“Mycotecture off Planet” Phase II Final Report

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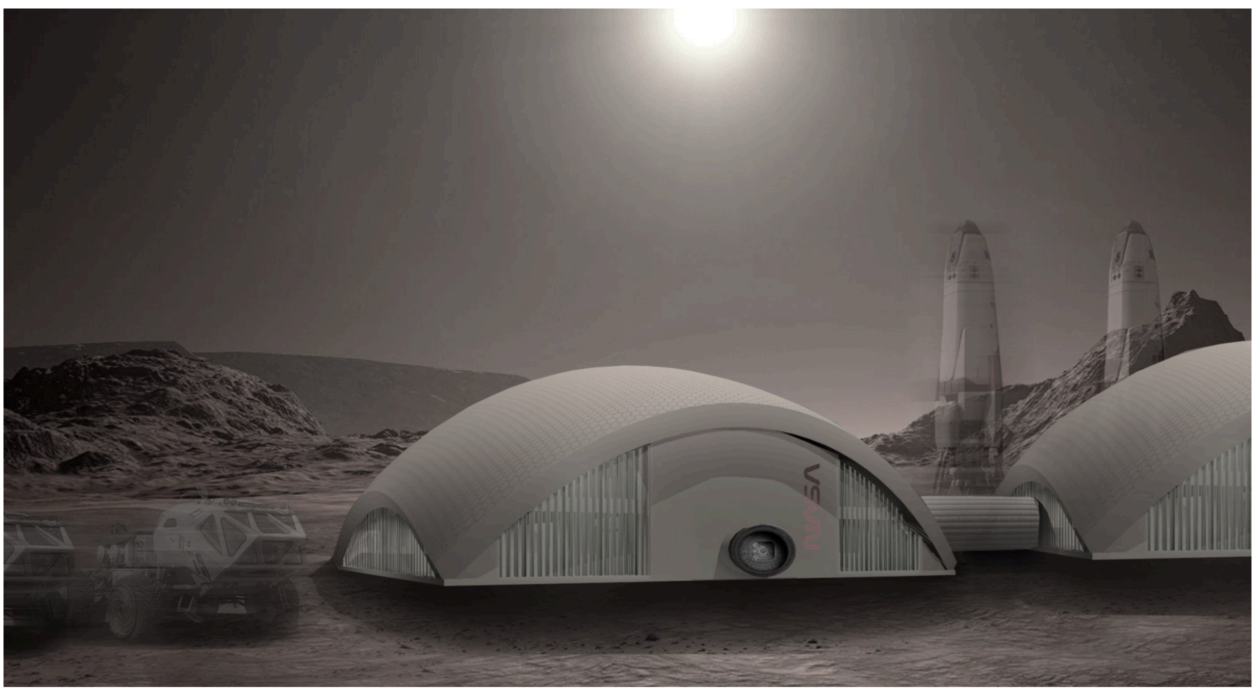

image courtesy of Co-I Chris Maurer, redhouse studio

(21-21NIACA2-P-0020) that was submitted to the 2021 NASA Innovative Advanced Concepts (NIAC) Phase II Solicitation, (80HQTR21NOA01-21NIAC_A2)

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2 Summary of key findings from Phase II

Assessed fungal/algal/bacterial mixtures. We tested several fungi, and none could survive temperature above 47 °C for 5 minutes, although our fungal reference materials are viable after 52 days of storage at -80 °C or slow drying at room temperature. Notably we did not use any cryoprotectants. We developed a reproducible method with increased throughput for producing mycelium materials from various fungi and substrates. The fungus we tested can grow in the presence of acetate and on food-grade powdered *Chlorella vulgaris* (green alga) as a carbon source. The green alga *Chalmydomonas reinhardtii* can grow in and within an agar hydrogel matrix. We produced bricks composed of a hydrogel matrix infused with living *Chalmydomonas reinhardti*i cells.

Regolith simulant composites for the in situ creation of structural mycelium-regolith components.. We demonstrated successful mycelium growth on inorganic substrate (silica-based sand) with minimum addition of organic nutrients (85/15 w/w%). We created a computational simulation tool that allows us to examine the autonomous assembly process under simulated extraterrestrial conditions, including atmospheric pressure and gravity. This tool facilitates the study of various component geometries, aiding in the selection of the most effective design. We demonstrated the bio-welding properties of these components, after being assembled, able to grow/fuse together, into cohesive habitat shell structure. We produced composite agar-based lunar regolith simulant hydrogel materials with and without fungi infused. Biowelding was tested for assembly of mycelium-regolith components.

Developed prototypes. We further developed designs that incorporate expandable bioreactor cells in the shells of inflatable structures. We prototyped several inflatable concepts in silicone scale models. We developed a melanin rich fungal / hydrogel combination in a working prototype that requires transit of less than 1% of the final mass (99% can be in situ derived mass). We prototyped a 4m x 4m scale model of inflatable architecture with Moonprint Solutions and successfully grew a mycelium dome on top.

Determination of the effect of lunar and martian simulation on materials. Using the planetary simulator at McMaster University, we showed that environmental testing of the biocomposites using the planetary simulator allows for assessment of material properties in lunar and martian conditions. We demonstrated that exposure to UV radiation leads to local damage of the hyphae. The combination of low temperature (-20 °C) and exposure to UV radiation appeared to have the most damage. We found that temperature cycling between -196 °C and room temperature resulted in mechanical damage, such as the twisting and breaking of hyphae. We showed that the efficacy of melanin-infused hydrogels as radiation protectors can be tested by measuring the change in absorbance spectra during the course of UV treatment in the planetary simulator.

Measure mechanical properties of prototypes before and after exposure to planetary simulator. By tuning different steps of production, we can change the mechanical properties of the mycelium biocomposites as they undergo compression.

1. Compacting mycelium biocomposite samples made from *Ganaderma lucidum* during the final step of production to make them denser, they can handle higher strengths when under a compressive load, even after being treated in cold temperatures (-196 ˚C and -20 ˚C) and with UV radiation.

2. A 30-day growth period has a higher ultimate compressive strength and Young's modulus (E) than a longer growth period (60 day and 120 day). While samples cut parallel to the direction of initial compaction had higher E-modulus, sample cut perpendicular had noticeably higher ultimate compressive strengths.
3. Mycelium biocomposites using synthetic martian regolith as a substrate, samples grown in low humidity had better mechanical properties, likely due to the high density of the regolith.
4. Adding fungi to lunar regolith simulant improved the mechanical properties of regolith undergoing compression. For example, E increased by ~41%.
5. While *Ganoderma lucidum* has a higher ultimate compressive strength, *Pleurotus ostreatus* has a higher E. Further testing must be done to see how environmental tests affect these species.

<u>Identification and analysis of potential enhancements to the mycelia</u>. The capabilities of the mycelia could be developed using bioengineering augmentation. In Phase II we focused on incorporating melanin-producing strains into the mycocomposite for radiation protection. *Ganaderma lucidum* and *Bacillus subtilis* were shown to grow in co-culture, leading the way for easily functionalizing the mycocomposite through engineering the B. subtilis.

<u>Detailed mycelium-based Moon habitat designs with tradeoffs and subsystem considerations.</u> Co-I Maurer designed a series of detailed architectural drawings using what he termed lunar optimized bioreactor enclosures. The LOBEs are designed to be made of custom drop-stitch materials that pairs the form developing capabilities of drop-stitch technology with the robustness of TPU coated Vectran® (liquid crystal polymer) material. Drop stitch materials are multi-layered air-tight weldable polymer coated fabrics that have "drop yarns'' or drop stitches fastened to the top and bottom. This allows the materials to maintain desired shapes when inflated that may be more orthogonal than standard inflated shapes This also creates a scenario that converts compressive forces into tensile forces by dispersing the forces weight action to internal pressure.

<u>Mission architecture: the Haldley Max 500 Day Design Reference Mission to the Apollo 15 Hadley-Apenine Region</u>. We utilized the 500-day Design Reference Mission Concept (DRM) to define the science objective and infrastructure requirements to support extended exploration missions to the Moon and Mars. We then identified the critical gaps that must be addressed during Phase III in infrastructure development for materials reducing upmass and further defining in situ construction inflatable enclosures, together to optimize the Moon-Mars feed-forward and scientific return. We conceptualized and drafted several archetypes per the Hadley Max 500 day mission. We presented these concepts to NASA Administrator Bill Nelson and RI Senator Jack Reed during their visit to our lab at Brown University. Working together with Apollo 15 Commander David R. Scott and numerous co-authors and students, we presented six contribution describing multiple aspects of the Hadley Max DRM at the 55th Lunar and Planetary Science Conference in Houston, and we are currently preparing a synthesis paper for submission to Astronautica Acta. Planned Phase III activities include more specific traverse designs linked to scientific goals and objectives for Hadley Max, improved definition of how these requirements map to human and robotic habitat designs and environments, mapping these concepts to the more stringent requirements of the South Circumpolar Artemis Zone, and exploring how lunar results and requirements will feed-forward to Mars.

Terrestrial applications. These included MYCOHAB, everyday aerogel products, BIOCYCLER, the CHILL, plates. We successfully completed the world's self-supporting mycelium composite structure in Namibia, patented the process for turning waste African bush into food and housing with MIT, and started a company called Mycohab that hopes to build millions more with this regenerative process. The company, process, and successful structure claims lineage to this NIAC research. Co-I Maurer founded a company called Biocycler that is developing a process of remediating and recycling organic construction and demolition (C&D) waste. part of our investigation into developing inflatable forms at various scales could lead to another terrestrial application. The Chill is a concept for temporary urban summer cooling stations that are highly insulative and 100% biodegradable. At the three Michelin star Azurmendi restaurant in Larrabetzu, Spain, a pioneer in sustainability, a novel approach was taken to harness the biotechnological capabilities of Aspergillus oryzae. A dining banquet was designed and tableware built by both Maurer and Massa, all made from the NIAC "Mycotecture off Planet" technology.

TRL level. During Phase 1 we raised the TRL to 2 by assessing the growth of three mycelial-producing strains on potential food substrates and analyzing their use off planet. We exited Phase II having completed TRL 3 for this complex, integrated system of inflatables, biological components, the need for them to survive, multiply and produce a material that has been tested. We designed prototypes and subsystems. We performed multiple proof-of-concepts analyzing mycomposite function with and without exposure to some relevant environments in a planetary simulator placing the technology in an appropriate context. In our Phase II report and publications we have documented analytical/experimental results on the fungal, fungal algal, fungal bacterial and inflatable components of the system validating prediction of key parameters

3 Introduction to the Phase II Report

A turtle carries its own habitat. While reliable, it costs energy in transporting mass. NASA makes the same trade-off when it transports habitats and other structures needed for humans and other applications on lunar and planetary surfaces “on the back” of its missions. During Phase 1, we identified a novel biology-based solution to the *in situ* production of usable components for space exploration: using fungal mycelial composites to grow structures off-planet, from habitats to furniture. In Phase 1, we delivered the biological strains needed to produce the mycelia appropriate to engineering uses, a fabrication process to form the mycelia into blocks and stools, an adhesive to join the blocks into components, and mechanical testing to characterize the strength of the parts. We developed architectural designs, and a mission architecture to implement the concept on Mars. The results have attracted wide attention from the press and the project, through the participation of the 2018 Stanford-Brown-RISD iGEM team, was nominated for best manufacturing project and best new composite part in the premier student synthetic biology competition.

Phase 2 continued to develop the Phase 1 concept, with our focus towards lunar habitats with a “feed forward to Mars” mindset. We continued to advance the technology by developing a novel prototype which uses the mycelia to fill a light-weight porous scaffold coated with nutrient hydrogel enclosed in plastic sheets ("bag") or a living cyanobacterial feedstock. Key technology parameters (choice of fungi, growth conditions and speed, pore size) were tested in the miniature prototypes. Selected prototype materials were tested for their mechanical properties, and then in a planetary simulator to assess resistance to specific challenging lunar and martian conditions and to gauge the degree to which developed capabilities can be delivered in those conditions. More complete structures, including infrastructure for a habitat like plumbing and air handling components, were conceptualized and work towards such designs was undertaken. A mission architecture that will be flexible, as is appropriate for the flexible nature of the components themselves, and the potential broad outlines of a systems engineering scheme for lunar implementation was created in response to those of our development goals that appear to be most viable. For Phase II we focused on a 500-day Hadley Max scenario. As we develop a pathway to implementation, key knowledge gaps in the technology, habitat design and mission architecture were identified in close step with developments in understanding human needs in such habitats and systems in the lunar context. This included an estimate of mass and other factors required for implementation as the design was refined from Phase 1. Finally, expanded our assessment of the use of this revolutionary fabrication and building approach for immediate terrestrial applications in stressed environments where rapid and low-cost applications to house people are in dire need; for example, in areas of dislocation due to war or environmental change, endemic poverty, and other adverse factors; and for furniture for earth and space.

We envision future enhancements to the mycelial structure; some will be explored now at no cost to the NIAC proposal. The capabilities of the mycelia will be developed using bioengineering augmentation; for example, production of useful polymers will be considered. We envision a future that includes the addition of cyanobacteria that can produce oxygen, bacteria that can provide sensing capabilities (e.g., sensing of environmentally relevant gasses like oxygen for crew health support), and decoration of the mycelia with proteins for assorted chemical transformation functions. Enhancements such as these can result in “living architecture" in the true sense of the word,because of sensitivity and near real-time adaptability to internal and external environmental changes.

What follows is the status of the deliverables from the Phase II proposal, publications, conference presentations, student involvement and public outreach derived from the project.

4 Deliverables

4.1 Prototypes

Prototypes were developed by the team in multiple facets. These prototypes include the development of architectural models both physical and digital, novel biomaterials, scaffold materials, nutrient hydrogels, integrated bioreactor development, as well as the integration of these parts including mass-balance calculations.

4.1.1 Architecture

The design concept of a deployable self-growing structure must take into consideration the viability of growing the constituent organisms at destination. Lunar and Martian environments are extreme compared to Earth. Life as we know it cannot live in the absence of liquid water, and thus it appears that there is no life on the Moon, and only the possibility in a few regions of Mars. The high levels of radiation on both bodies further diminish these possibilities. The solution is to use the example of the only living organisms to go to the Moon and return alive as our model. Twelve American men have walked on the Moon and when they did they were protected from the lunar environment by their A7L spacesuits designed and produced by ILC Dover. These suits and subsequent EVA mobility units have made space conditions bearable by creating an enclosure that mimics clement Earth environments in atmosphere, temperature, and pressure. The same requirements exist for our microorganisms. The architectural designs produced by this team link these enclosures that are analogous to biological cells with an analog circulatory system (Fig. 1). These cells, which we call Lunar Optimized Bio-reactor Enclosures (LOBEs), act like space suits for the production of biomaterials or what we also call "bioterials" (to distinguish from biomedical devices) within the inflated scaffolding. The LOBEs are linked together and are transported in folded inflatable structures to create performant living shells that replace the air and water in the cavities as they grow-in-place.

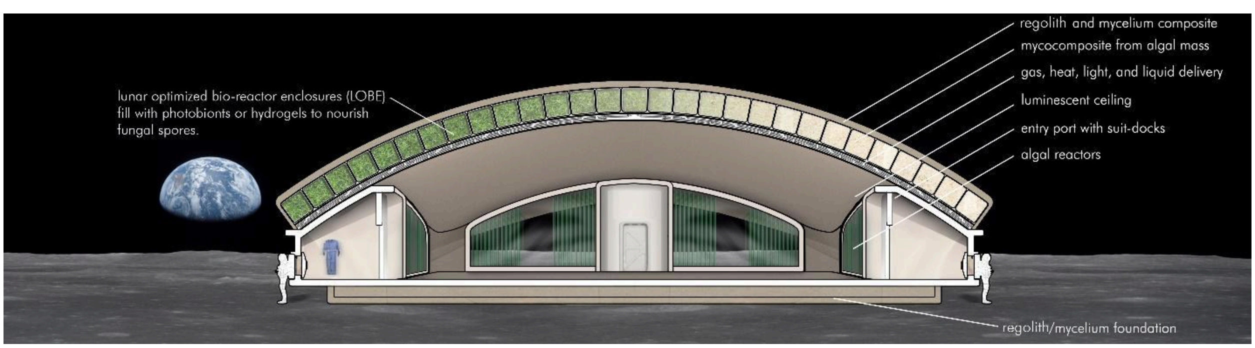

Figure 1. Section through prototypical habitat showing lunar optimized bioreactor enclosures (LOBEs) from left to right first growing photosynthetic organisms that are then converted into a composite mycomaterial by the growth of fungal mycelium.

4.1.2 Digital Prototypes

The architectural team created dozens of prototype designs utilizing the "mycotecture off planet" concept to align with the eight typologies of Co-I Head's Hadley Max 500-Day Design Reference Mission concept. More information on the 500 day mission can be found in section 4.5. The prototypes are as follows: 1. Landing pads, 2. Initial Base Structure, 3. Evolutionary Base Structure, 4. Remote Rover Garages, 5. Rover Round House, 6. Remote Science Bases, 7. Pony Express Stations, and 8. Robotic Rover Partnerships. Several are shown in Fig. 2.

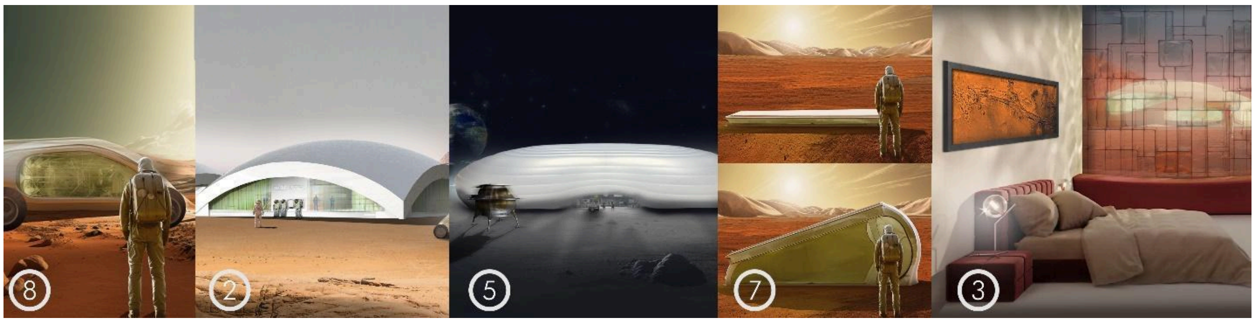

Figure 2. Digital models based on Head's Hadley Max 500-Day Hadley Max Design Reference Mission concept outlined in section 4.5. 8. Robotic Rover Partnerships; 2. Initial Base Structure; 5. Rover Round House; 7. Pony Express Stations; 3. Evolutionary Base Structure.

4.1.3 Physical Architectural Models

The team utilized parametric software to develop deployable concepts including the use of inflatable and elastic skins, folding models inspired by origami, and auxetic expansion based on metamaterial concepts. These concepts were converted into rapid prototypes using various materials including poured silicone, 3d printed polylactic acid, and fused inflatable fabrics by redhouse studios. These models can demonstrate the Grow in Place (GIP) concept that utilizes simple circulatory systems to nourish simulated LOBEs (SLOBEs)

Silicone models were made using 3d printed forms to cast silicone resin membrane (Fig. 3). The models utilize interior air chambers, form building air chambers, and SLOBE chambers. The interior chambers represent the living space of off-planet habitats. The form-building air chambers can define the shape of the structure irrespective of air pressures below and above. The calculus of form building air chambers becomes more complex with differential pressures off planet, but they serve here to help advance terrestrial applications of biotectural form-making. Computer modeling and vacuum testing including that of McMaster's planetary simulator could take this research further to help define form building air chambers within the lunar context of extreme pressure and temperature differentials, and subsequently Mars. These air cavities would ideally be filled with aerogels that would act as thermal insulators. We illustrate in section 4.4 that aerogels produced *in situ* can provide superior insulation and be produced with very little material transport, and resource use. SLOBE chambers demonstrate the tessellation options that work with simulated circulatory systems. These LOBEs simulated could be filled with hydrogel substrates for growing radiation attenuating mycelium species (melanin-rich fungi) that, as discussed in section 4.1.6, can convert ionizing radiation into benign forms of energy and may even serve as shields from particle radiation such as galactic cosmic radiation (GCR). These LOBEs serve as stand-ins for the two phase GIP system that utilize the growth of photosynthetic organisms to produce fungal substrates *in situ*. In most cases we developed the former model of filling SLOBEs with nutrient rich hydrogels that could be produced with 1%-4% materials transported from Earth and 96-99% *in situ* derived water. If materials transported from Earth were sterilized and transported properly, no further sterilization would be needed after mixing *in situ* but for this simulation the gels were autoclaved within the silicone molds. After the molds were cooled, liquid cultures of mycelium were added via sterile syringe. Various genera were tested including *Ganoderma, Aspergillus,* and *Cryptococcus* and effectively filled the SLOBES.

Figure 3: Silicon models, all less than 750 cm^2, the chambers of inflatables are connected by small holes punched in the silicone interior sidewalls and use bike tire nozzles or small silicone tube inlets that are inflatable by "ball-pump" with needles. They represent several of the Hadley Max DRM archetypes as well as some experimental forms to test new possibilities. Redhouse studios.

