este dominio tras la independencia del país.

Palma esculpe personajes que personifican las fallas y vicios de la aristocracia de su tiempo. Con un tono irónico burlón, los desafía, los pone de cabeza y los obliga a mirarse en el espejo de la burla. En este duelo literario, la ironía es tanto su espada como su escudo, permitiéndole expresar sus ideas liberales y continuar participando en el escenario político de modo más pasivo y defensivo. La función “enfática” de la ironía lo dota de precisión y contundencia, mientras que la “subversiva” y la “autoprotectora” le ofrecen un velo de seguridad para expresar sus convicciones sin incurrir en la persecución directa de las élites. “La camisa de Margarita” se erige, por lo tanto, como un testimonio inquebrantable de la complejidad y la relación dinámica que subyacen en la ironía según la definición de Linda Hutcheon.

El resultado es un relato desternillante de la sociedad peruana del siglo XIX, donde los poderosos son despojados de su solemnidad y presentados como caricatos de la historia. La obra de Palma no solo es una celebración de la identidad peruana, sino también un grito de resistencia, un llamado a la crítica y una prueba viviente de que la literatura puede ser un instrumento de cambio, incluso cuando se presenta en el disfraz de la comedia. Su legado perdura como un recordatorio del importante rol de la sátira como fuerza poderosa para desafiar la norma establecida y subvertir estructuras de poder.

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THE AUTOMATIC GENERATION OF GAME ENVIRONMENTS FOR THE PURPOSE OF TRAINING ARTIFICIALLY INTELLIGENT AGENTS

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ABSTRACT

With the rise of self-driving cars and humanoid robots, it has become important to validate the performance of AI agents in simulated environments. In particular, simulated agents need diverse environments to evaluate their skills. This presents an opportunity to use automated methods to generate training data. The purpose of this study is to compare the effects of training AI agents on various mixtures of algorithmically-generated and AI-generated environments under various test conditions. Inside a simulated environment, AI agents were trained using reinforcement learning on different mixtures of artificially generated environments. The results show that the agent trained on a mixture of AI-generated and algorithmically-generated levels performed best, while the AI trained on purely AI generated levels performed worst. These findings show that using data from a mixture of artificial sources may improve the overall performance of trained AI agents when faced with limited data availability.

INTRODUCTION AND BACKGROUND

In recent years, machine learning-based Artificial Intelligence (AI) has exploded in popularity as a method of generating new creations and performing complex tasks with limited human involvement. For example, in language processing, OpenAI's ChatGPT can find errors in code and correct them, write essays, and explain concepts (OpenAI, 2022), and computers have been "taught" to play complex games at the level of world champions by exposing them to millions of actual games (Silver et al., 2016). The latter demonstrates that in order to perform complex tasks, AI platforms require extensive training, and such training often requires extremely large amounts of data or simulations of actual events. For example, versions of ChatGPT-3 were trained on over 300 billion tokens (with about 0.7 words per token) worth of data (Brown et al., 2020). The ChatGPT example highlights a major challenge with AI training—the size of the dataset required to teach an AI to perform these

complex tasks is restricted by data availability which can restrict the ability of the technology involved to perform the desired tasks (Brown et al., 2020). Video games, like language processing, have adopted the use of AI in many facets of gameplay and design, in order to create a more immersive and lifelike environment in-game. For example, AI is often used to control non-player characters to act as enemies for a more interactive experience (Skinner & Walmsley, 2019). Generative AI can also be used to create levels for tile or grid-based games such as *Super Mario Brothers* (Awiszus, Schubert, & Rosenhahn, 2020). However, as with ChatGPT, a large number of simulations are required for training which may restrict the use of the AI technology. This data often takes the form of simulation environments, like game levels, which may not be available or may be limited when developing the AI protocol, and can be difficult or expensive to produce. As such, it may not be practical for humans to create these datasets manually. An option would be to use algorithms for developing levels procedurally or using other AI to generate those environments in order to reduce the cost of AI training.

This work compares the effectiveness of an AI agent trained in environments created by other AI and AI agents trained in procedurally generated environments. More specifically, it tests whether there are performance differences between AI trained on AI generated data, AI trained on procedurally generated data, and AI trained on both procedurally-generated data and AI-generated data.

MATERIALS AND METHODS

In this study, we first generated environments, or levels, using procedural generation. Then, we trained a generative Long Short-Term Memory, or LSTM, neural network to create environmental levels based on those procedurally generated levels. Lastly, we trained reinforcement learning-based agents on various mixtures of the two types of generated data and compare the trained agent's proficiency in completing a task put before them.

Procedural Level Generation

As noted, to train an AI, one needs training data. This research uses two separate methods for creating the dataset: procedural generation and generative AI. The first AI that was trained was a LSTM neural network used to generate the game environments. To create the dataset

for this AI, the Unity Game Engine was used as it features a useful toolkit for machine learning agent training (Juliani et al., 2018). This toolkit has been used in the past to create intelligent agents that perform complex tasks, such as ones used to solve mazes (Hung, Truong, & Hung, 2022). Each level generated was composed of a square grid-based tilemap. In such a tilemap, each of the square grid spaces, called tiles, are set to hold a certain sprite, or image-based game asset. Together, these tiles form the level as a whole. The levels were based on tilemaps that were 70 tiles wide and 70 tiles tall, for a total of 4900 tiles per level. Each level started out with all tiles in the tilemap being set to hold empty space. The tilemaps were then populated with a four-step approach utilizing procedural generation, a form of content generation that uses algorithms to create useful data.

First, the general terrain was created using a smoothed random walk algorithm adapted from Ethan Bruins' work with Unity Technologies (Bruins & Technologies, 2018). In such an algorithm, starting at a randomly chosen height within chosen bounds, the height of the level is changed either up or down by one grid space vertically after a certain number of spaces are passed horizontally, with the exception of if the change in height would send the vertical height of the level outside the chosen bounds for level height. The minimum length was set at the same vertical height to be six spaces horizontally, and the bounds were set to not allow the algorithm to either raise the level beyond three-fourths of the level's total height or below one-third of the total height to prevent camera errors as the level was played. All tiles below or equal to the algorithm's chosen height at any horizontal position were set to land tiles, while those above this position contained empty space.

Next, the tiles at the surface level of the previously generated tilemap were changed to hold surface tiles, such as grass, and inclines were added at the positions in which the height of the level changed to make the level look more cohesive. After that, obstacles were added to the level to make it more difficult. A random value between zero and three, inclusive, was chosen to hold how many obstacles would be placed, and then valid positions among the flat sections of the level would be chosen to add these obstacles. These obstacles would be raised parts of the terrain for the player to jump over. Lastly, the level was completed by adding stretches of flat land on the left and right sides of the level, and

placing a flag that completed the level when touched by the player. Once these steps were followed, a level for the dataset was complete. An image of such a procedurally generated level is in Figure 1. The art for each tile was adapted from the “Platformer Art Deluxe” package by Kenney (Kenney, 2014). Gameplay starts on the flat part of the left side of the level, and the level is completed when the player reaches the flag at the right side of the level.