Larger scale inflatables were created to test the performance of larger slabs of mycelium composite materials (Fig. 4). Mycelium composite was grown in thirty-six 60 cm x 60 cm x 10 cm living mycelium composite panels and laid in a grid pattern on an inflatable dome fabricated by the team with Moonprint Solutions led by David Cadogan, who became a member of the team after the proposal was funded. The inflatable model utilized drop stitch side walls that maintained stiff arches at 34 kPa and a clear vinyl lenticular dome at 2 kPa that supported the weight of the mycelium composite (0.8 g/cm^3). The living mycelium panels were covered with a lightweight polymer sheet and HEPA filtered air was piped under the sheet to maintain fresh air exchange and combat moisture build-up on the polymer sheet. The living mycelium panels fused, or "bio-welded" together on the surface of the inflatable dome over a period of 4 weeks to form a monolithic dome. The drying process took much longer and after an additional six weeks, the underside of the dome read 30%+ on the hygrometer – a dry material in this environment would read less than 5%. This drying time could be expedited by including a heating membrane or gas exchange membrane under the mycelium material above the inflatable surface. While this is not an accurate representation of the current mycotecture off-planet proposal, the creation of monolithic structures using inflatables could be invaluable to off-planet concepts as well as terrestrial concepts; see section 4.7 for more information on terrestrial concepts, specifically the "Chill" concept that proposes super-insulated biodegradable cooling stations for urban centers suffering with seasonal excess heat.
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Figure 4. Photos of living prototype production. From left to right: "Alien" making mycopanels, inflated dome form before panels are placed, placing the living material on the dome, NIAC mid-review as the mycelium dome had freshly started growing, full mycelium dome weeks after mid-review.

The team developed a system for compacting the mycomaterial cells to increase the density, increase material strength, and to discharge water. The system works by including an inflatable baffle in with the lunar optimized bioreactor cell. The baffle as designed has the ability to compact the biocomposites within LOBEs at pressures of 100 kPa using an air-compressor. This could be further refined to achieve much higher pressures. Common methods of thermo-mechanical densification of wood use mechanical pressure at temperatures of 130 -190 °C to loosen the lignin bonds of ligno-cellulosic materials and align the resulting cellulosic particles to form strengths of 200 - 300 % the natural strength of seasoned wood.[1]

4.1.4 Furniture Models

In addition to habitats, our team proposes that mission furniture, fixtures, and equipment (FFE) can be created using the mycotectural and bioterial processes, which would save mass upmass costs on more than just building materials. We have developed several different methods and designs for furniture that can be grown at destination (Fig. 5). Exterior equipment could use LOBE technologies, but as most of the FFE are within the habitat, they do not need special environmental enclosures and can be grown within the conditioned structure in bioreactor enclosure cells (BECs). If the FFE are pre-wired to the structures, they can make use of the building's circulatory systems for nutrients, gas exchange, and water supply.

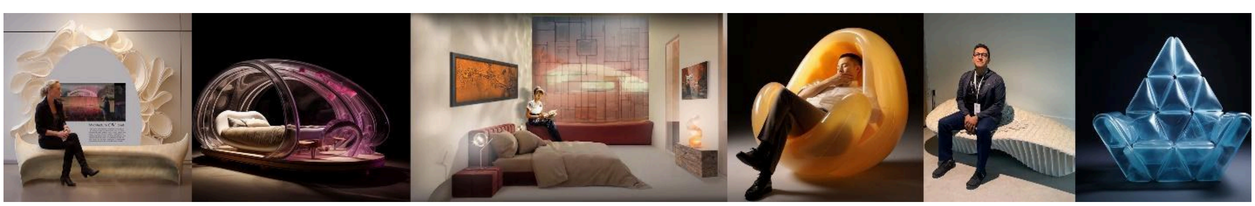

Figure 5. Furniture models. From left to right, bench made from mycoterial, concept for bed that has melanin-rich mycomaterial shield, interior render with grown in place furniture, concept for grow in place chair, mycobench at biofabricate 2022, concept for grow-in-place chair.

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[1] Cabral, J.P., Kafle, B., Subhani, M. et al. 2022. Densification of timber: a review on the process, material properties, and application. *J Wood Sci* 68, 20. https://doi.org/10.1186/s10086-022-02028-3

Moonprint solutions (David Cadogan) worked with redhouse studios (Chris Maurer) to suggest inflatables to create the furniture. Polyurethane coated drop-thread material is preferable to PVC as PVC has a higher chance of releasing volatiles. The edges of the drop-thread will be closed with a polyurethane coated Nylon fabric. Moonprint will seal polyurethane fittings to the inflatable to connect it to hoses that feed the reaction. They have not completely worked out the flow channels for water and gasses to support the bioprocess, but favor tubes inside the drop-thread. The tubes would have a permeable wall to support transmission of molecules while still containing the fluid mass.

4.1.5 Integration of Mycotecture to the Models

The physical models not only served as studies models, but also as the forms for mycomaterial production. With this delivery system in mind, we have developed many possibilities for creating mycomaterials *in situ,* and have begun testing their laboratory analogs for durability.

Hydrogels serve as good substrates for the growth of mycelium as they can be rich in nutrients and use very little upmass from the Earth. Sodium alginate is a hydrophilic hydrogel derived from marine brown algae that can have up to 99.9% water. For this application, that means that 99.9% of the substrate material could be *in situ*-derived material.

4.1.6 Melanin Infused Hydrogels

Melanin, a group of pigments primarily derived from the amino acids tyrosine or cysteine, provides protection from radiation for a wide variety of organisms, including human skin where production is induced by exposure to UV radiation. Melanin is believed to protect from ionizing radiation due to its shell structure. According to the "local-oder-global-disorder" model of melanin, layers of nanostructure bonding create spherical structures called melanin granules.[2] Researchers have noted that the 200 nm melanin spheres are composed of 0.5-30 nm nanospheres.[3],[4] This fractal-like scaling of structure exists in many natural systems. The radio-protective nature of melanin is thought to be a function of its chemical composition and its spatial configuration. Its spherical-shell structure enables melanin to protect through Compton scattering and free radical scavenging.[5] This ability transcends the life of the organism that grew the melanin, further pointing to the structure as a key factor over metabolic

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[2] Cordero RJB, Camacho E, Casadevall A. 2020. Melanization in *Cryptococcus neoformans* Requires Complex Regulation. mBio. 11(1):e03313-19. doi: 10.1128/mBio.03313-19

[3] Camacho E, Vij R, Chrissian C, Prados-Rosales R, Gil D, O'Meally RN, Cordero RJB, Cole RN, McCaffery JM, Stark RE, Casadevall A. 2019. The structural unit of melanin in the cell wall of the fungal pathogen Cryptococcus neoformans. *J Biol Chem.* 294(27):10471-10489. doi: 10.1074/jbc.RA119.008684

[4] Lemaster JE, Jeevarathinam AS, Kumar A, Chandrasekar B, Chen F, Jokerst JV. Synthesis of Ultrasmall. 2019. Synthetic Melanin Nanoparticles by UV Irradiation in Acidic and Neutral Conditions. *ACS Appl Bio Mater.* 2(10):4667-4674. doi: 10.1021/acsabm.9b00747. Epub 2019 Sep 26. PMID: 31930189; PMCID: PMC6953903.

[5] Revskaya E, Chu P, Howell RC, Schweitzer AD, Bryan RA, Harris M, Gerfen G, Jiang Z, Jandl T, Kim K, Ting LM, Sellers RS, Dadachova E, Casadevall A. 2012. Compton scattering by internal shields based on melanin-containing mushrooms provides protection of gastrointestinal tract from ionizing radiation. *Cancer Biother Radiopharm.* 27(9):570-6. doi: 10.1089/cbr.2012.1318

processes. The fractal pattern is continued at another scale as the melanin granules organize in concentric layers within the cell walls of the organisms that produce them.[6]

Mycelia often form skins on the surfaces of substrates to help lock-in moisture and protect their territory.[7] Further, methods of developing shell structures exist in hydrogel creation whereby sodium alginate (a component of the cell wall of marine brown algae) is cross-linked with a source of calcium to create a gel skin or shell that can encapsulate a liquid.[8] This method of hydrogel spherification can be used to create microscopic spheres of nutrient rich material that would allow the growth of 2-20 µm mycelial hyphae with 200 nm melanin granules within a 1 µm cell wall that are composed of 1-30 nm nanospheres. When converted to aerogels these structures should remain suspended in air by the aerogel matrix, making them lightweight (Fig. 6). Their continued efficacy as insulators and radiation protectors will be tested in future research with UV radiation exposure at McMaster's planetary simulator by Rheinstadter and Krivic and thermal conductivity at Stanford by Senesky and Kornegay.

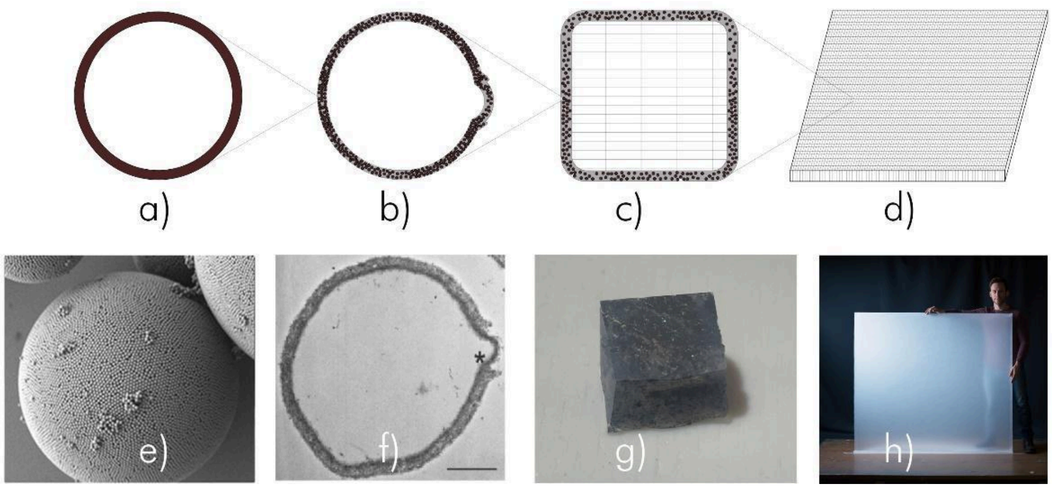

Figure 6. Nature inspired radiation shielding aerogels: scaling the nestled sphere structure of melanin for radio-protection and strength. a) melanin nanospheres of 1 nm diameter composing a melanin granule shell of 200 nm, b) melanin granules of 200 nm forming concentric rings within the 1 µm cell wall of a 10 µm cell, c)10 µm melanized fungal cells with in the "shell" of the 1 mm hydrogel/aerogel spheres/cells, d) aggregation of hundreds of thousands of aerogel-shells into a usable product to be determined by this convergence, e)

[6] Camacho E, Vij R, Chrissian C, Prados-Rosales R, Gil D, O'Meally RN, Cordero RJB, Cole RN, McCaffery JM, Stark RE, Casadevall A. 2019. The structural unit of melanin in the cell wall of the fungal pathogen *Cryptococcus neoformans. J Biol Chem.* 5;294(27):10471-10489. doi: 10.1074/jbc.RA119.008684.
[7] Houette, T.; Maurer, C.; Niewiarowski, R.; Gruber, P. 2022. Growth and Mechanical Characterization of Mycelium-Based Composites towards Future Bioremediation and Food Production in the Material Manufacturing Cycle. *Biomimetics* 7, 103. https://doi.org/10.3390/biomimetics7030103
[8] de Farias YB, Coutinho AK, Assis RQ, Rios AO. 2020. Biodegradable sodium alginate films incorporated with norbixin salts. *J Food Process Eng.* 43:e13345. https://doi.org/10.1111/jfpe.13345

TEM image of melanin granule [9] *f) image of cell with melanin granules in cell wall* [10]*, g) 1 cm^3 melanized hydrogel made by our team from sodium alginate crosslinked with calcium lactate,* C. neoformans *melanin provided by MelaTech LLC is creating a shell around the gel, AI generated image of aerogel material composed of microparticles of melanized gel spheres.*

A formulation was made to test the efficacy of melanin suspended within hydrogels as a radiation shield. Sodium alginate (1% solution) cross linked with calcium lactate (1% solution) was mixed with melanin (0.1% wet weight) extracted from *Cryptococcus neoformes* by Melatech LLC. (Baltimore, MD). The samples are currently being tested at McMaster University (Fig. 7) in their planetary simulator (see section 4.3 for a description of the simulator.)

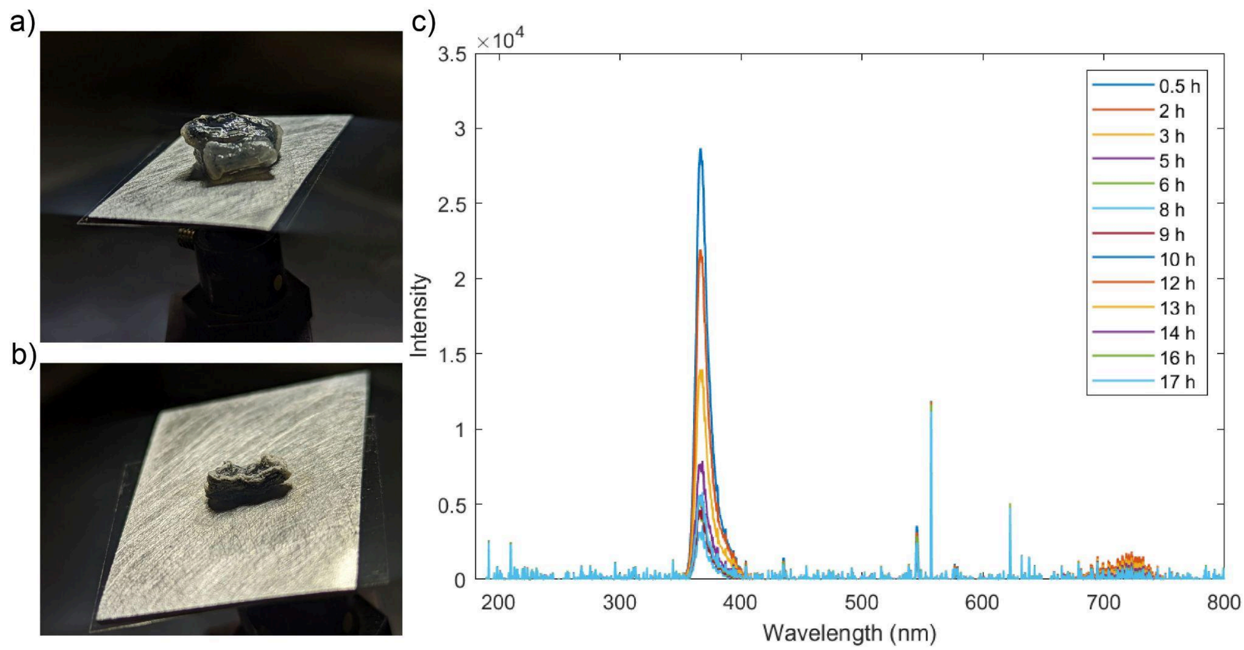

Figure 7. Melanin absorbance spectra over the course of the dry cycle. a) An aluminum sheet with a hole cut-out in the center was placed on top of a spectrometer connected inside of the Planetary Simulator. A melanin-infused hydrogel was placed directly on top of the hole and was allowed to dry while being exposed to UVA, UVB, and UVC. b) The dried-out melanin-infused hydrogel. c) The absorbance spectra was measured at different time points as the sample dried. Preliminary data from McMaster University on UV radiation absorbance shows promise, more research is underway.

Photosynthetic organisms including plants, cyanobacteria and micro and macroalgae, could be grown in liquids within the LOBEs. As light would be hard to moderate in lunar and martian

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[9] Ming Xiao et al. 2017. Bioinspired bright noniridescent photonic melanin supraballs.Sci. Adv.3,e1701151 .DOI:10.1126/sciadv.1701151

[10] Eisenman HC, Nosanchuk JD, Webber JB, Emerson RJ, Camesano TA, Casadevall A. 2005. Microstructure of cell wall-associated melanin in the human pathogenic fungus *Cryptococcus neoformans. Biochemistry.* 15;44(10):3683-93. doi: 10.1021/bi047731m

contexts, LED fixtures could be embedded within LOBEs. We tested various photosynthetic organisms including the filamentous cyanobacterium *Spirulina*, as well as several species of algae including *Chlorella, Chaetomorpha,* and *Macrocystis* as members of fungal biocomposites (Fig. 8).

Figure 8. Algal/fungal biocomposites. From left to right, Chlorella *newly inoculated with liquid mycelium,* Chlorella/Ganoderma *composite,* Macrocytis/Ganoderma *composite,* Spirulina/Ganoderma *composite, Close up of* Ganoderma *hyphae running on* Chlorella sp.*, microscopy of* Ganoderma *on Spirulina.*

4.1.7 Biotechnology and Bioengineering

Engineering living materials to specification will require the measurement of many parameters for many samples in order to determine the most performant materials for a given application.[11, 12, 13, 14] For example, genetic enhancements of fungal components will require experimental laboratory evolution or forward engineering. Both methods benefit from replicable and high-throughput experimentation to elucidate the ideal parameter set for a given application. Likewise, determining the most performant fungal-algal co-culture would benefit from replicable and high-throughput experimentation for pairing various natural or genetically-enhanced fungi-algae combinations. To build a foundation for bioengineering, we prototyped methods for basic laboratory culture and assessment necessary for increased throughput bioengineering workflows with various fungi and algae, although the constraints of the NIAC funding did not allow the sort of high-throughput experimentation that is currently being applied to industry.

First, we characterized the morphology and growth performance of various fungi on different substrates. We then characterized the survivability at various temperatures of a select group of fungi. Second, using a subset of the most performant fungi, we prototyped various laboratory culture form factors that we hypothesized would be useful for reproducibility and increased throughput. Third, we prototyped various composite materials composed of combinations of agar-based hydrogels, simulated lunar regolith and simulated Martian regolith. Finally, we prototyped various laboratory culture methods for fungi-algae pairings to enable increased

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[11] McBee, R.M., Lucht, M., Mukhitov, N. et al. 2022. Engineering living and regenerative fungal–bacterial biocomposite structures. *Nat. Mater.* 21, 471–478 https://doi.org/10.1038/s41563-021-01123-y
[12] Zou, G., Nielsen, J.B., and Wei, Y. 2023. Harnessing synthetic biology for mushroom farming. Trends in biotechnology. vol. 41, ISSUE 4, p480-483, https://doi.org/10.1016/j.tibtech.2022.10.001
[13] Q. Wang, Z. Hu, Z. Li, T. Liu, G. Bian, 2023. Exploring the Application and Prospects of Synthetic Biology in Engineered Living Materials. *Adv. Mater.* 2305828. https://doi.org/10.1002/adma.202305828
[14] Charles Jo, Jing Zhang, Jenny M. Tam, George M. Church, Ahmad S. Khalil, Daniel Segrè, Tzu-Chieh Tang, 2023. Unlocking the magic in mycelium: Using synthetic biology to optimize filamentous fungi for biomanufacturing and sustainability, *Materials Today Bio*, vol. 19, 100560, https://doi.org/10.1016/j.mtbio.2023.100560

reproducibility and throughput. These methods will be critical for further biotechnology and bioengineering development.

To identify fungi with rapid growth and the ability to grow on potential waste byproducts, such as lignocellulosic materials, algae as a carbon source, and electrochemically-produced carbon sources, we grew various fungi on a standard potato dextrose agar (PDA) medium and various nutrient sources (Fig. 9). We used a simple radial extension measurement[15] to determine which fungi grow the fastest (Fig 10). We also grew these fungi on lignocellulosic materials and qualitatively assessed their growth and morphology. The fungi we tested are all relevant to future space missions. *Pleurotus ostreatus* is a culinary mushroom that has also been used for mycelium materials. *Ganoderma lucidum* is a medicinal mushroom that has also been used for mycelium materials. *Neurospora crassa* is a model fungus that has long been used for genetic studies and benefits from genetic engineering tools. Recently, it has also garnered increased interest as an alternative protein source. *Aspergillus oryzae* is a culinary fungus that has had some genetic engineering tools developed for use in food and materials. *Aspergillus niger* has long been used for industrial production and benefits from a wide array of genetic engineering tools and techniques. Further, it forms conidia that contain melanin in their cell wall, which may provide resistance to salinity and desiccation as well as UV radiation.[16] Importantly, conventional mycelium materials work with wood-degrading fungi, fungi that are able to digest and bind lignocellulosic materials, known as white-rot fungi predominantly in the Basidiomycota division. *Neurospora spp.* and *Aspergillus spp.* reside in the Ascomycota and are typically not able to digest the lignin in lignocellulosic materials, which has traditionally precluded their use as strains for mycelium materials production. Nevertheless, we included these fungi in our initial assessments as they are fast growers, they are gaining interest as for producing engineering living materials and they may still be useful for other space exploration applications, and it may be feasible to enhance their lignin-degrading abilities via genetic engineering.[17] We found that all fungi could grow on simple PDA media. The fungi exhibited differential radial extension rates on PDA and various morphologies on lignocellulosic substrates.

[15] Perez, R., Luccioni, M., Kamakaka, R. et al. 2020. Enabling community-based metrology for wood-degrading fungi. *Fungal Biol Biotechnol* 7, 2. https://doi.org/10.1186/s40694-020-00092-2
[16] Segers FJJ, Wösten HAB, Dijksterhuis J. 2018. *Aspergillus niger* mutants affected in conidial pigmentation do not have an increased susceptibility to water stress during growth at low water activity. Lett Appl Microbiol. 66(3):238-243. doi: 10.1111/lam.12846
[17] Li, Zhen Wei, Jianyao Jia, Qing Xu, Hao Liu, Chao Zhong, He Huang, 2023. Engineered living materials grown from programmable Aspergillus niger mycelial pellets, *Materials Today Bio*, Vol 19, 100545, https://doi.org/10.1016/j.mtbio.2023.100545

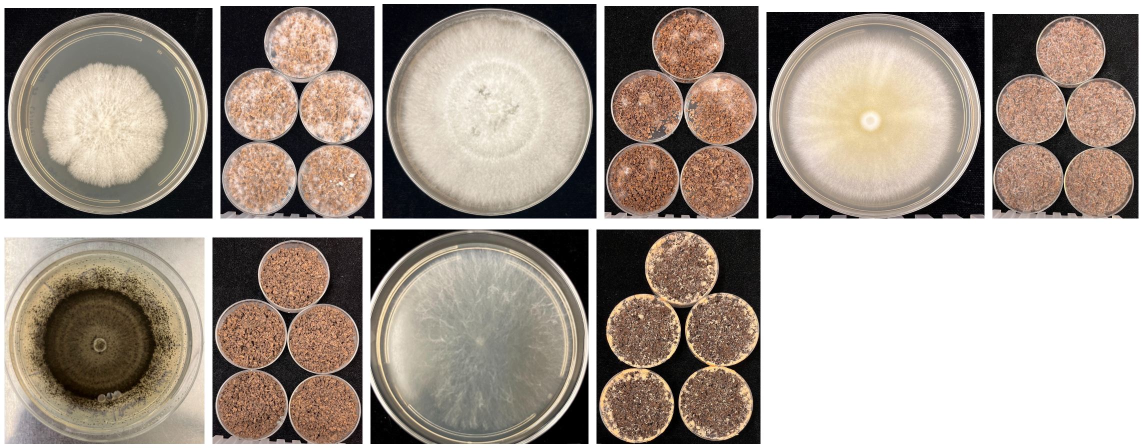
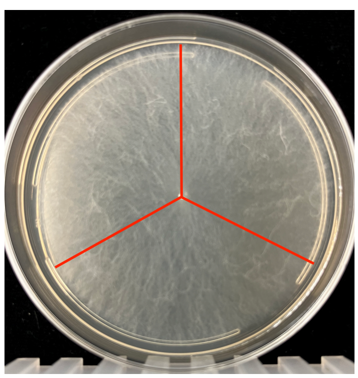


Figure 9. Morphology of various fungi tested. Representative images of cultures of Pleurotus ostreatus *(top left),* Ganoderma lucidum *(top middle),* Aspergillus oryzae *(top right),* Aspergillus niger *(bottom left),* Neurospora crassa *(top right). We grew the fungi on potato dextrose agar and a commercially-available lignocellulosic solid substrate. Each strain exhibited unique morphologies. Notably the white-rot fungi demonstrating the slowest radial extension rates but a superior ability to grow on the lignocellulosic substrates.*



	Radial Extension (cm/day)
Pleurotus ostreatus	0.691167
Ganoderma spp.	0.974833
Aspergillus oryzae	0.988500
Neurospora crassa	4.792500

Figure 10. Fungi radial extension performance on agar nutrient substrates. We measured radial extension rates for all the fungi we tested. Radial extension was measured by culturing the fungal mycelium for each strain for five days and measuring the radius of the mycelium along three axes (left). We assumed a linear model to calculate the daily extension rate (n=5, right).

Temperatures on the lunar and martian surface can fluctuate rapidly beyond conditions that are viable for living organisms. It is important to understand how temperature might affect the viability of our living materials, so that we can understand the limitations of the materials, mitigate them, and engineer improvements. To this end, we then sought to test the viability of select fungi when exposed to various extreme temperatures. The fungi we selected typically grow in mesophilic conditions at temperatures ranging from 20 - 30 °C. Furthermore, traditional mycelium materials production often involves a postprocessing step where the living materials are baked at low temperature, 50 °C or above, for at least 24 hours. To test the upper temperature range of the fungi, we grew them in a liquid potato dextrose medium and exposed aliquots of the cultures to various elevated temperatures for 5 minutes. We then plated the aliquots of each sample onto plates in the form of a spot assay. We found that all fungi could grow at 37 °C, *A. oryzae* exhibited some growth at 47 °C, and no fungi survived temperatures above 47 °C in this experiment (Fig. 11).

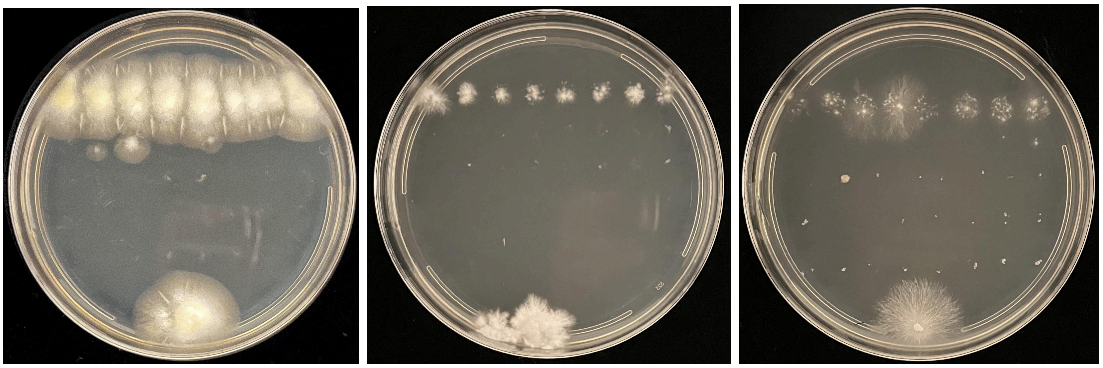


Figure 11. Fungal survivability at elevated temperatures. Spot assay of liquid cultures of A. oryzae, P. ostreatus, Ganoderma spp. *after heat treatment in the thermocycler. 37 °C top row, 47 °C (second from top), 57 °C (third from top), 77 °C (fourth from top), 97 °C (bottom row), positive control at room temp (bottom center spot). No fungi survived temperatures above 47 °C .*

It is commonly known to mycologists that fungi can survive storage at reduced temperatures, such as 4 °C, but require cryoprotectants when stored below freezing (Fig. 12). Cryopreservation of fungal strains is an active area of research with emphasis on the long-term genetic effects of cryopreservation and subsequent genetic drift.[18] Here we sought to understand the viability of mycelium materials upon exposure to reduced temperatures relevant to space exploration, such as temperatures below 0 °C. We produced mycelium materials from *Ganoderma spp.*, stored them at -80 °C for 5 days, removed them from cryo-storage, cultured them at 30 °C, and subsequently assessed their viability. We found that the mycelium materials we produced and stored at -80 °C without cryoprotectant remained viable upon subsequent culture at 30 °C. Though this requires further study, our initial results are promising with respect to living materials that may be exposed to extreme temperatures in space or on the surface of the Moon or Mars and compliment evidence in the literature for the ability for mycelium materials to revive and self-repair after desiccation.[19] (https://onlinelibrary.wiley.com/doi/10.1002/adfm.202301875).

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[18] Kenta Sakurai, Munemitsu Yuasa, Syoko Ohji, Akira Hosoyama, Masanori Sato, Nobuyuki Fujita, and Hiroko Kawasaki. 2019. Gene Mutations in *Ganoderma lucidum* During Long-Term Preservation by Repeated Subculturing. *Biopreservation and Biobanking.* 395-400. http://doi.org/10.1089/bio.2018.0149
[19] Elise Elsacker, Meng Zhang, Martyn Dade-Robertson. 2023. Fungal Engineered Living Materials: The Viability of Pure Mycelium Materials with Self-Healing Functionalities. *Advanced Functional Materials* 33(29) DOI: 10.1002/adfm.202301875

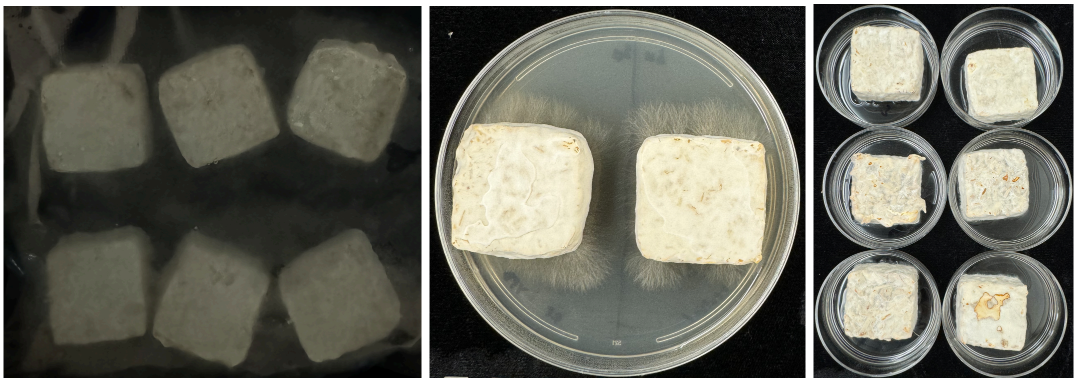


Figure 12. Viability of mycelium materials at low temperatures and desiccated at room temperature and humidity. We grew mycelium materials, froze them at -80C for 52 days without cryoprotectant. The materials remained viable after cold storage.

Mycelium materials are typically grown in batch-fed reactions, in sizes greater than several centimeters in length and in sample sizes ranging in the tens of samples. The ability for mycelium materials to be produced at large scales is a feature that is touted as a superior attribute of the production process. While large-scale samples are useful for constructing final objects, prototypes and life-sized models, the ability to interrogate the parameter space of possible mycelium materials is required in order to advance mycelium materials beyond prototypes, novelty items and art. To engineer mycelium materials with advanced capabilities we need improved laboratory techniques that enable reproducible and high-throughput experimentation and data generation for hundreds to thousands of samples. Only then will we be able to make use of predictive artificial intelligence models for designing materials for new applications. While high-throughput methods exist for liquid culture of fungi and for unicellular organisms, there is a need for analogous materials, measures and methods for solid-state culture systems. To lay the foundation for such laboratory techniques we prototyped various methods for high-throughput culture and characterization of mycelium materials. First, we attempted to adapt existing laboratory consumables to grow mycelium materials. We found that we could qualitatively assess differential growth of various fungi and substrate pairs but downstream processing, such as drying and material recovery, proved difficult. We then sought to develop a continuously-fed system with a form factor that would enable facile release of the grown materials. A continuously-fed system could enable more rapid growth and continuous harvesting of the materials, and mitigate some of the current challenges with batch-fed cast systems currently used for producing mycelium materials.[20] Our initial results were promising as we observed some fungal growth in the system, but contamination proved to be a major obstacle when working with large amounts of liquid media in large open containers. Finally, we opted to expand on traditional mycelium materials production methods by scaling down the size of the samples grown by an order of magnitude (Fig. 13). We used off-the shelf forms made of silicone that can be packed with pre-grown mycelium material, spawn, and that enable facile recovery of the grown sample. Using this form factor we are able to grow an order of magnitude more samples, enabling us to vary many more parameters and test many more conditions.

[20] He Q, Peng H, Sheng M, Hu S, Qiu J, Gu J. 2019. Humidity Control Strategies for Solid-State Fermentation: Capillary Water Supply by Water-Retention Materials and Negative-Pressure Auto-controlled Irrigation. *Front Bioeng Biotechnol.* 7:263. doi: 10.3389/fbioe.2019.00263

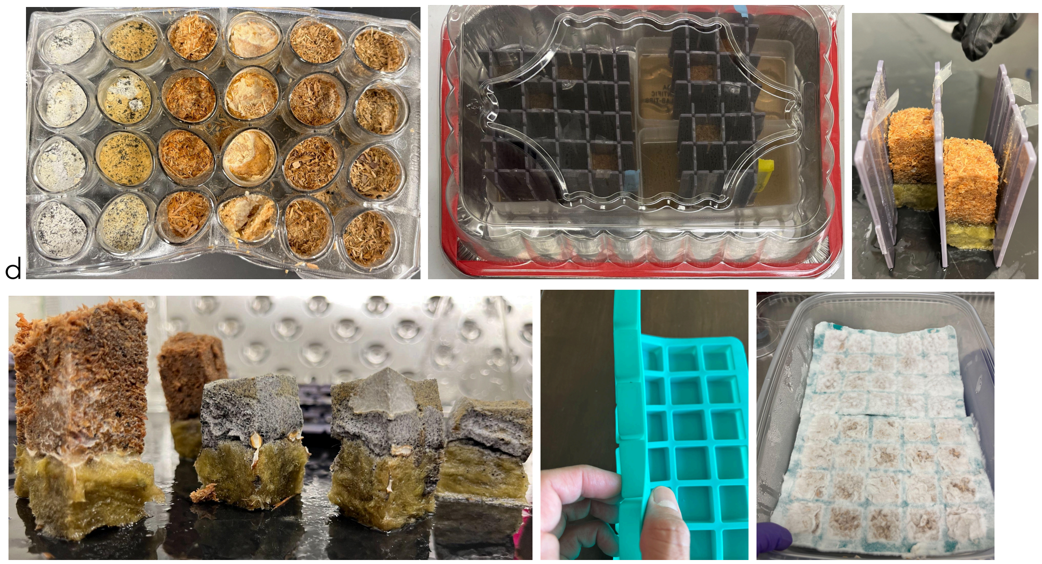


Figure 13. Increasing material production throughput. We prototyped various form factors to increase the reproducibility and throughput of material production. We found that a continuous culture system could enable the continuous production of products but the system requires further optimization. We ultimately moved forward with a silicone form system that enabled us to routinely produce an order of magnitude more materials.

We further expanded on our characterization of our culture bank of fungi. We tested the ability of the fungi to grow spawn, the pre-grown starter culture that conventionally is used to inoculate a final substrate mix prior to casting mycelium materials in their final forms (Fig. 14). We grew *P. ostreatus*, *Ganoderma spp.*, *N. crassa*, *A. oryzae*, and *A. niger* on a commercially available hardwood solid substrate. We found that *P. ostreatus* and *Ganoderma spp.* could readily myceliate, grow on and bind the substrate. *N. crassa*, *A. oryzae*, and *A. niger* exhibited differential growth on the substrate, but markedly less than the Basidiomycete strains and none of them, with the exception of *A. oryzae*, could appreciably bind the substrate together as the the Basidiomycetes do. *For this reason, we decided to proceed with the Basidiomycetes for further studies.*

Figure 14. Spawn of various fungi tested. We tested the ability of various fungi to produce the seed culture for material production, spawn. We found that Ganoderma sp. and P. ostreatus were best able to produce viable spawn, followed by A. oryzae, N. crassa and A. niger.

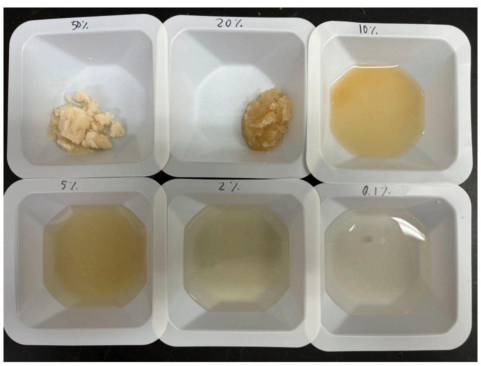
To demonstrate our ability to grow large numbers of materials we used our silicone forms and a select group of fungi to grow mycelium materials using a commercially available substrate and our in-house substrate recipe. Furthermore, we used a commercially available mycelium material kit from Ecovative LLC. as a reference strain and substrate. We adapted methods from various literature sources to produce samples for subsequent characterization.[21,22] We were able to produce and test the material properties of hundreds of samples (Fig. 15). We found varying morphologies and material properties among different fungi and substrate combinations. Using the reference strain and substrate, we were able to develop a reference standard for material morphology and mechanical properties. These materials, measures and methods will be critical for reproducibility, reliable production and tuning the parameters of mycelium materials for specific applications.

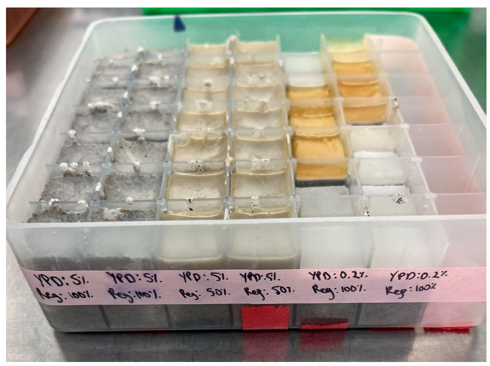
Figure 15. Lignocellulosic biomass-based mycelium materials. We developed a system to explore various species and substrate combinations for material production. We used a commercially available mycelium material as a reference material for developing our own in-house strain and substrate.

A major goal for us is to realize a living material composed of a synthetic symbiosis between fungi and photosynthetic organisms, such as algae, obviating the need to launch even dehydrated lignocellulosic materials. Hydrogels and aerogels are key aspects of some of our designs. In some embodiments, these hydrogels and aerogels may be, in part, composed of lunar or martian regolith. Thus, it is important for us to understand the performance of fungi and algae in the context of these materials. While there has been extensive work done with

[21] Pohl, C., Schmidt, B., Nunez Guitar, T. et al. 2022. Establishment of the basidiomycete Fomes fomentarius for the production of composite materials. *Fungal Biol Biotechno*l 9, 4. https://doi.org/10.1186/s40694-022-00133-y
[22] Elsacker E, Vandelook S, Brancart J, Peeters E, De Laet L. 2019. Mechanical, physical and chemical characterisation of mycelium-based composites with different types of lignocellulosic substrates. *PLoS One.* 14(7):e0213954. doi: 10.1371/journal.pone.0213954

algae in hydrogels, less is known about how fungi respond to hydrogels and how fungal-algal materials might be developed.[23] Like traditional mycelium materials, there is a need for basic laboratory culture and bioengineering techniques to systematically explore the parameter space of potential materials. To begin to address this need, we prototyped materials and methods for synthesizing various hydrogel-based fungal composite materials containing simulated lunar or martian regolith (Fig. 16 and 17). First, we specified an agar-based hydrogel, owing to its biocompatibility and availability, and determined a range of viable compositions that enabled material production, such as castability. We determined an ideal inclusion rate for simulated lunar regolith, produced materials and characterized their mechanical properties. We then iterated on our process to investigate alternative form factors, casting and production methods. We used our most performant methods to produce fungal composite materials using simulated lunar regolith. In some versions, we mixed fungi into the materials before casting. In others, we grew fungal skins on the outside of the materials. We found that the fungi we tested can grow in and on agar-hydrogel materials containing simulated lunar regolith (Fig. 16). Additionally, we found that the fungi we tested can grow on simulated Martian regolith that has been moistened with nutrient broth (Fig. 17).





[23] Gantenbein, S., Colucci, E., Käch, J. et al. 2023. Three-dimensional printing of mycelium hydrogels into living complex materials. *Nat. Mater.* 22, 128–134. https://doi.org/10.1038/s41563-022-01429-5

Figure 16. Agar hydrogel-based simulated lunar regolith materials and fungal composites.

We prototyped materials made from simulated lunar regolith, agar and fungi. First, we determined the maximum workable concentration of agar and simulant (top left and right). We found that the subsequent materials maintained their shape (center left). We then prototyped an alternative form that enabled more facile production and variations in material properties, such as fungal skins (center) and porosity (center right). Baking produced brick-like materials (bottom left). We found that the *Ganoderma sp.* that we tested was not able to significantly bind together the nutrient-infused lunar regolith simulant without the presence of agar. More studies are needed to definitely explore the geo biotechnical engineering capabilities of fungi.

Figure 17. Fungi can grow on nutrient-supplemented simulated martian regolith. Various fungi grown on simulated martian regolith supplemented with liquid PDY nutrient. Left at time zero and right after 2 weeks of growth. Top row, left to right: G. lucidum *strain A, wells 1-3;* G. lucidum *strain B, wells 4-6. Bottom row, left to right:* A. oryzae*, wells 7-9;* P. ostreatus, *wells 10-12*.

The most promising fungus, *Ganoderma sp.*, we tested and used for subsequent studies has historically been genetically intractable. Genetic tractability is essential for functional studies, bioengineering and establishing a synthetic symbiosis with an autotrophic organism. We imagine scenarios that require engineered material functionality, e.g., remediation of toxic *in situ* resources. While we are working to build tools to readily engineer fungi, we also explored the possibility of using more genetically tractable organisms as biological amendment applications that enable new material functionality.[24] Toward this end, we co-cultured an engineered strain of *Bacillus subtilis* with *Ganoderma sp*. on agar plates and qualitatively observed their interaction via fluorescence microscopy (Fig. 18 and 19). We hypothesized that the two organisms could coexist based on their probable interaction as soil-dwelling organisms in the wild. First, we grew the organisms together on a modified version of PDA, a potato sucrose agar (PSA), and yeast peptone dextrose agar (YPD) and observed their interactions by eye (Fig. 18). We observed a color change in the fungal mycelium when the organisms were grown on PDA, regardless of contact between the organisms, that we did not observe on YPD. Additionally, the fungus exhibited stunted growth on PSA. We sampled regions of the fungal mycelium that were visibly in contact with the bacteria and regions that were not, and we checked for the presence of fluorescent bacteria or protein via fluorescence microscopy (Fig. 19).

[24] Meng D, Mukhitov N, Neitzey D, Lucht M, Schaak DD, Voigt CA. 2021. Rapid and simultaneous screening of pathway designs and chassis organisms, applied to engineered living materials. *Metab Eng.* 66:308-318. doi: 10.1016/j.ymben.2021.01.006. Epub 2021 Jan 16. PMID: 33460821.