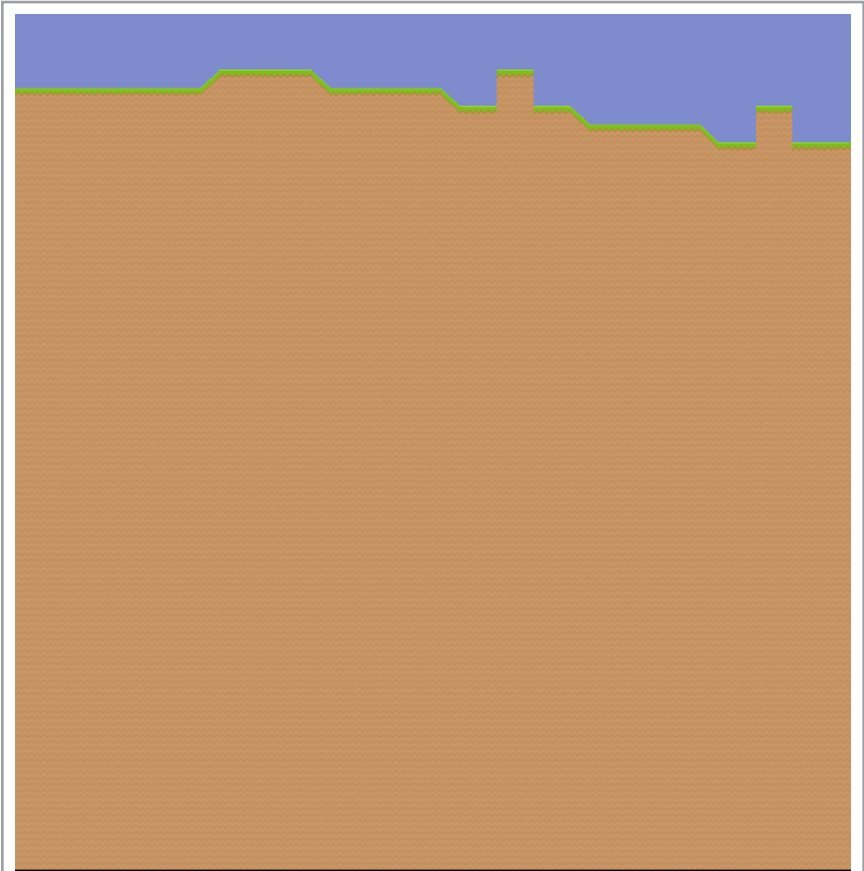


Figure 1. Procedurally generated level. This is an example of a procedurally generated level using a smoothed random walk algorithm with raised sections added

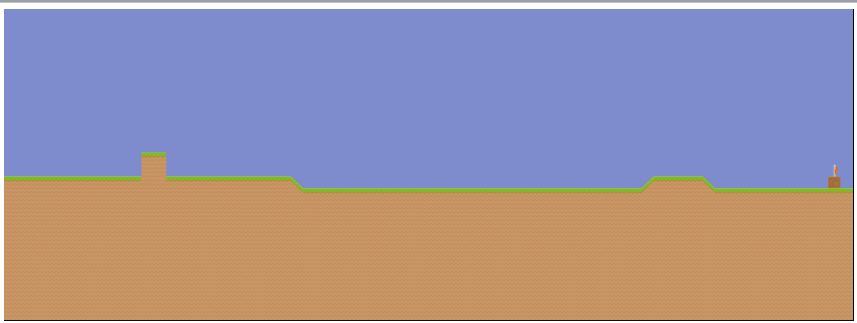


Figure 4. LSTM-generated level in Unity. This is a level from the Long-Short Term Memory Network in Unity.

AI Agent Training

The last step in this research work was the training and testing of AI Agents on these levels. According to Bansall (2023), an AI agent is a “...computer program or system that is designed to perceive its environment, make decisions and take actions to achieve a specific goal or set of goals.” In the case of this project, the AI agents were the AI used to play the levels. To make these AI agents functional, they first had to be trained. To test the effectiveness of different methods of generating data, three separate AI agents were trained using Unity’s ML-Agents toolkit (Juliani et al., 2018). The first was trained only on procedurally generated levels. The second was trained only on LSTM- generated levels. The last was trained on a mix of both. Each AI was trained using the same parameters, including the same number of maximum training steps: 500,000. During training, the AI agents received an 86-pixel by 64-pixel camera feed as input. As output, during gameplay the agents would choose if they would jump, if they would move, and which direction they would move in if they chose to move. All three agents were trained with a reinforcement-learning technique, where rewards and punishments guided the behavior of agents who work to maximize their reward value. The agents were rewarded for heading towards the goal and reaching the goal and punished for moving away from the goal and taking up time. After training, each of the AI agents were tested on 100 different levels in three different categories: 100 levels made purely through procedural generation, 100 levels made purely through LSTM, and 100 levels from both. In the category where levels from both the LSTM and the procedural

generation script were used, the level was randomly selected to be from either the LSTM-generated levels or the procedurally-generated levels with equal probability. In all cases, the levels the AI agents trained on were not the same as the ones they were evaluated on, as all evaluation levels were newly generated. The time the agents took to complete a level in each test was recorded, and the performance in these tests was then compared.