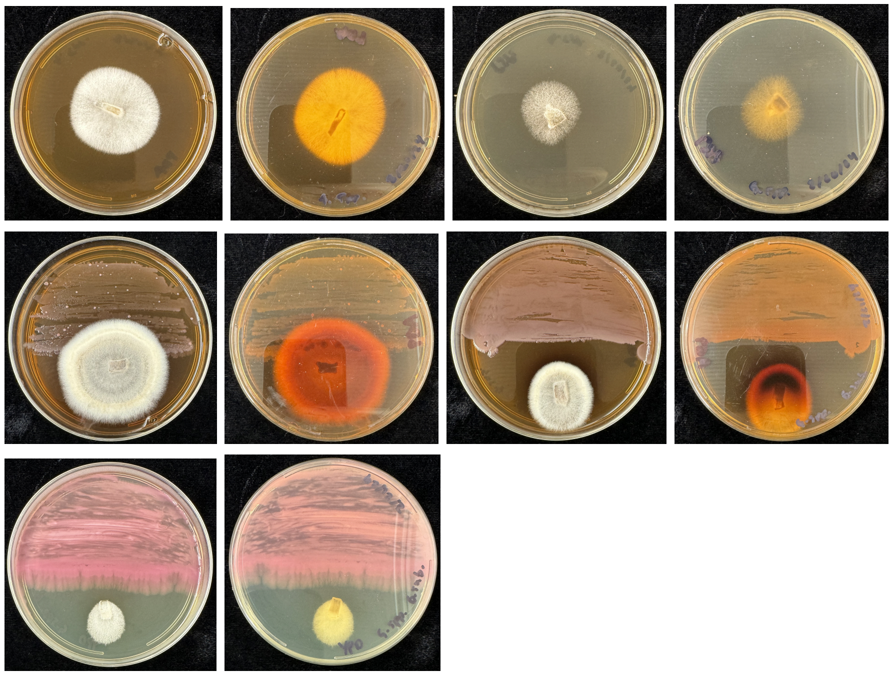


Figure 18. Co-culture of Ganoderma sp. *with Bacillus subtilis on various substrates. We grew our reference strain of Ganoderma sp. in coculture with a strain of B. subtilis that was engineered to express a red fluorescent protein. We first grew the fungus on media that had been modified to contain all of the nutrients reportedly required by B. subtilis to produce bacterial levan in its biofilms (top left and middle left, modified PDA; top right and middle right, potato sucrose agar (PSA).*

We used fluorescence microscopy to qualitatively assess the ability for the fungus and bacteria to coexist (Fig. 19). First, we assessed the morphology and autofluorescence of the fungus without bacteria. We observed that the fungus was autofluorescent and produced chlamydospore-like structures. The presence of chlamydospores may explain the ability for the fungal materials to maintain viability after cryo-storage and slow drying at room temperature but further studies are needed to definitively determine the source of the regenerative abilities of the materials. We also observed what appeared to be accumulation of the red fluorescent protein in the fungal mycelium regardless of direct contact with the bacteria. Finally, we sampled tissue from the leading edge of fungal colonies that were in contact with bacteria, leading edges of the same colony opposite the edge in contact and that were not directly in contact with the bacteria, and from colonies that were not in direct contact with the bacteria but that exhibited a color change. We observed fluorescent, motile bacteria in samples taken from leading edges of fungal colonies that were in direct contact with the bacteria. In some samples, we also observed fluorescent, motile bacteria in samples taken from the leading edges opposite leading edges of colonies that were in direct contact with the bacteria, albeit to a lesser extent. Samples from colored colonies that were not in direct contact with the bacteria appeared red in color and exhibited increased autofluorescence. Further studies are needed to determine the source of the color change in the fungal colonies, potential accumulation of protein in fungal mycelium and the ability for the fungus and bacteria to coexist.

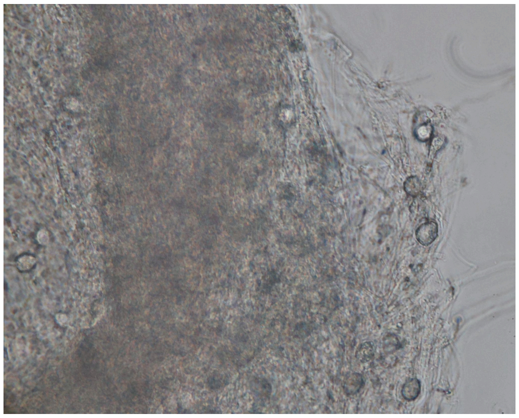
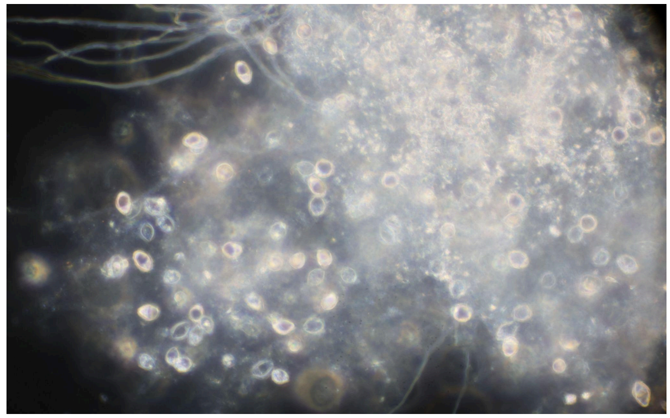
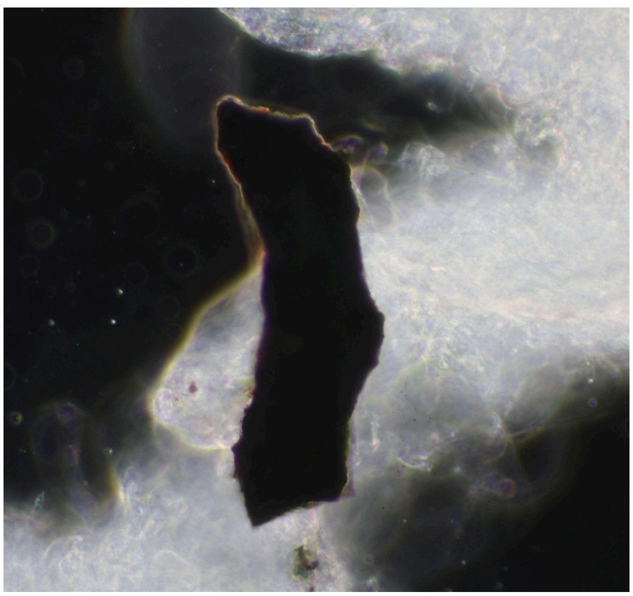
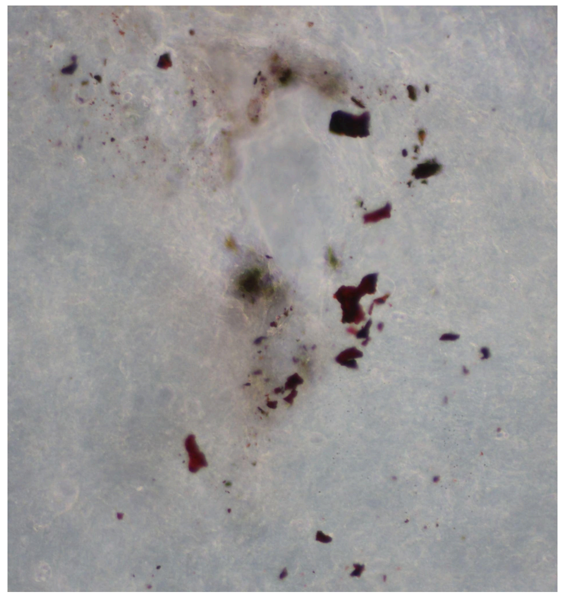
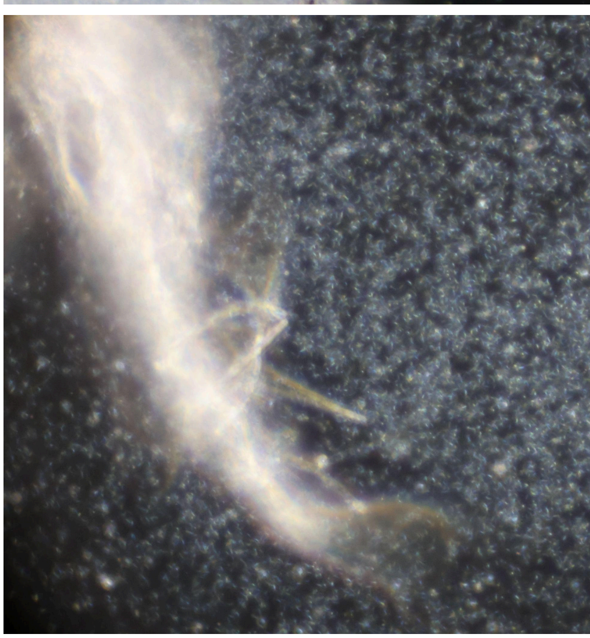







Figure 19. Microscopy of samples from fungal-bacterial co-culture. The fungus we tested produced chlamydospore-like structures when grown on modified PDA (40x brightfield, top left; 40x phase contrast, top right). We observed large flakes of red material in samples taken from fungal colonies that were growing on plates with bacteria but that were not in direct contact with bacteria (10x phase contrast, center left and right). Further studies are needed to determine the identity and source of the red material. Finally, we observed fluorescent, motile bacteria in samples taken from leading edges of fungal colonies that were in direct contact with bacteria (40x phase contrast, bottom left; 40x Cy3, bottom right). Taken together, our results suggest that the fungus and bacteria we tested may be amenable to co-culture but further studies are needed to quantify the interactions we observed.

Toward our ultimate goal of producing a living material composed of fungi, algae and hydrogels, we prototyped algae-infused hydrogel materials. We hypothesized that the unicellular green alga, *Chlamydomonas reinhardtii,* could grow within the hydrogels made from a common algae medium, Tris acetate phosphate (TAP) medium. We grew *C. reinhardtii*, washed the cells and mixed the cells with a TAP agar solution. After mixing, we filled forms with with the alga-hydrogel mixture and let the material set. Once the agar set, the bricks were removed from the mold and incubated in a light room at room temperature for 8 days. We found that *C. reinhardtii* can grow on and within the hydrogel (Fig. 20).

Figure 20. Algal hydrogel bricks. Through qualitative assessment, we found that the alga Chlamydomonas reinhardtii *can grow within hydrogel bricks. This finding is important for our ultimate goal of producing living materials composed of symbiotic interactions between algae and fungi.*

Lunar and Martian regolith do not contain organic carbon and thus by themselves do not provide the nutrients necessary to sustain life. Therefore, most studies with simulated or real lunar regolith supplement the regolith with a nutrient source, such as glucose-based media for microbes or mineral nutrients for plants. Some evidence suggests that plants grow better on simulated rather than real lunar regolith, showing the limitations of simulated regoliths for such studies. Beyond our own studies, there is a general need for simulants with higher chemical fidelity to real lunar regolith for use in biological experimentation.

Our ultimate strategy is to enable a living material based on a symbiosis between a heterotrophic fungus and an autotrophic organism such that the autotroph provides nutrients, e.g., carbon, for the heterotroph, thereby addressing the need to add nutrients for the heterotrophic fungus. Even in this scenario, the autotroph will need to be supplied with mineral nutrients and it may need additional carbon sources depending on the solar radiation amounts and overall structural designs. A complementary way to produce carbon, e.g., acetate, is via electrosynthesis, technology under active development for biomanufacturing on Earth. Through an artificial photosynthetic system, CO_2 can be captured and converted into acetate, at an efficiency rate higher than what most crop plants can capture carbon https://www.nature.com/articles/s43016-022-00530-x . If fungi can grow using acetate, a carbon source that can be potentially synthesized *in situ*, it would reduce the load of nutrients required for growth sent from Earth, and thus the overall upmass costs, and provide a level of redundancy in the event that we cannot establish a symbiotic system or disruptions in autotrophic growth. Here, we investigated the ability for a fungus and an alga to grow on acetate as a carbon source (Fig. 21). First, we grew a fungus and an alga on acetate individually. We assessed the ability for *Aspergillus oryzae* to grow in the presence of acetate and as a sole carbon source. We found qualitative evidence that the fungus we tested can grow on acetate as a sole carbon source, albeit to a much lesser extent than growth on medium without acetate and medium containing dextrose and acetate. We then assessed whether our alga could grow on increased concentrations of acetate. TAP medium, a common growth medium for algae, already contains acetate so we were unsurprised to find qualitative evidence that the alga could grow in the presence of increased levels of acetate, with clear growth inhibition at 5% acetate and above. The fact that the fungus and alga can grow in the presence of acetate and as a sole carbon source is promising initial result, as this form of redundancy can help to de-risk our ultimate goal of creating living materials for habitats in the event that we are unable to establish a self-contained symbiotic system.

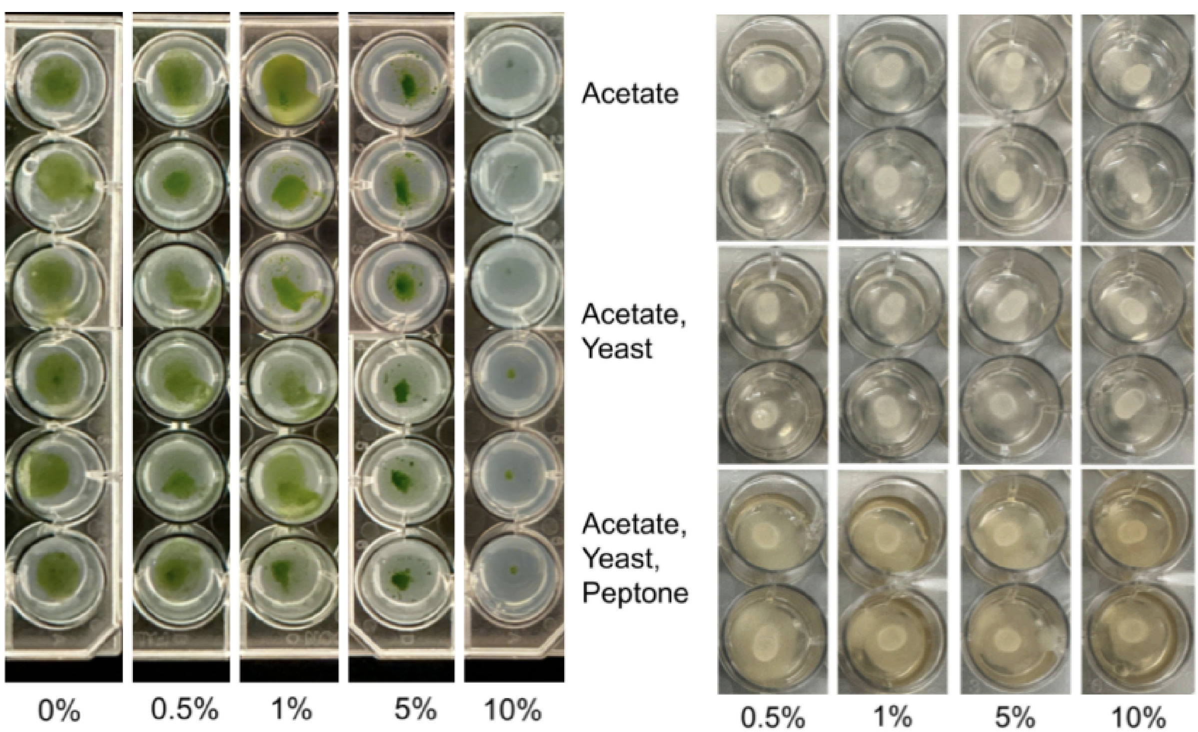


Figure 21: Growth of a Chlamydomonas reinhardtii *and* Aspergillus oryzae *on acetate. We assessed the ability for* C. reinhardtii *and* A. oryzae *to grow in the presence of acetate. We were unsurprised to find that* C. reinhardtii *can grow in the presence of acetate (0%-5%) as the*

TAP medium already contains acetate (left). We found that 10% acetate content significantly inhibited the growth of C. reinhardtii. *We found that* A. oryzae *can grow in the presence of acetate (0.5%-10%, right). We observed the most significant growth when the medium was supplemented with yeast and peptone.*

We then assessed the ability of a fungus to grow on algae as a direct carbon source (Fig. 22). We grew the fungus on algal-medium prepared from food-grade Chlorella vulgaris powder or dried *Chaetomorpha sp.* whole cell biomass. *Chlorella* medium was prepared by mixing a powder solution with combinations of 1% yeast, 2% peptone, and 2% dextrose. *Chaetomorpha* medium was prepared by baking whole cell biomass overnight, grinding with a mortar and pestle to obtain a coarse powder with large pieces of intact *Chaetomorpha* biomass, and resuspended in sterile water to make a 5% solution. Experiments were run for 5 days. We found that *Ganoderma sp.* can grow on *Chlorella vulgaris* powder as a carbon source. Conversely, fungal growth was significantly inhibited on the *Chaetomorpha* medium. Of note, the fungus grew on the *Chlorella* independently but grew denser when supplemented with other nutrients.

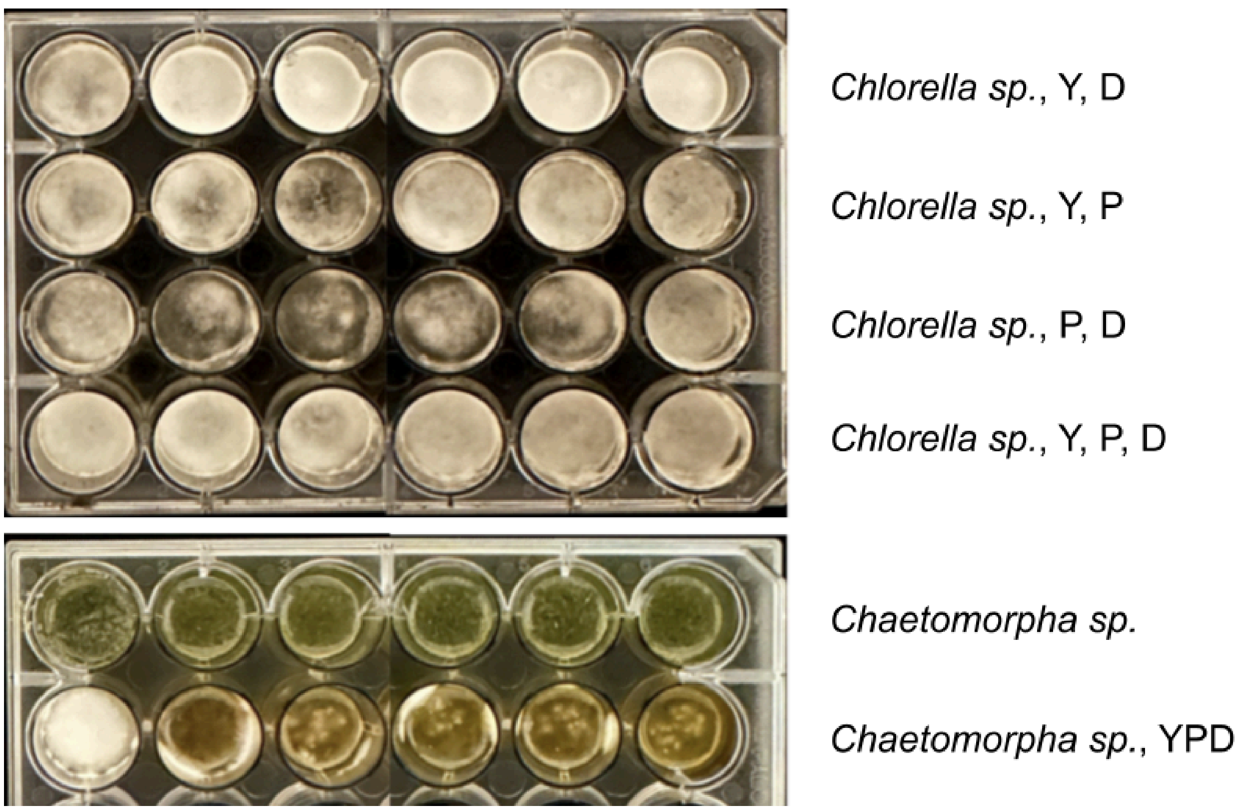


Figure 22. Growth of Ganoderma sp. *on algae as a sole carbon source. We grew a fungus on a food-grade powder of* Chlorella vulgaris *in combination with other nutrients, yeast extract (Y), dextrose (D), and peptone (P) (top). Our preliminary studies showed that* Ganoderma sp. *could not grow on* C. vulgaris *powder alone (data not shown) but was not significantly inhibited by the presence of* C. vulgaris *powder. We were unsurprised to find that the fungus exhibited significantly more growth in the presence of dextrose. Conversely, we found that the fungus*

could not grow on Chaetomorpha sp. *alone and was significantly inhibited by the presence of* Chaetomorpha sp. *when grown in nutrient rich YPD broth (bottom).*

Species of algae and fungi form symbiotic relationships in nature known as lichen. A symbiosis between a fungi and a photosynthetic organism, such as an alga, could reduce upmass costs and enable living materials that can self-regenerate. Since, lichens are notoriously hard to cultivate and grow very slowly, many groups have explored the establishment of synthetic symbiosis between fungi and algae.[25] To this end, we tested the ability of *Pleurotus ostreatus* and *Chlamydomonas reinhardtii* to grow in co-culture (Fig. 23). A first step toward establishing a synthetic symbiosis is to screen for fungus-alga pairs that can support further engineering and evolution through co-culture. To co-culture these two organisms it is necessary to determine a growth medium that supports the growth of both organisms. We grew the organisms in TAP media, used to culture *Chlamydomonas* and PDY: 2% dextrose, 0.1% potato starch, 0.15% yeast, and TAP + PDY (1:1), in broth and agar format. We found that *C. reinhardtii* was able to grow on all media combinations. We found that *P. ostreatus* was able to grow on PDY, as expected, and the TAP+PDY but not the TAP medium alone. These results indicate that *C. reinhardtii* and *P. ostreatus* can be co-cultured on PDY and TAP+PDY. Furthermore, the wells with both the alga and fungus are dominated by fungus in PDY and the alga in TAP. However, in PDY, the fungus overgrew the alga. We were able to prototype a high-throughput method for pairing fungi and algae that we intend to use to search for viable pairings and to engineer synthetic symbioses. Further studies are needed to determine the optimal strains, cell densities, media compositions, and environmental conditions necessary to establish a synthetic symbiosis between a fungus and an alga.

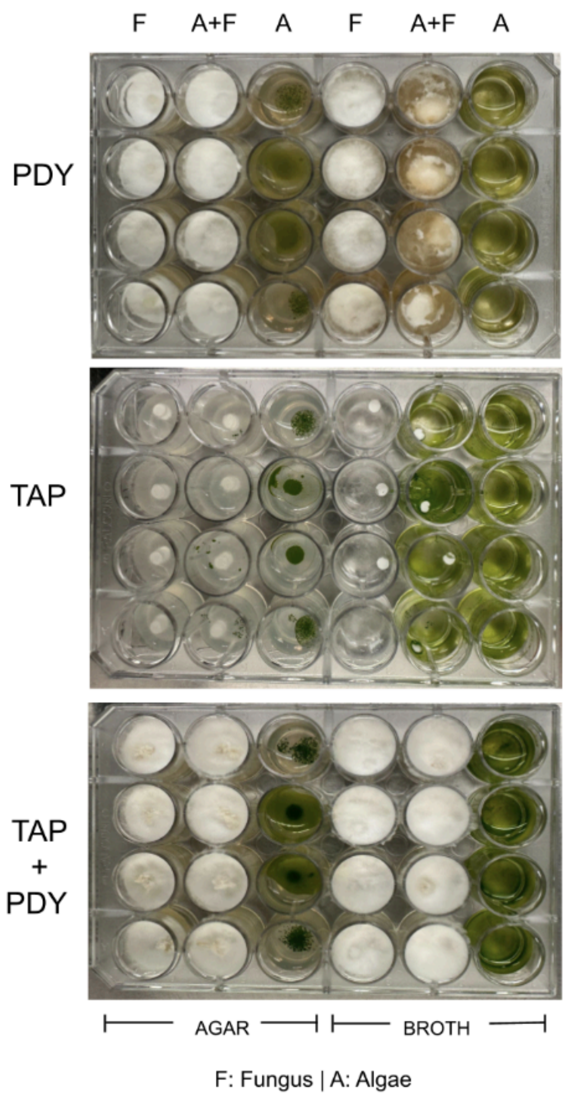


Figure 23. Co-culturing a fungi and algae to establish a synthetic lichen for engineered living materials and space exploration. We established a method for increased throughput screening of fungus-alga candidate pairs. We grew P. ostreatus *and* C. reinhardtii *individually and in co-culture on PDY, TAP, and TAP + PDY medium in both a broth and agar format. We found that the alga could grow on all media tested and that the fungus could not grow on TAP alone. Additionally, we found that the fungus overgrew the alga in co-culture with the exception of co-culture on TAP media.*

[25] Arjun Khakhar. 2023. A roadmap for the creation of synthetic lichen, *Biochemical and Biophysical Research Communications*, vol. 654, Pages 87-93, https://doi.org/10.1016/j.bbrc.2023.02.079

4.1.8 Regolith-mycelium composites

As part of her Ph.D. thesis at the University of Newcastle, Monika Lipińska focused on the production of regolith-mycelium composites. The traditional biomanufacturing process for creating mycelium-based composites involves growing mycelium on an organic substrate, for example, made of agricultural waste such as rice husks or straw. The mycelium grows into the substrate, forming a network of fibers that bind the substrate residual particles together. Often the mycelium and substrate mixture is molded into the desired shape and then allowed to grow and solidify into a dense, lightweight, and durable material. The growth process needs optimal conditions, such as controlled temperature and humidity, and the presence of oxygen. Mycelium-based composites are known for their unique properties, such as their biodegradability, fire resistance, and thermal insulation. Melanin-rich mycelium could provide radiation protection for the habitat, and the self-healing properties could help maintain the habitat structure in case of any structural damage. Mycelium-based composites are also highly customizable, as the growth process can be controlled to create different textures, shapes, and densities.[26]

As the mycelium can be grown in any quantity required, the use of mycelium for building martian habitats improves the flexibility and reliability of the building process. Our approach for the creation of the structural components (mycelium-based composites used for the construction of the habitat) in martian conditions utilizes the traditional biomanufacturing process, and it is adapted for resource-limited conditions (Fig. 24). This approach is a follow-up study to the "Regolith biocomposite" concept introduced within Biological growth as an alternative approach to on and off-Earth construction paper[27] (Fig. 25). The challenges associated with adapting the construction system to new, Martian conditions include physical and chemical aspects such as access to organic materials (biomass), access to oxygen, proper temperatures, etc. In this study, we are focusing on addressing only one of these limitations - the access to and the use of biomass - as we work on developing a proof of concept. However, in the future, all the limitations should be taken into consideration.

[26] Elsacker, E., Vandelook, S., Brancart, J., Peeters, E. and De Laet, L. 2019. Mechanical, physical and chemical characterisation of mycelium-based composites with different types of lignocellulosic substrates. *PLoS ONE*; Haneef, M., Ceseracciu, L., Canale, C., Bayer, I.S., Heredia-Guerrero, J.A. and Athanassiou, A., 2017. Advanced Materials from Fungal Mycelium: Fabrication and Tuning of Physical Properties, *Scientific Reports*, pp.1–11; Islam, M.R., Tudryn, G., Bucinell, R., Schadler, L. and Picu, R.C., 2017. Morphology and mechanics of fungal mycelium, *Scientific Reports*, 7(1), pp.1–12; Jones, M., Mautner, A., Luenco, S., Bismarck, A. and John, S., 2020. Engineered mycelium composite construction materials from fungal biorefineries: A critical review. *Materials and Design*, 187, pp.108397; Elsacker, E., Zhang, M. and Dade-Robertson, M., 2023. Fungal Engineered Living Materials: The Viability of Pure Mycelium Materials with Self-Healing Functionalities. *Advanced Functional Materials*, 2301875, pp.1–16.
[27] Brandić Lipińska, M., Maurer, C., Cadogan, D., Head, J., Dade-Robertson, M., Paulino-Lima, I.G., Liu, C., Morrow, R., Senesky, D.G., Theodoridou, M., Rheinstädter, M.C., Zhang, M. and Rothschild, L.J., 2022. Biological growth as an alternative approach to on and off-Earth construction. *Frontiers in Built Environment*, pp.1–17.

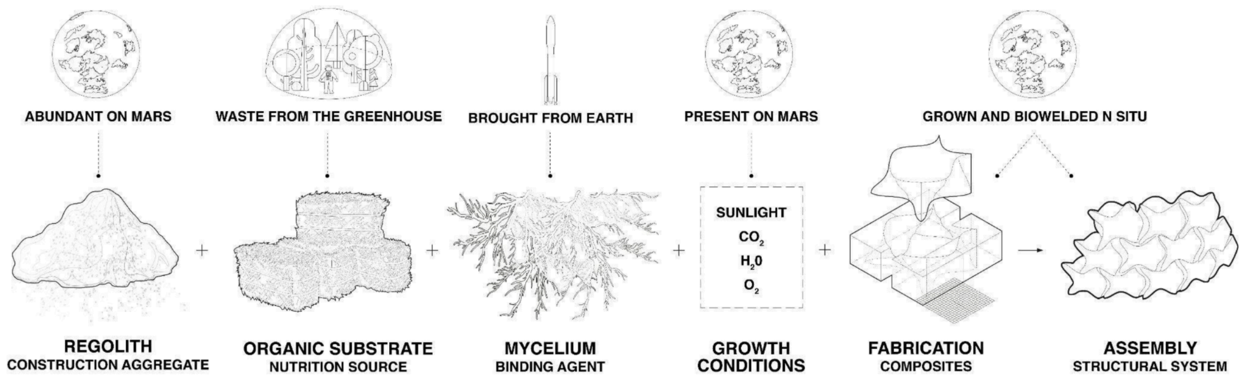


Figure 24. Components needed to build a mycelium-based structure on Mars

In addition to its abundance, utilizing the inorganic surface material (regolith) as the primary mass of the components to create hybrid composites provides further benefits. Composites created using inorganic materials have the potential to last longer than traditional mycelium-based composites, With traditional organic biocomposites, the growth of the mycelium has to be stopped at a certain point, when the mycelium network is sufficiently developed for the whole composite to be strong enough, but also the organic material giving structural strength is not digested to the point that it weakens the structural performance of the component.[28] In the case of creating mycelium-based regolith composites, with a predominantly inorganic material, the mycelium can be kept alive, without the risk of it digesting the whole substrate and weakening the strength. These regolith biocomposites can be dried and later reactivated (e.g., by increased humidity) to promote further growth, enabling other functions and properties like bio-welding, or biosensing.

[28] Elsacker, E. Vanden, 2021. "MYCELIUM MATTERS: An interdisciplinary exploration of the fabrication and properties of mycelium-based materials," *VUBPRESS*.

Figure 25. Cylindrical mycelium-sand composites, comprising 85% w/w sand to biomass (mycelium pre-grown on beechwood). Left: A basic cylindrical column, Right: A cylindrical column with an arch, exploring the feasibility of introducing openings and diverse shapes into the components.

4.2 Determine time, conditions and infrastructure for deployment based on prototypes.

4.2.1 Deployment parameters based on silicon prototypes

The silicon prototypes produced by redhouse (section 4.1.3; Fig. 3) were used as a basis for these estimates. Full colonization by *G. lucidum* occurs within 60 days, with some indication that other species may be faster at colonization. The material tests at Stanford demonstrated that the materials are stronger at 30 days than 60 days. We hypothesize that this is because there is more woody material left at 30 days which contributes to the strength. Interestingly, strength increased from 60 to 120 days but 30 was still the strongest. We do not have data on the insulation value or radiation attenuation at 30 / 60 / 120 days yet but this would be a great experiment for phase iii. These are ultimately the metrics we need as the structural performance of our design is in the fabrics.

The melanin-rich fungi on agar would take 7-30 days to fully colonize layered cells. Fig. 26 shows a partially colonized silicon model.

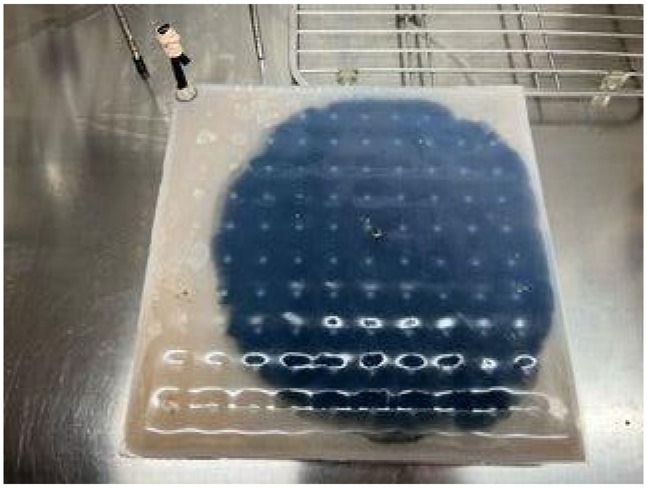


Fig. 26. Black fungi colonizing agar in a sealed silicone architectural model.

Moonprint Solutions (David Cadogan) suggests fabricating the inflatable with polyurethane coated drop-thread material. The edges of the drop-thread will be closed with a polyurethane coated Nylon fabric. Moonprint will seal polyurethane fittings to the inflatable to connect it to hoses that feed the reaction. They have not completely worked out the flow channels for water and gasses to support the bioprocess, but favor tubes inside the drop-thread. The tubes would have a permeable wall to support transmission of molecules while still containing the fluid mass. However, a multilayer insulation blanket and external cover that provides environmental protection will be required. Moonprint Solutions has conducted tests indicating that a fluoropolymer coated fabric would be appropriate. Essentially this describes the construction of a space suit. The fluoropolymers are capable in high and low temperatures and provide excellent dust resistance.

4.2.2 The assembly of the regolith-mycelium composites: growth of components

To investigate mycelium growth on the inorganic substrate, a series of experiments were conducted that proved it is a viable approach. The main observations are described in the next section - Scales of interactions. The conducted experiments aimed to determine whether mycelium can grow on inorganic aggregate, with minimal addition of nutrients (usually between 10-15% of the mass of the biocomposites), and whether mycelium can bind the loose aggregates together, creating stable structures. The aggregate used in the experiments was fine silica sand at 250-300 µm. The fungal species used in the experiments was *Ganoderma lucidum*. The mycelium spawn was first populated on Petri dishes containing malt extract agar and later grown on sterilized beechwood and millet in filter bags, allowing for fresh air exchange whilst keeping contaminants out. The selected source of nutrients for the initial experiments was malt extract, millet, and 0.2-1.25 mm beechwood sawdust. The experiments initially took place on Petri dishes allowing for an understanding of material interaction. Transitioning to larger-scale - columns made out of acrylic molds (diameter of 9 cm, height of 18 cm) allowed for exploration of the biocomposites fabrication process and its characteristics. Finally, more complex shapes created with 3D-printed molds were tested for composite bio-welding properties and structural interactions.

During the experiments, moving across different scales from Petri dishes, columns, and more complex forms, three types of processes were observed - mycelium internal network, surface binding, and bio-welding. Each of these observations was made at different stages of project development and brings certain qualities to the developed system. The first process (Fig. 27.1)

- internal network - is on the material level and was explored during the Petri-dish experiments. The mycelium hyphae, which are life-sustaining filamentous, branching filaments, grow in between the sand grains, surrounding them and binding individual sand particles together. This interaction provides stabilization of the component, transferring loose aggregate into a consistent form. The second process (Fig. 27.2) - surface binding - is on the composite level and was observed with the column experiments. Mycelium creates a hyphal tissue on the surface of the composite, providing graded surface binding. It holds the composite despite the density of the mycelium internal network and strengthens it. The third process (Fig. 27.3), bio-welding, is on the structural level. The structural strength depends on the size and shape of the biocomposites, the interaction between them (force distribution), and the bio-welding process.

Figure 27. Three scales of the interaction.

From the empirical analysis of the samples, it is visible that the mycelium network is the densest on the top of the sample, where it had the most exposure to oxygen. However, in the successful specimens, the mycelium network is observable throughout the whole structure. The microstructural imagery shows the interaction between mycelium hyphae and the sand particles; the mycelium hyphae tangle around the sand grains, creating a dense "net" which is holding the sand particles together.

Mycelium-based regolith composites are not homogeneous structures. The biocomposites are mostly held by a mycelium layer that is created on the surface due to water condensation and contact with oxygen. The mycelium layer is also not homogeneous; it is thicker in some places and thinner in others. Below this layer, inside the composite, the mycelium network is much less developed. However, the composite keeps the structural integrity. Since the mycelium network doesn't need to be grown evenly within the whole biocomposites to keep the load-bearing capacity, the creation of the surface binding contributes to the aim of minimizing the biomass needed to produce biocomposites. In the approach where the mycelium layer is developed on the surface but not that much inside, the biocomposites could also enable the creation of bigger components, normally limited due to oxygen availability. Additionally, it could be interesting to further investigate the compression strengths of the components and the mycelium outer layer specifically.

The process of bio-welding (Fig. 28) dictates requirements for establishing the geometry of the components (for example to promote adhesion, the elements should have maximized surface-to-surface contact area) as well as growth conditions. The bio-welding process can be accelerated by adjusting the conditions in the controlled environment (growing chamber) such as temperature, humidity, and nutrient availability [1],[29] both in laboratory conditions, as well as in an enclosed chamber deployed on Mars. Such a growing chamber would promote faster mycelium growth at the same time providing protection from any forward and backward contamination (planetary protection).

The process of bio-welding is intricately connected to two other processes: internal network formation and surface binding. The ability to achieve a certain sectional depth, which is dependent on the growth limitations of mycelium, determines the possible size and shape of the final product. As a result, all three processes have interdependent requirements and are integral components of structure creation.

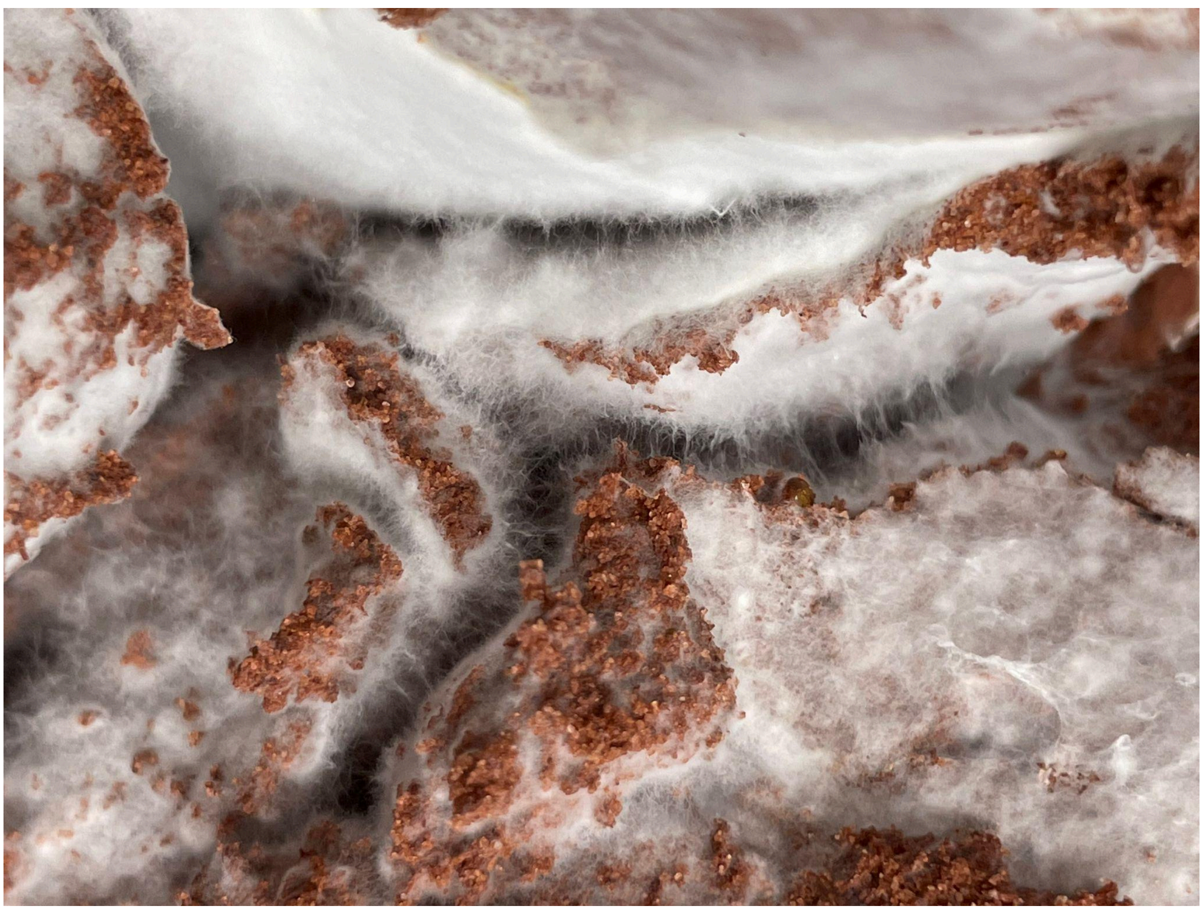


Figure 28. Mycelium bioweIding separated parts of broken composite.

[29] Modanloo, B., Ghazvinian, A., Matini, M. and Andaroodi, E. 2021. Tilted arch; implementation of additive manufacturing and bio-welding of mycelium-based composites. *Biomimetics*, 6(4).

4.2.3 The assembly of the regolith-mycelium composites: biowelding

In the first phase of the construction process, the assembly, the elements would be held together by their geometry, which is designed to allow for the jamming of the elements, either with temporary scaffolding or without the need for scaffolding. As the components come into contact, they begin to stack together, forming a system of increasing size and complexity. The jammed elements would form a habitat shell structure, shielding the interior from harsh martian conditions and providing protection from physical damage and harmful radiation. By aleatory assembling (dropping or piling) pre-grown structural elements, the process of assembling the structure would conserve energy due to the elimination of the requirement for intricate assembly and complicated robotic operation. In case of any errors during the assembly process, the "dropping" robots could do manual adjustments to the structure, however, the process is envisioned to have a high tolerance for failure, due to the aleatory nature of the assembly process. The reduced gravitational pull in the environment could enable the assembly of larger structures without the need for external intervention or detailed assembly operations. The dropping and self-assembling of the elements would be the first phase in the construction process, followed by consolidation of the structure through the process of bio-welding.

The consolidation of the jammed elements forming a structure is performed by bio-welding. Bio-welding is a process in which the natural bonding properties of mycelium create strong, cohesive joints between two or more pieces of mycelium-based materials. The process involves the continuation or initiation of mycelium growth within the composites, along with the growth of mycelium on the surface of the components. This enables the fusion of adjacent composites through the expansion of mycelium (Fig. 29).

Figure 29. Bio-welded mycelium-sand composites. Right: Preliminary bio-welding trials involving interlocking mycelium-sand components (85% w/w sand to biomass, with mycelium pre-grown on beechwood), cultivated outside the molds. Left: mycelium-sand cylindrical components of identical inorganic-to-organic ratio, cultivated within the molds, resulting in a more robustly developed mycelium layer

With the bio-welding process, the aleatory-assembled composites over time would grow together, creating solid and potentially sealed structures, providing stable structural shells for habitat and other surface structures.

To find the optimal geometry for the structural composites, three main aspects were identified that must be considered: 1) The Bio-welding process, 2) The Assembly process, and 3) The Fabrication process (Fig. 30).

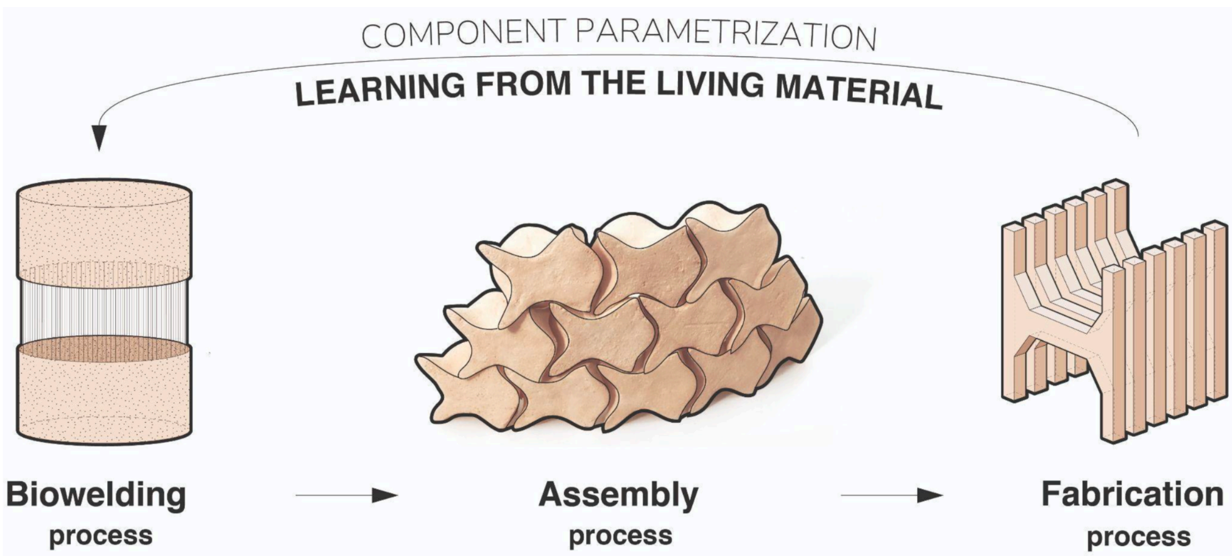


Figure 30. Component parametrization. Three aspects must be considered when looking for the optimal shape for the structural biocomposites.