RESULTS

The results show that the AI agents trained only on LSTM-generated levels performed materially worse in each of the three categories tested than the other AI agents. Not only was it slower, but it also had a much higher standard deviation of time taken to complete levels than all of the other agents tested across all three categories. The best-performing agent across all three categories was the one trained both on levels generated by the LSTM and levels generated by procedural generation, and it also had the lowest standard deviation of time taken to complete a level across all three categories. In all three categories, the second-best performing AI agent was the one trained only on procedurally generated levels, and this agent also had the second-lowest standard deviation of time taken to complete a level across all three categories. However, the difference between the first- and second-best agent was marginal compared to the difference between the second- and third-best. The results are summarized in Table 2 and Table 3. In summary, the best-performing agent was the one trained on both procedurally generated levels and LSTM-generated levels, while the second-best was the one trained on just procedurally generated levels, with the worst agent being the one trained on only LSTM-generated levels.

	Procedurally Generated Levels Completion Time (seconds)	LSTM-Generated Levels Completion Time (seconds)	Mixed Levels Completion Time (seconds)
LSTM AI	16.0	15.16	15.20
Procedural Generation AI	12.74	13.11	12.64
Mixed Dataset AI	12.67	12.80	12.48

Table 2. Average time per level in seconds.

	Procedurally Generated Levels Completion Time (seconds)	LSTM-Generated Levels Completion Time (seconds)	Mixed Levels Completion Time (seconds)
LSTM AI	4.19	3.58	3.58
Procedural Generation AI	1.27	1.43	1.11
Mixed Dataset AI	0.93	1.23	0.99

Table 3. Standard deviation in average time per level in seconds.

DISCUSSION

The results show that while training an AI agent only on AI-generated levels may reduce performance significantly, training an AI agent on a dataset of mixed AI-generated and procedurally generated levels may increase performance. The results are somewhat surprising, as one could reasonably expect that the agent trained on procedurally generated levels would perform best on procedurally-generated levels, and the one trained on AI-generated levels would perform best on AI-generated levels. The fact that the agent trained on the dataset including both AI-generated and procedurally generated levels performed the best in all categories was unexpected. Perhaps training on both procedurally generated and AI-generated data trained the agent in more diverse environments, which could lead to better model performance. Alternatively, perhaps the addition of the new data prevented the agent from overfitting, where an AI becomes overly-trained on the training data to the point of losing its ability to generalize to new situations. Future research may help to isolate the actual cause of this improvement and find the best mixture of data sources to maximize agent performance.

CONCLUSION

In this study, AI-generated data showed promise in improving the effectiveness of AI agents. While exclusive use of AI-generated data was not shown to be optimal, mixed use of AI-generated data and other methods of generating data was shown to improve the performance of the trained agent. This research indicates that it may well become commonplace in the future to train AI agents in such mixed environments. Future research may indicate the precise reasons why this improvement occurs with

mixed training data, combine additional data sources (including human-generated data), and find the optimal ratio of data from different sources to maximize agent performance. These findings may apply more broadly than just in game agent training, as “...it is immensely cheaper to develop and test AI in a created environment with thousands of instances, than to build robots and have them do thousands of tests,” (Skinner & Walmsley, 2019). For example, with self-driving cars, “...it is much easier to just train the AI through a driving computer game rather than risk damaging the hardware and injuring people,” (Skinner & Walmsley, 2019). Training the AI agent controlling self-driving cars may be done in AI-generated or procedurally generated environments, and thus these findings may apply. Similarly, for surgical robots, the agents controlling the robots may be trained in virtual environments to prevent the harming of patients during real application, and so these findings may apply once more (Bourdillon et al., 2022). As AI permeates human life, training AI agents will become an increasingly large issue. As such, understanding the consequences of training AI agents in AI-generated environments and procedurally generated environments will only increase in importance in the future.