The bio-welding process includes all geometrical constraints derived from the biological aspects of the creation of the mycelium-based regolith composites. The examples of the parameters include the maximum size of the composite, driven mainly by the need for oxygen, and the optimal volume-to-surface area ratio. On top of that, this aspect includes the constraint derived from the bio-welding process, like maximizing the contact area, or the quality of the contact surface (smooth vs rough, etc).

The second aspect of finding the optimal geometry is derived from the aggregation and assembly process. The geometry of the structural elements should allow for the stacking and jamming of the elements leading to the self-assembly into the complex structural system, as opposed to requiring precise assembly operations. This aspect includes a study of the connectivity between elements, points of contact vs surfaces of contact, and the general force distribution. It also includes the analyses and simulations of the behavior of the construction elements in lunar or Martian gravity conditions. The last aspect is considering the fabrication of the biocomposites. The objective of the entire construction process is to conserve energy and resources while maximizing automation.

Constructing the entire habitat structure requires a significant number of elements, and therefore, the composite fabrication should be also optimized and suitable for mass production. For example, complex geometries with multiple axes of interlocking can complicate the de-molding and therefore fabrication process. Additionally, the properties of the material itself dictate certain constraints on the geometry, for example, the biocomponents cannot be too thin. All three aspects are equally important in the development of the optimal geometry for the construction seeds.

4.3 Identification and analysis of potential enhancements to the mycelia.

We envisioned enhancements to the mycelial structure. The capabilities of the mycelia could be developed using bioengineering augmentation; for example, production of useful polymers will be considered. We envisioned a future that includes the addition of cyanobacteria that can produce oxygen, bacteria that can provide sensing capabilities (e.g., sensing of environmentally relevant gases like oxygen for crew health support), and decoration of the mycelia with proteins for assorted chemical transformation functions. Enhancements such as these can result in "living architecture" in the true sense of the word,because of sensitivity and near real-time adaptability to internal and external environmental changes.

<u>Electromagnetic radiation shielding through the use of melanin.</u> In Phase 1 we proposed that using natural melanin-producing strains would aid in the absorption of UV radiation as melanin absorbs UV radiation.[30] This work is reported above in section 4.1.6.

<u>Embedded biosensors.</u> With the addition of *B. subtilis* to the mycelium biocomposite, we envisioned a straightforward path to an embedded biosensor. To that end, Co-I Anil Wipat proposed to bio-engineer *B. subtilis* and test it for sensing capabilities of O_2 concentration, light and pressure, all of which would be useful for assessing habitat integrity. Unfortunately, health issues resulted in retirement so this work was not pursued. However, the work presented here testing the ability of *B. subtilis* to grow with the fungal mycelia has put the team in an excellent position to continue to pursue this goal (see section 4.1.7)..

<u>Metal binding</u>. We developed a copper-binding biofilter from mycelia by linking a metal-binding domain - chitin binding domain (CBD) fusion protein to mycelia. Our prototype removed >92% of the copper from a >300 µM aqueous solution within 30 min. One patent was filed,[31] and a paper published[32] on the filter, work which leveraged results from Rothschild's

[30] Dadachova, E., Bryan, R.A., Howell, R.C., Schweitzer, A.D., Aisen, P., Nosanchuk, J.D. & Casadevall, A. 2007. The radioprotective properties of fungal melanin are a function of its chemical composition, stable radical presence and spatial arrangement. *Pigment Cell Melanoma Res*. 21: 192–9.
[31] "Functionalizing biological substrates with bioengineered peptides to bind targeted molecules for utilization in water filtration applications" U.S. Patent Appl. No. 17/073,226, filed 10/16/20
[32] Urbina, ... & Rothschild, L.J. 2019. Urban biomining: A new approach to bioengineering surfaces for reclaiming and recycling metals from e-waste. *Scientific Reports*, Group 9:16422

prior Phase I NIAC "Urban biomining meets printable electronics" (2017). Towards the end of the period of performance of the Phase II alternate funds were awarded to continue this work.

4.4 Determination of effect of two week lunar and martian simulation on the materials.

The stability of the mycelium-based biocomposites under Mars and lunar conditions were tested in the Planetary Simulator in the Origins of Life Laboratory at McMaster University, Canada under the direction of Co-I Maikel Rheinstadter. The planetary simulator (Angstrom Engineering, Hamilton, Ontario, Canada, Fig. 31) is a one-of-a kind, custom-built environmental chamber that allows for the simultaneous control of temperature (-30°C to 130°C), relative humidity (0 – 100% RH), radiation from UV to far infrared (185 – 1,000 nm), pressure (0.8 – 760 Torr), and gas composition. The simulator is equipped with light bulbs (30 W/m^2) that produce UVC and UVB radiation and LED arrays (300 W/m^2) capable of generating radiation in the UVA, visible, infrared, and far-infrared range. The atmospheric gas composition may consist of up to four different gas species that are introduced into the chamber through gas lines. The relative humidity is controlled by splitting the gas flow into two paths: 1) one fraction of the gas is bubbled through a water reservoir where it undergoes gas humidification to introduce "wet" gas into the chamber and 2) the remaining "dry" gas flows directly to the chamber. All parameters can be controlled simultaneously by a computer interface through the Aeres integrated recipe driven software platform. The recipes can be set up to mimic cyclic changes in all parameters to simulate, for instance, day-night and seasonal changes.

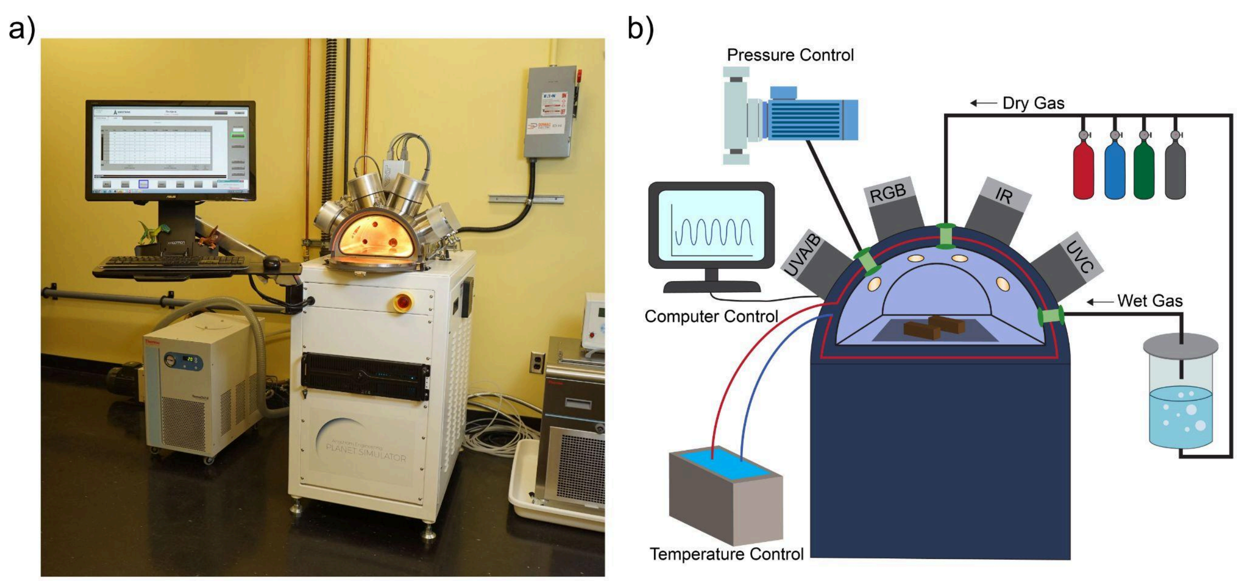


Figure 31. The planetary simulator is an environmental chamber capable of simultaneously controlling temperature, humidity, pressure, radiation, and atmospheric composition. (b) Schematic of the planetary simulator. The inner volume of the chamber is about 0.011 m^3 (0.4 ft^3.) All parameters are controlled simultaneously by a computer interface.

Stability testing was done on baked and unbaked *Ganoderma* pre-grown on millet (10% of mass) grown on sand (90% of mass). All samples were cut into 1 cm x 7 cm blocks, as shown in Fig. 32a and b. When exposed to vacuum, as shown in Fig. 32c, there is an initial, rapid increase in the humidity inside the planet simulator indicating a residual water content. There was also a steady, linear increase of chamber pressure during the 24 h of the experiment that can be attributed to a slow degassing process over time. Fig. 32d shows a photo of the samples in the simulator under UV exposure. The degassing can be seen in part e.

To investigate the response of the biocomposites to exposure to UV radiation and different temperatures, samples were exposed to UVA, UVB, and UVC in the planetary simulator under vacuum conditions (1 Torr) at different temperatures. The surface temperature of Mars ranges from -153 °C to 20 °C. To achieve the lower extremum, samples were exposed to liquid nitrogen at -196 °C in one of two experimental conditions: 1) samples were completely submerged in the liquid nitrogen for 3 days or 2) samples were dipped in and out of the liquid nitrogen for a total of 10 cycles.

Additional samples were placed in the planetary simulator for 3 days at either -20 °C or 30 °C under vacuum conditions with exposure to UVA, UVB, and UVC. All samples were imaged using a Nikon Eclipse LV100 ND microscope equipped with a Nikon DS-Ri2 camera with a resolution of 4908 x 3264 pixels and pixel size of 7.3 x 7.3 μm.

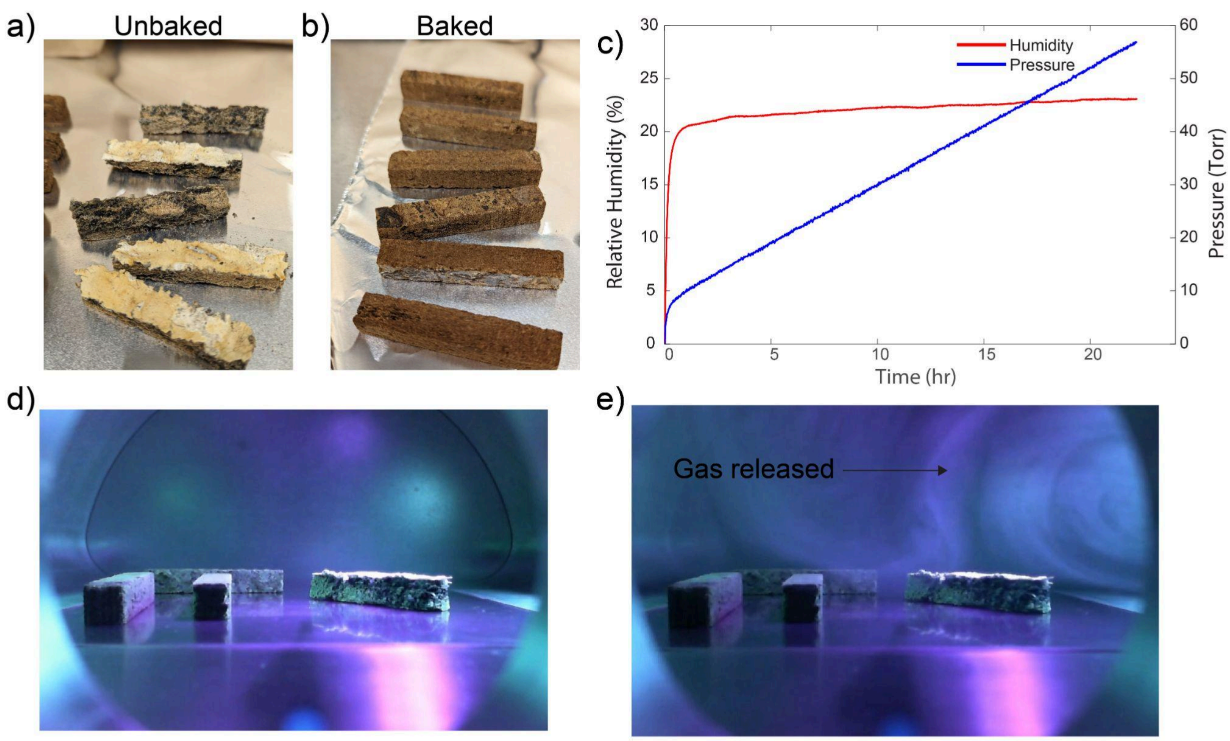
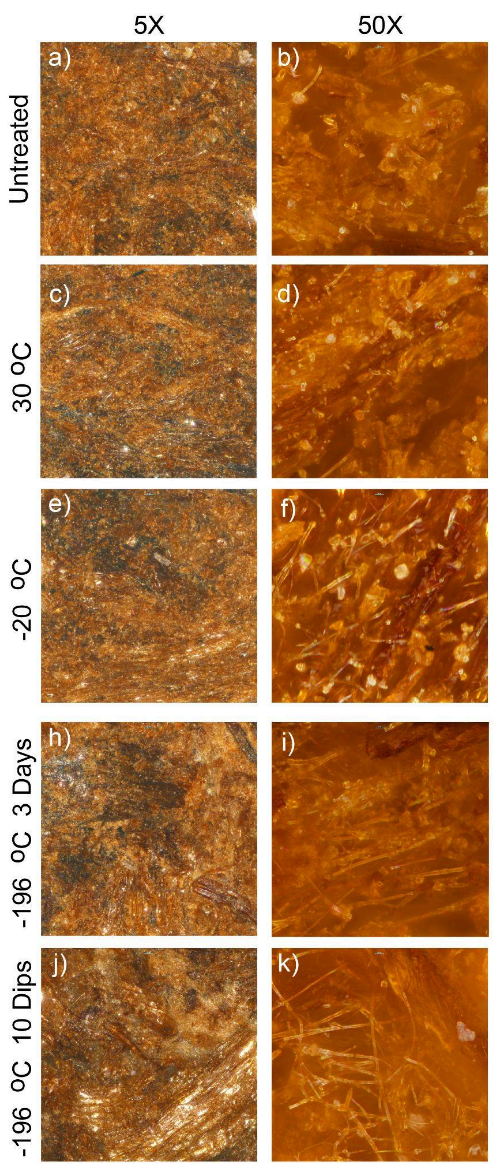


Figure 32. The effect of vacuum and UV radiation on Ganaderma. (a) Unbaked and (b) baked Ganoderma *pre-grown on millet (10% of mass) and grown on sand (90% of mass). (c) Mycelium based biocomposites were placed in the planetary simulator for 24 h under vacuum conditions and exposure to UVA, UVB, and UVC. The change in humidity and pressure was recorded over*

simulation time. (d) Samples in the planetary simulator at 0 h. (e) Samples undergo degassing upon exposure to UV radiation.

The resulting images of the mycelium-based biocomposites following treatment for all samples are shown in Fig. 33. The untreated sample in Figure 33a at 5x magnification shows a relatively uniform surface of the biocomposite. Hyphae become visible at a higher magnification of 50x in 33b and show a random orientation. After 72 h of exposure to UV radiation at 30 °C (Fig. 33 c and d), the surface appears to be more ordered with the hyphae taking a preferred orientation. While UV exposure at a lower temperature of -20 °C appears to have no structural effect on the biocomposites at low magnification (5x) (Fig. 33 e), the hyphae appear to be "cut" at higher magnification.

Exposure of the biocomposites to constant very low temperatures of -196 °C seems to lead to a more ordered and pronounced surface with pronounced hyphae shown in Fig. 33 h and i. Cycling between -196 °C and room temperature, however, led to twisting and breaking of the hyphae under high magnification (Figure 33 j and k).



UV radiation and temperature seem to have distinct effects on the biocomposites. While the effect of UV radiation appears to be local damage to the hyphae leading to breaking and cutting, temperature cycling seems to lead to more mechanical damage including twisting and stretching of those structures. We note that the effect of UV radiation appears to be larger at lower temperatures and that the hyphae can somehow "heal" and compensate for the UV induced damage at higher temperatures.

Figure 33. Microscope images of all samples. Images were taken under brightfield at 5x and 50x magnification. While the untreated sample (a and b) shows a uniform surface, hyphae become visible at high magnification. UV radiation at 30 °C seems to have an ordering effect on the surface and hyphae in c and d. Damage to hyphae is visible in e and f when exposed to UV radiation at low temperatures of -20 °C. While a constant low temperature of -196 °C in h and i led to a more ordered and pronounced structure, mechanical damage is visible in j and k after cycling between -196 °C and room temperature for 10 times.

4.5 Measure mechanical properties of prototypes before & after exposure to the planetary simulator.

4.5.1 Background

Understanding the mechanical properties of mycelium bio-composites (myco-composites) is crucial to understanding how the material can withstand the test of space. Thus mechanical testing of the materials was conducted at Stanford University under the direction of Co-I Debbie Senesky. Five different experiments were conducted for this report.

<u>1. Understanding environmental effects on myco-composites.</u> We looked at how liquid nitrogen and UV radiation affected the mechanical properties of myco-composites. *Ganoderma lucidum* was our mycelium strain and a mix of sawdust as our substrate, to create the myco-composites at redhouse studio. Once the myco-composite was grown (30 days of growth on hardwood sawdust), we allowed some of the specimens to dry in air (from 60% hydration to 5-10% hydration) to expel excess water. The other specimens were compressed and baked at high temperature and pressure (10 tons over 300 cm^2 baked at 160 °C). All were cut into smaller samples. Then the samples were sent to McMaster University for the environmental tests (as described in section 4.4) before being sent to Stanford University for mechanical testing.

<u>2. Understanding growth period effects.</u> In this experiment, we looked at how changing the growth period affected the mechanical properties of myco-composites. We used the same mycelium strain and substrate as in the previous experiment, to create the myco-composites at redhouse studio. However, we looked at three different growth periods: 30, 60, and 120 days. The specimens were then compressed and baked at 160 °C. They too were cut into smaller samples, both perpendicular and parallel to the direction of compaction, to understand myco-composites' anisotropic mechanical properties. The samples were then sent to Stanford University for mechanical testing.

<u>3. Understanding humidity effects using synthetic Martian regolith.</u> In this experiment, we looked at how humidity affects the growth of mycelium in myco-composites and its mechanical properties. We used the same mycelium strain, but this time used a mix of silica-based sand, iron oxide, flour and beechwood pre-grown with mycelium, to create the myco-composites at Newcastle University by Monika Lipińska. Sets of specimens were either grown at a lower (25%) or higher humidity (50%). Once grown, they were sent to Stanford University for mechanical testing.

Sample preparation:

- The mycelium (spawn) was mixed with autoclaved beechwood (beechwood + distilled water (150% mass of dry beechwood)) and grown for 7 days in growing chamber (Temperature between 24-27°C)
- The pregrown biomass (beechwood-mycelium mix) was blended (to make sure mycelium is distributed evenly) and mixed with autoclaved sand, flour and iron oxide (see Table 1)..
- The mixture was put into acrylic molds and kept in a growing chamber for 10 days of growth.

- High humidity: When demolded, the samples were kept in a growth bag, featuring a 0.2 micron filter patch to allow FAE (fresh air exchange) whilst keeping contaminants out, for an additional 2 days (ensuring higher humidity environment).
- The samples were air-dried. To make sure they were dry, according to the standard, if the weight of the sample didn't change more than 0.01% over 24h it means the sample was dry.

Diameter [cm]	Height [cm]	Volume [cm^3]	Inoculation	Biomass [g/1000g of sand]	Water content [g/1000g of sand]	Nutrition Additive: Flour [g/1000g of sand]	Iron Oxide g/1000g of sand	Starting density [kg/m^3]	Temperature in growing chamber
7.5	15	662.3	spawn	200	80	10	10	1.4	24-27C

Table I. Summary of martian regolith sample preparation described in this section.

4. Understanding lunar regolith effects

In this experiment, we looked at how using lunar regolith as a substrate affected the mechanical properties of myco-composites. We also used *G. lucidum* spawn (catalog #)M9726 from Urban Farm). Three sets of samples were looked at: (1) samples filled with only lunar regolith simulant, (2) samples filled with lunar regolith and agar as the nutrient source, and (3) samples filled with regolith and agar, dipped in *G. lucidum* fungi. These specimens were grown at NASA Ames before they were sent to Stanford University for mechanical testing.

5. Understanding fungal species, substrate, and environmental effects (preliminary tests)

In this experiment, we tuned different parts of the production and post-treatment to understand how they each affect the mechanical properties of myco-composites. In our initial batch, we looked at two different types of fungal species, *Pleurotus ostreatus* and *Ganoderma lucidum*, both grown on sawdust at NASA Ames. Once grown, we did compression tests with no post-treatments to have a baseline of their properties at Stanford. We then sent additional samples to McMaster to undergo the same environmental tests as in Experiment 1. Finally, those samples were sent to Stanford for mechanical testing whose results were compared with the samples not given a post-treatment.

4.5.2 Method

For all of the above experiments, we performed uniaxial compression testing on the myco-composites using Stanford University: Soft Materials Facility's Instron Machine 5560. Compression testing measures fundamental parameters that determine specimen behavior under compressive load. As the Instron compresses the specimen at a uniform rate, it collects data on both the force exerted on the load and the overall deformation. Though the specimens of each experiment had different dimensions, we attempted to keep the Instron's strain rate (the rate at which the specimen is being deformed) at 1% of length. We also attempted to keep the height-to-width ratio at a minimum of 2:1[33]. We were then able to create stress/strain curves from that data using the following equations:

$$\textit{Strain}, \varepsilon_c, \textit{load deformation}:$$

$$\varepsilon_c = \frac{\Delta l}{l_i}$$

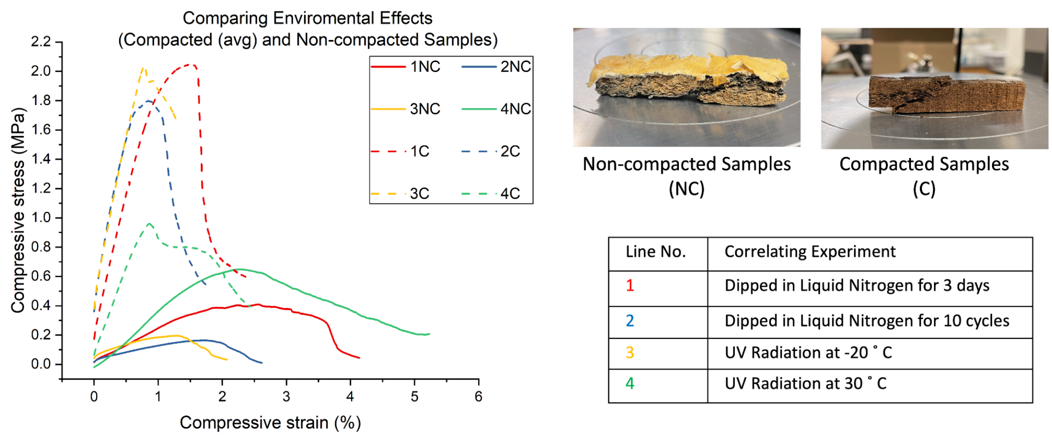
$$\textit{Stress}, \sigma_c, \textit{force applied to specimen per area}:$$

$$\sigma_c = \frac{F}{A}$$

Where the change in length is the specimen's initial length, F is the force exerted, and A is the cross-sectional area at which force is being exerted.

4.5.3 Results

1. Understanding environmental effects on myco-composites



Line No.	Correlating Experiment
1	Dipped in Liquid Nitrogen for 3 days
2	Dipped in Liquid Nitrogen for 10 cycles
3	UV Radiation at -20 ˚ C
4	UV Radiation at 30 ˚ C

Figure 34. (a) Stress-strain curves for each environmental test, curves for compacted, C, samples were averaged to produce curves in this figure. (b) Images of non-compacted, NC, and compacted, C, samples after compression tests. (c) Description of each environmental tests and its corresponding numbers on the stress-strain curves.

Fig. 34 compares the stress/strain curves for each of the environmental tests and for compacted and non-compacted (NC) samples. The overall compressive strength, as well as Young's modulus (E-modulus) was higher for the compacted (C) samples. This is mainly due to the fact that those samples are much denser than the non-compacted. For these compacted samples, we can see that the overall compressive strength and E-modulus were significantly higher for the sample that sat in colder temperatures for long periods of time (i.e., dipped in

[33] Houette, T., Maurer, C., Niewiarowski, R., and Gruber, P. 2022. Growth and Mechanical Characterization of Mycelium-Based Composites towards Future Bioremediation and Food Production in the Material Manufacturing Cycle. *Biomimetics*, 7(3).

liquid nitrogen or radiation at -20 ˚C). For the non-compact, the opposite occurred, as the graph shows higher strength and E-modulus.

2. Understanding growth period effects

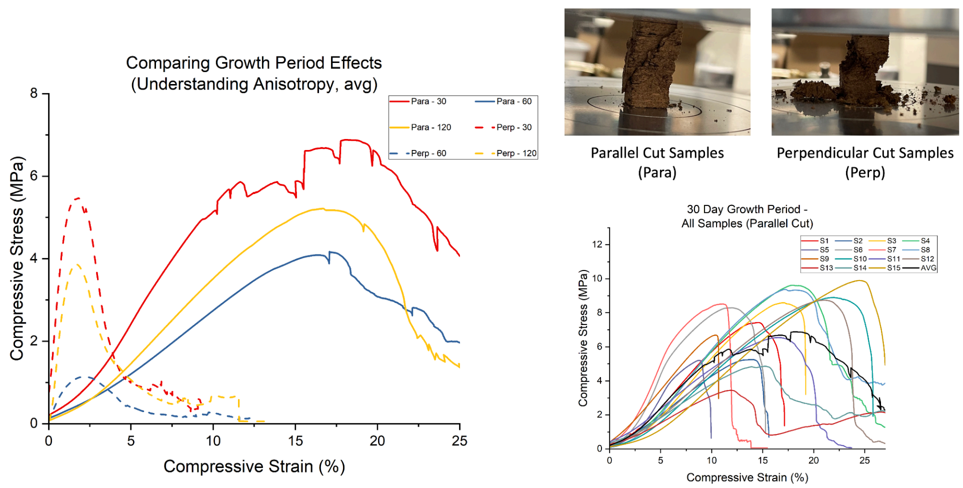


Figure 35. (a) Averaged stress-strain curves for 30, 60, and 90 day growth, for both parallel and perpendicular cuts. (b) Images of parallel cut and perpendicular cut samples after compression tests. (c) Stress-strain curves for all 15 samples grown for 30 days.

Fig. 35 compares the stress/strain curves for varying growth periods, as well as how anistropy factors into the myco-composite's mechanical properties. Regardless of the directional cut, we notice that samples grown for 30 days have the largest overall strength and E-modulus, while samples grown for 60 days had the lowest. We also found that while samples cut parallel to direction of initial compaction had higher E-modulus, sample cut perpendicular had noticeably higher ultimate compressive strengths.

3. Understanding humidity effects

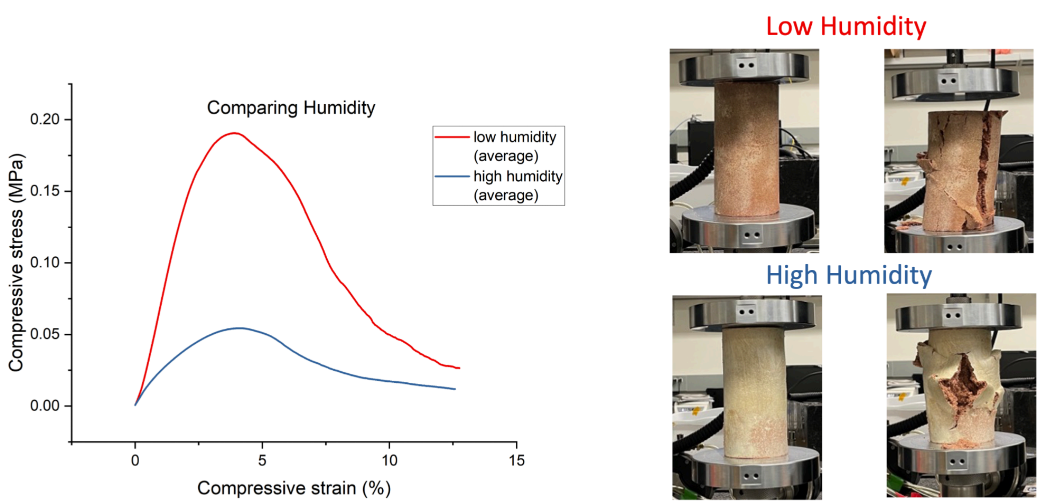


Figure 36 (a) Averaged stress-strain curves for low and high humidity growth using synthetic Martian regolith. (b) Images of low and high humidity grown samples, before and after compression tests.

Fig. 36 compares the stress/strain curves of our high- and low-humidity samples. Overall, we can see that low-humidity samples are tougher, stiffer (with their higher Young's modulus), and have a larger compressive strength, while the high-humidity samples are more ductile.

4. Understanding lunar regolith effects

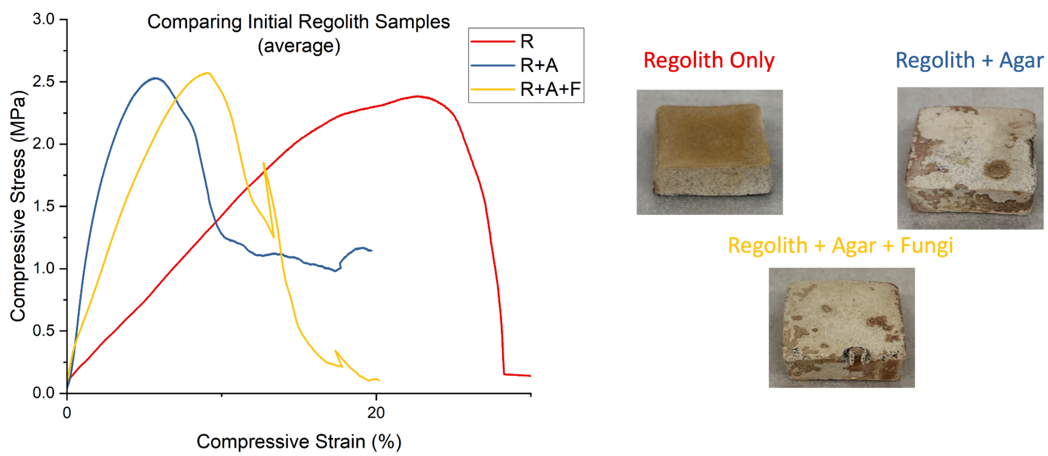


Figure 37. (a) Averaged stress-strain curves for samples grown using lunar regolith. (b) Images of each sample before compression tests corresponding to the curves.

Figure 37 compares the stress/strain curves of our lunar regolith samples. Each of the curves above come from an average of 5 samples. Though variations between the ultimate compressive strength are not drastically different, we still see that the samples dipped in fungi have a slightly larger strength and a larger E as well.

5. Understanding fungal species, substrate, and environmental effects (preliminary tests)

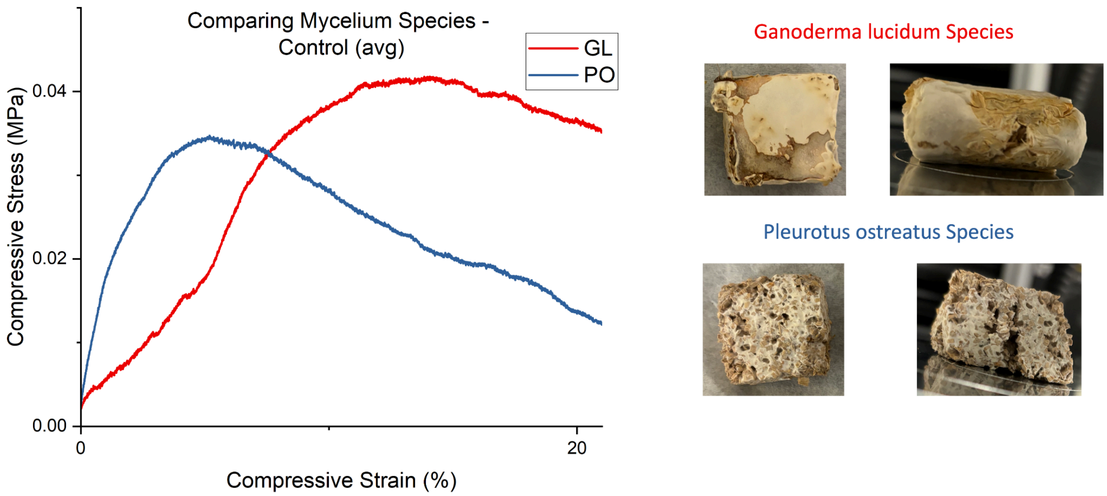


Figure 38. (a) Averaged stress-strain curves for Ganoderma lucidum *(G-l) and* Pleurotus ostreatus *(P-o) samples. (b) Images of respective samples, before and after compression tests.*

Fig. 38 compares the stress/strain curves of our different fungal species grown and not yet exposed to radiation or cold temperatures. Again, although variations between the mechanical are not drastically different, we still see that the G-l has a larger ultimate compressive strength while P-o has the larger E-modulus. It is important to note that the growth conditions were not identical between both species.

4.5.4 Discussion

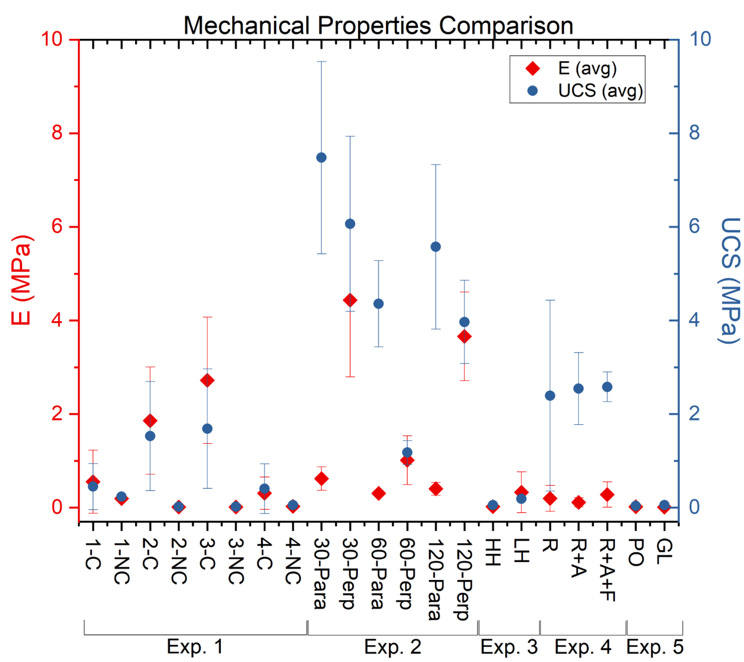


Figure 39. A comparison of mechanical properties (Young's modulus, E, and Ultimate Compressive Strength, UCS) between all five experiments.

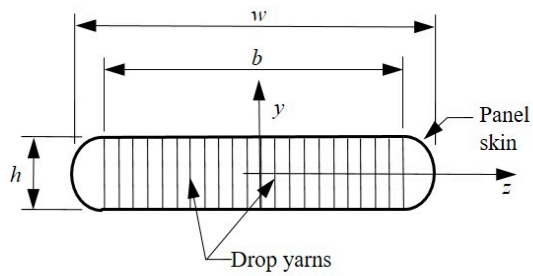
Figure 39 is a plot and table comparing some of the mechanical properties for all five experiments. For most of our experiments, the E-modulus tends to be within 0.5 MPa of each other. The exceptions to this are for samples that were compacted then exposed to cold temperatures (Experiment 1: 1C, 2C, 3C), and for samples that we compacted, then cut perpendicular to the direction of compaction (Experiment 2: 30-Perp, 60-Perp, 120-Perp). *The ultimate compressive strength for experiments varied a lot more, though samples from Experiment 1 had significantly higher strength, due to compaction, and therefore increase in density. Further, it is interesting to note the ultimate compressive strength of the samples in Experiment 4 (lunar regolith substrate) had higher strengths than that of Experiment 3 (synthetic martian substrate), most likely due to the grain-size of lunar regolith being larger than martian regolith.*

4.6 Detailed mycelium-based lunar habitat designs with tradeoffs and subsystem considerations.

To be able to grow a building like a living organism, we look to nature for examples. This process of learning from nature to complete engineering goals is called biomimicry. Biological systems are incredibly complex and work at many scales, from the nanoscopic conversion of ATP to ADP within cells that powers all life to the macro level of intercooperation of symbiotic animals. We are essentially designing an organism that, like many other organisms, have rich microbiomes, and that can live on the Moon. The organism needs to provide healthy habitats for its constituent microorganisms that are fed by circulatory systems of nutrient exchange and immunity delivery, respiratory systems which exchange gasses, and digestive systems that can supply new food material and expel waste material.

The system Co-I Maurer designed has a series of analog cells termed LOBEs for lunar optimized bioreactor enclosures. The LOBEs are designed to be made of custom drop-stitch materials that pairs the form developing capabilities of drop-stitch technology with the robustness of TPU coated Vectran® (liquid crystal polymer) material. Drop stitch materials are multi-layered air-tight weldable polymer coated fabrics that have "drop yarns'' or drop stitches fastened to the top and bottom. This allows the materials to maintain desired shapes when inflated that may be more orthogonal than standard inflated shapes, that is, spheres, cylinders, and torii etc. This also creates a scenario that converts compressive forces into tensile forces by dispersing the forces weight action to internal pressure (Fig. 40). TPU coated fabrics have very high tensile strengths and resist pressures up to 275 kPa.[34]

[34] Cavallaro, PV, Hart, CJ, & Sadegh, AM. 2013. Mechanics of Air-Inflated Drop-Stitch Fabric Panels Subject to Bending Loads." *Proceedings of the ASME 2013 International Mechanical Engineering Congress and Exposition.* Volume 9: Mechanics of Solids, Structures and Fluids. San Diego, California, USA. November 15–21, 2013. V009T10A055. ASME.



$$M_w = \frac{2I_{zz}}{\hbar A_s}(P+F)$$

Defines the bending moment, M_w, for a panel subjected to an external axial force, F (compression negative), and bent about the *z*-axis. I_{zz} is the moment of inertia (second moment of area) of the panel, A_s is the perimeter defined by the fabric skin,

Figure 40. Conversion of axial force to bending moment, image and equation taken from [35]. Drop stitch and other horizontal reinforcing features would enable flat surface for fenestration and openings.

By converting an exterior shell made of drop stitch fabrics that can handle high pressure differentials, the interior to exterior pressure balance can be controlled with light weight materials (Fig. 41). The inflated LOBEs will push against the interior pressure and resist the resulting outward thrust of the negative pressure of the lunar atmosphere. While the pressure inside the ISS is the same as the earth's atmosphere (1 bar) the interior environments of these structures could be less than Earth's atmospheric pressure if a higher percentage of oxygen is present.[36]

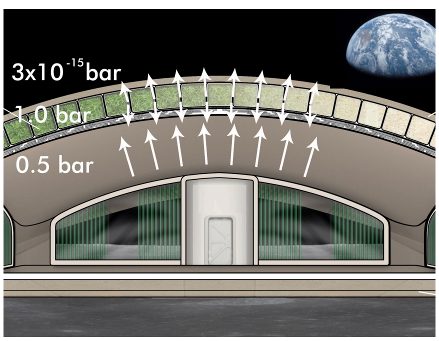


Figure 41. This figure shows the outward facing forces based on pressure differentials between the interior and exterior environments. In contrast to Earth construction where the weight of buildings cause downward forces (that require materials with high compressive strengths), Martian and lunar architecture will have pressure from the interior environment pushing outward (which will require materials with high tensile strength.)

LOBEs (Fig. 42) can support all the requirements for life including gasses with high levels of carbon dioxide for photosynthesis organisms and oxygen for fungal organisms supplied by drop stitch ductwork, heat traced membranes that warm the interior to the proper temperature for growth and arrest growth as needed, LED embedded membranes to supply light within proper circadian rhythms, nutrient supply channels to feed the organisms, and liquid circulation to allow the delivery and extraction of water and solutions.

[35] Davids, W.G. 2023. Behavior of Inflatable Drop-Stitch Fabric Panels Subjected to Bending and Compression. *Materials 16*, 6919. https://doi.org/10.3390/ma16216919
[36] George, JA. 1998. Suited for Space Walking, National Aeronautics and Space Administration Office of Human Resources and Education. Education Division Washington, DC. www.nasa.gov/wp-content/uploads/2009/07/143159main_Suited_for_Spacewalking.pdf?emrc=db3414

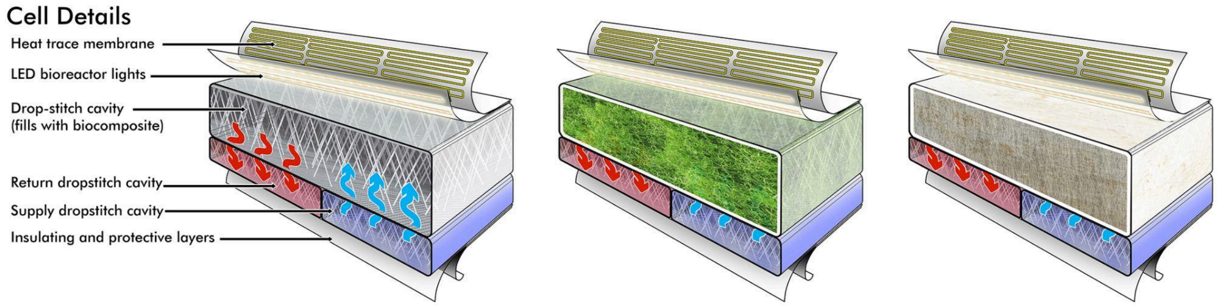
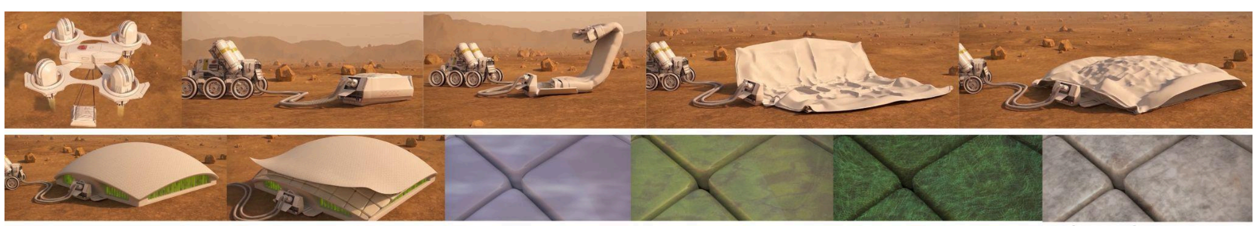


*Figure 42. LOBE details. From left to right, a single LOBE that would be within a network of others acts biomimetically as a living cell and architectonically as a masonry unit. The cell is connected to networks of hoses and ducts to supply its inhabitants (microorganisms) with the resources for life. The LOBE can be filled with in situ resourced water and carbon dioxide that activates the embedded photosynthetic organisms (*Chlorella sp. *and others). As the organisms metabolize, they create oxygen and biomass that become the feedstock for fungal life to begin. Liquid fungal cultures are time released and the fungi metabolize the biomass into a composite material that has performative features such as thermal insulation and radiation attenuation.*

Several archetypes were developed (see prototypes section) but two will be discussed here in depth. The Initial base structure (IBS) building number 2 on the 500 day mission roadmap outlined in the report.



youtu.be/xUk4YQTo1oY

Figure 43. Initial Base Structure (IBS). From left to right, a lander delivers the IBS to lunar or martian surface where it is met by rovers that can supply in situ resourced water, gasses, and loose regolith. The deployable structure grows as its bioreactors (LOBEs) fill with water and CO2 enabling photosynthetic organisms to create biomass within. Time released fungal spores then grow to convert the photosynthetic biomass into a bio-performative composite.