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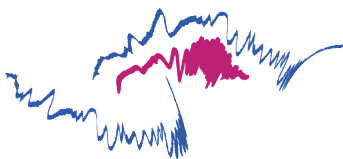
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AUTHOR BIOGRAPHIES



David Harbaugh & Marissa Shank

David Harbaugh is a recent graduate from the Wilkes Honors College, where he pursued his studies in Biological Chemistry. During his undergraduate journey, he had the privilege of working under the mentorship of esteemed professors, including Dr. Joseph Kissil, Dr. Catherine Trivigno, and Dr. Jordan Merritt. David's passion for scientific exploration and research, particularly in cancer therapy development, has shaped his academic pursuits. With a deep commitment to contributing to the field, he aspires to leverage his knowledge and skills to make a positive impact on cancer treatment and patient outcomes.

Marissa Shank is a graduate of Florida Atlantic University's Wilkes Honors College. She graduated with a Bachelor of Science in Biological and Physical Sciences. Marissa's interest in research was found under the guidance of Dr. Jordan Merritt through a Course-based Undergraduate Research Experience (CURE). Marissa has presented her CURE research at the 12th Annual Undergraduate Research Symposium and the 20th Annual Wilkes Honors College Symposium.

NOTE: David Harbaugh and Marissa Shank completed the research project and submitted it with equal contribution.



Alysa Suissa, Marcos Klingler, & Jake Pearman

Alysa Suissa was born and raised in Miami and currently is an undergraduate senior at FAU. She is pursuing a Bachelor's in Biological Sciences. She has plans of continuing to the Biomedical Master's Program at FAU. Alysa plans to pursue a career within the medical industry. She is doing research with Dr. Zhang focusing on genetics and plant biotechnology. Alysa's current research involves the insertion of a human gene into plants through chloroplast transformation.

Marcos Klingler was born and raised in South Florida, and recently graduated from FAU with a Bachelor's in Biological Sciences. Having a previous publication doing a metagenomic study, Marcos is pursuing a career in research focusing on genetic engineering and biotechnology. He has worked in Dr. Zhang's lab for almost two years at FAU.

Jake Pearman is a Florida Atlantic University alumnus who graduated in 2023 with his Bachelor's in Mechanical and Aerospace Engineering. During college, Jake founded and ran the Aerospace Experimental Association, where he organized FAU's first CubeSat and High Altitude Balloon missions. Furthermore, upon graduation, Jake commissioned from the University of Miami as a United States Air Force officer and is currently serving as a pilot. He is also an industry advisory board member for FAU COECS, helping to establish the aerospace program and center.



Amparo Godoy Pastore

Amparo Godoy Pastore is a fourth-year undergraduate student at Florida Atlantic University's H. L. Wilkes Honors College in Jupiter, Florida. Originally from Buenos Aires, Argentina, she moved to the United States to pursue a B. A. in Interdisciplinary Mathematical Science with a minor in Spanish Literature, combining her two passions. In her pursuit of knowledge, Amparo found herself captivated by the works of Latin American literary masters such as Ricardo Palma.

Isaac Dash

Isaac Dash is a Florida Atlantic University High School student working as an undergraduate researcher at the Machine Perception and Cognitive Robotics (MPCR) lab under the mentorship of Dr. William Hahn. Isaac has been interested in machine learning and artificial intelligence for several years and has been researching the topic as part of the MPCR lab. He presented related research, titled "Automatic Generation of Virtual Learning Environments using Generative Adversarial Neural Networks", as an oral presentation at the 12th Annual FAU Broward Student Research Symposium in 2022.

NOTES

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