The IBS (Fig. 43, and 44) is a 15 m x15 m three bedroom deployable structure with 170 m^2 of living space and 460 m^3 of interior pressurized volume. The 170 m^2 interior could be arranged in a number of configurations but the plan presented here is for a 3 bedroom, 2 full bath with large common space for work, cooking, living, and flex space for exercise. The design opts for large spaces, including bedrooms, and ample fenestration for psychological comfort in contrast to the ISS and other space structures. The isolation, delay in communications, reduced gravity, and inability to routinely walk outside can lead to mental health stress with effects including, sleep deprivation, visual disturbances, depression, and anorexia that can in part be mitigated by having larger private spaces.[37] The work/lab and kitchen could be compartmentalized but are left open in the following plan and envisioned as a garden with many plants used for making food and providing biophilic stimuli.

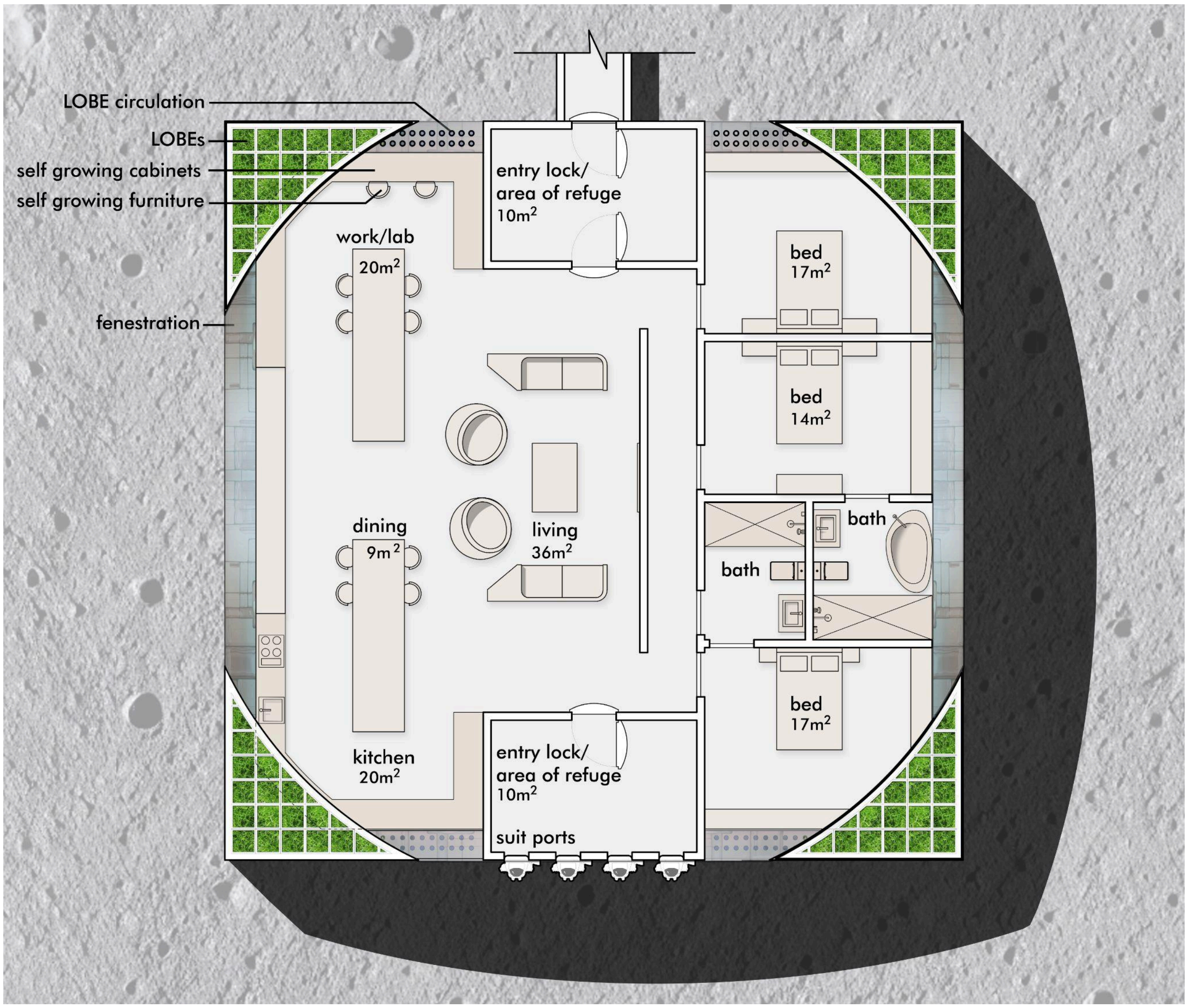


Figure 44.The above floor plan for the Initial Base Structure shows ample living space not normally associated with space architecture. This should not be considered a luxury as personal space is closely correlated with psychological comfort. Entry locks at either end provide pressure redundancies for areas of refuge and could be equipped with suit ports or passage to connected buildings.

The outer shell is an air supported grid of LOBEs that allow pre-seeded organisms to grow within the network creating biomass. The multilayered exterior shell has 237 m^3 of LOBE space

[37] Arone A, Ivaldi T, Loganovsky K, Palermo S, Parra E, Flamini W, Marazziti D. 2021. The Burden of Space Exploration on the Mental Health of Astronauts: A Narrative Review. *Clin Neuropsychiatry.* 18(5):237-246. doi: 10.36131/cnfioritieditore20210502.

to create a 50 cm thick biomass shell. With 10 cm^3 of supplied *Chlorella sorokiniana* and a doubling time of 4.44 days the 237 m^3 LOBEs could be filled with biomass within 26 days. [38]

$$Nt=N0\times(2(t/Td))$$

where: *Nt* is the final population size, *N0* is the initial population size, *t* is the time elapsed, and *Td* is the doubling time.

$$t=\log2\,(23.7\times10{*}6)\times4.44 = 25.7\text{days}$$

Materials: Many different biomaterials, or bioterials, are are available for different applications within the building. Some developed by our team are optimized for strength, some are for comfort, and some have bioperformative features like the attenuation of ionizing radiation. The below chart compares some of the ISRU materials that are currently being proposed for space missions with our materials optimized for strength. These were tested at Stanford University's Xlab and published in *Frontiers* (Table II). The chart shows that mycomaterials developed by this team have structural properties that outperform concrete, sintered regolith, and other ISRU materials.

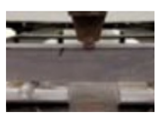
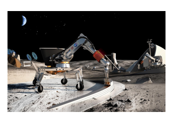
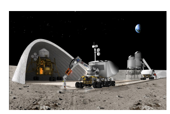
Material	Compressed Regolith	Sintered Basalt	Sulfur Concrete	Lunar Regolith	Ice	Mycoterial
Project/paper	Chow et al.,	PICES - HI	NASA - Khoshnevis	NASA - Khoshnevis	Ice House - SEArch+	Rothschild et al., NIAC
Image						
Compression Strength	40 MPa	206 MPa	53.5 MPa	17.24 MPa	3 MPa	26 MPa
Modulus of Elasticity					5100 MPa	275 MPa
R-value (per inch)		0.05			0.45	3
Temperature to produce		1400°C	130°C	1025°C	>0°C	20°C

Table II. Material comparison with materials under consideration for ISRU construction. Reproduced from [39]

[38] Anıl Tevfik Koçer, Benan İnan, Didem Özçimen, İskender Gökalp. 2023. A study of microalgae cultivation in hydrothermal carbonization process water: Nutrient recycling, characterization and process design, Environmental Technology & Innovation, vol. 30, 103048, ISSN 2352-1864, https://doi.org/10.1016/j.eti.2023.103048.

[39] Brandić Lipińska Monika, Maurer Chris, Cadogan Dave, Head James, Dade-Robertson Martyn, Paulino-Lima Ivan Glaucio, Liu Chen, Morrow Ruth, Senesky Debbie G., Theodoridou Magdalini,

While structural integrity is an important factor, it is not the most important, at least not compression strength. Lunar gravity is 17% that of Earth, which martian gravity is ~38%. Further, there are no wind loads or water/snow loads that control most of Earth's structural design criteria. The main factors to consider are the ability to resist the outward force of a pressurized interior, the thermal insulation, and radiation protection.

As it pertains to tensile strength of the exterior and interior pressurized cells, it is important to note the tensile strength of the polymer coated fabrics. Vectran® claims tensile strengths of 3.2 GPa, or more than twice that of titanium, more than one and half times stainless steel, sixteen times that of aluminum, and, very importantly, 350 times that of sintered regolith.[40, 41] Several layers of this inflatable material are incorporated in the design for redundancy and by nature of the multiple cavity system.

Thermal Insulation

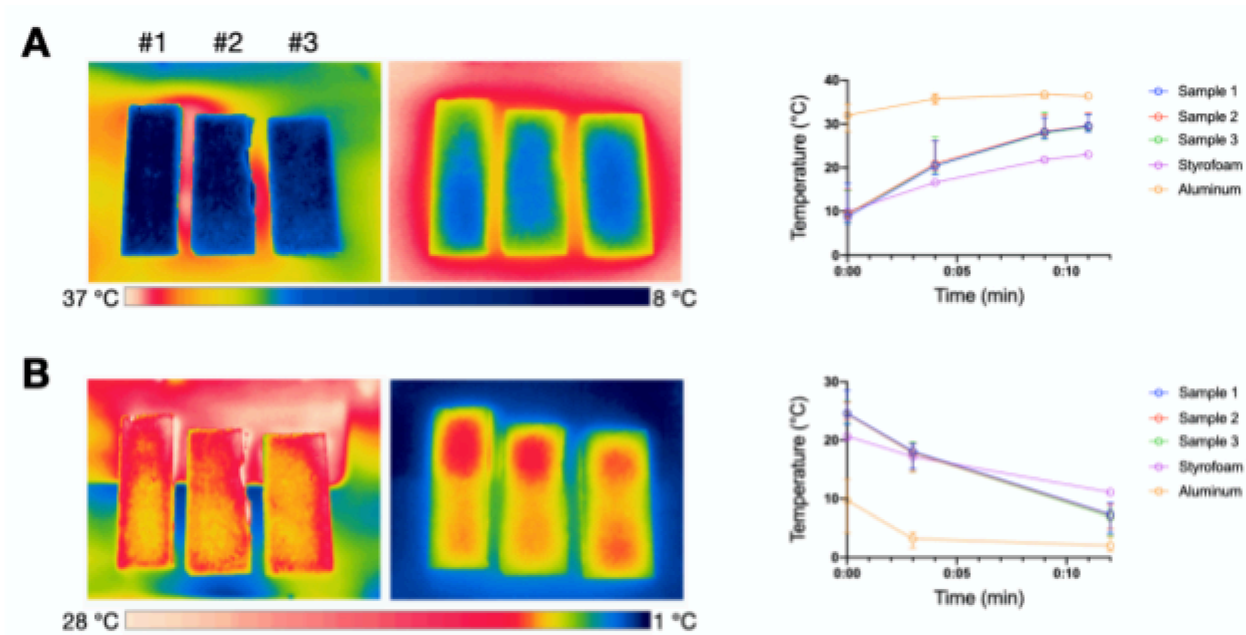


Fig. 45. Temperature change of compressed mycelium samples during incubation at 37 and 4 °C. (A) Shows thermal images of samples using a FLIR E96 microbolometer at 0 and 10 minutes after irradiation. Adjacent graph shows the average apparent temperature values of compress

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Rheinstädter Maikel C., Zhang Meng, Rothschild Lynn J. 2022. Biological growth as an alternative approach to on and off-Earth construction, Frontiers in Built Environment, vol. 8, DOI 10.3389/fbuil.2022.965145

[40] Vectram product data sheet. https://fibrxl.com/wp-content/uploads/2020/07/FibrXL-PDS-performance-0720-DEF-Vectran.pdf
[41] Zhao, H., Meng, L., Li, S. et al. 2022. Development of lunar regolith composite and structure via laser-assisted sintering. *Front. Mech. Eng.* 17, 6. https://doi.org/10.1007/s11465-021-0662-2

mycelium, and blocks of aluminum and styrofoam for reference. Following incubation at 37 °C, samples were placed inside a 4 °C cold room and panel (B) shows the thermal images at 0 and 10 minutes of cold incubation.

While the polymer coated inflatable fabrics are responsible for making the airtight shell and resisting tension, the bioterial palette is responsible for the other important features of thermal insulation and ionizing radiation resistance (Fig. 45). Lunar temperatures in the south pole region designated in our 500 day plan swing from +53 °C to -203 °C.[42] For this reason, we have begun testing biochemically infused aerogels. Aerogels are widely regarded as superior insulation materials due to their microcavities that trap air. Aerogels can have densities of ~0.003 g/cm^3 and very low conductivity ~0.01 W/mK.[43] Compared to industry leaders of rigid polyurethane foam (0.028 W/mK) and fiberglass batt insulation (0.36 W/mK) aerogels are two to three times as effective and have the added benefit of being able to be made easily *in situ.*

Aerogels could be an answer to radiation protection as well. Our team is developing melanin infused aerogels to combat ionizing electromagnetic radiation such as UV, X-ray, and Gamma. The 2020 readings of China's Chang E4 Lander radiation dosimetry measured an average dose equivalent of 1369 μSv/day on the surface of the Moon compared to 0.017 μSv/day average American on Earth.[44] [45] See section 4.1.6 for a discussion of how melanin is believed to protect from ionizing radiation. A preliminary report by Averesch et al. on the cultivation of the dematiaceous fungus *Cladosporium sphaerospermum* aboard the ISS and the effects of ionizing radiation posited that melanin enriched regolith would need 20% less material than regolith alone and 70% less than water. [46]

Fenestration. The need for fenestration for psychological comfort and performance is widely documented, and windows with views were found to enhance work and well-being in a number of ways including increasing job satisfaction, interest value of the job, perceptions of self-productivity, perceptions of physical working conditions, and life satisfaction.[47] The concept of "biophilia" is prevalent in architectural design that aspires to give inhabitants a connection to nature.[48] While there is no life to be seen in lunar and martian environments,

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[42] Mahoney, Erin. 2022. Moon's South Pole is Full of Mystery, Science, Intrigue. NASA/GSFC/Arizona State University.
[43] Prakash C. Thapliyal, Kirti Singh, 2014. Aerogels as Promising Thermal Insulating Materials: An Overview, *Journal of Materials*, vol. 2014, Article ID 127049, 10 pages. https://doi.org/10.1155/2014/127049
[44] Shenyi Zhang et al., 2020. First measurements of the radiation dose on the lunar surface.Sci. Adv.6,eaaz1334. DOI:10.1126/sciadv.aaz1334
[45] https://www.nrc.gov/about-nrc/radiation/around-us/doses-daily-lives.html
[46] Averesch, Nils & Shunk, Graham & Kern, Christoph. 2022. Cultivation of the Dematiaceous Fungus *Cladosporium sphaerospermum* Aboard the International Space Station and Effects of Ionizing Radiation. *Frontiers in Microbiology*. 13. 877625. 10.3389/fmicb.2022.877625.
[47] Farley, K. & Veitch, Jennifer. 2001. A Room with a View: A Review of the Effects of Windows on Work and Well-Being. DOI: 10.4224/20378971
[48] Won Hee Ko, Stefano Schiavon, Hui Zhang, Lindsay T. Graham, Gail Brager, Iris Mauss, Yu-Wen Lin, 2020. The impact of a view from a window on thermal comfort, emotion, and cognitive performance, *Building and Environment*, vol. 175,106779, ISSN 0360-1323,

view of the natural landscapes coupled with the biological systems at work within the living structure could serve this biophilic purpose. See Fig. 46 for an example.

Figure 46. Proposed interior of bedroom. Furniture and fixtures could be made of a combination of inflatable and grown-in-place bioterials. The same technology that powers the growth of the structure could be utilized to create comfortable furniture. Image by redhouse studios.

Window units can be made in several ways. Clear flexible polymers could be made compliant using modified drop stitch technology or other horizontal reinforcement. The resulting cavity could be filled with radiation attenuating gasses or liquids such as hydrogen or water, or ideally aerogels that are augmented with radiation-attenuating biochemicals . Another method for creating compliant cavities was illustrated beautifully in the 2008 Beijing Olympic National Aquatics Building also known as the Water Cube (Fig. 47). The Water cube was made of a steel space frame that supported 0.2 mm ETFE (Ethylene Tetrafluoroethylene) "pillows".[49] Other examples of this pneumatic fenestration technology exist, notably the Grimshaw designed buildings, National Space Center (UK) and the Eden Project. ETFE is highly UV resistant, allows 90-95% of visible light to penetrate, and can be used multiple layers to develop high insulation values.[50] These cavities would, in our applications, be filled with transparent gasses, liquids, or aerogels in order to augment the thermal insulation and radiation attenuation.

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[49] https://www.arup.com/projects/chinese-national-aquatics-center
[50] LeCuyer, Annette. ETFE: Technology and Design, Berlin, Boston: Birkhäuser, 2008. https://doi.org/10.1007/978-3-7643-8624-5

Figure 47. Examples of ETFE "pillows". From left to right: National Aquatics Building, Beijing 2008, The Eden Project Cornwall, England, 2000, The National Space Centre Belgrave, UK, 2001, Cleveland Metroparks Zoo Rainforest Dome, Cleveland, OH

<u>Mechanical, Electrical, Plumbing, and Life Support Systems</u> Utility supply is assumed to be by others. At the time of deployment we expect that NASA would have the infrastructure to be able to deliver water, power, and communications via hook-ups that can be embedded in the deployable package via autonomous systems like rovers (Fig. 48).

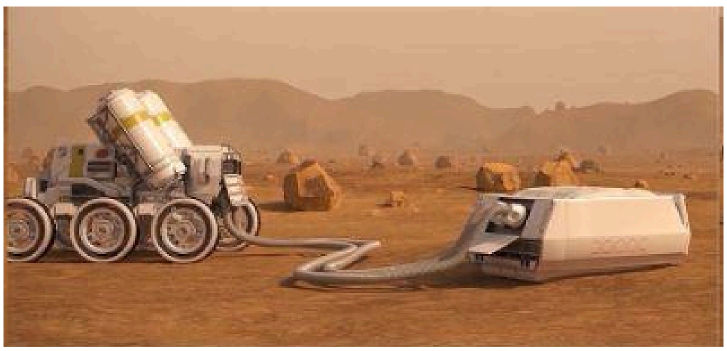

Figure 48. Illustration of rover providing water to the mycotecture habitat to initiate deployment.

The MEP systems can be prewired and pre-ducted using foldable tubes and wires that when pressurized take form. Likewise, even plumbing fixtures could be made of the same grow-in-place technology as the habitat itself (Fig. 49).

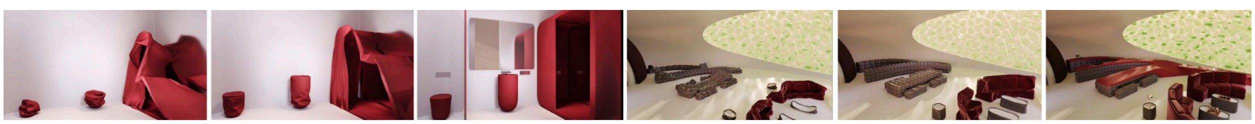

Figure 49. From left to right, a bathroom growing in place where fixtures are pre-piped for use and for manufacture. The toilet sink and shower enclosure are made of robust polymers that fill with bioterials that harden in situ. *A living space that grows in place by the same method - everything including the kitchen sink.*

The algal reactors and circulatory system is designed to make use of flexible tubing that stiffens when pressurized. Figures 49 and 50 shows such tubing actuating the vertical movement of a (relatively) heavy beam. This is essentially hydraulic lift and we can see the ability of the circulatory system to do multiple tasks. In addition to supplying LOBEs with nutrients and moving biomass throughout the building the stiffening force is used to create form and reinforce laterally, while exposing the process for monitoring, solar radiation gain, and biophilic enjoyment.

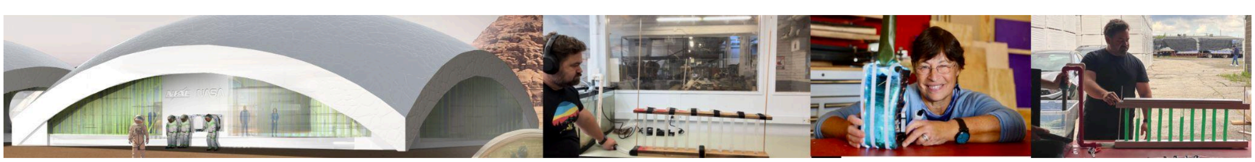

Figure 50. From left to right, an image of the IBS with bioreactors and suitorts, test of water actuator, sample dropstich bioreactor with lights embedded, algal fluid actuated hydraulic lift at NIAC midreview September 15, 2022.

While not specifically designed for this iteration, the biological processes involved in creating bioterials could have the added benefit of biobased life support systems. Biobased life support systems were investigated in another NIAC research termed WaterWalls by Cohen et al. in 2012. Water Walls life support architecture (WWLSA) was developed to serve multiple purposes including, CO_2 scrubbing, oxygen production, wastewater treatment, human solid waste treatment, and radiation treatment. The team proposed doing this using algal bioreactors as the walls as shown in Figure 51.

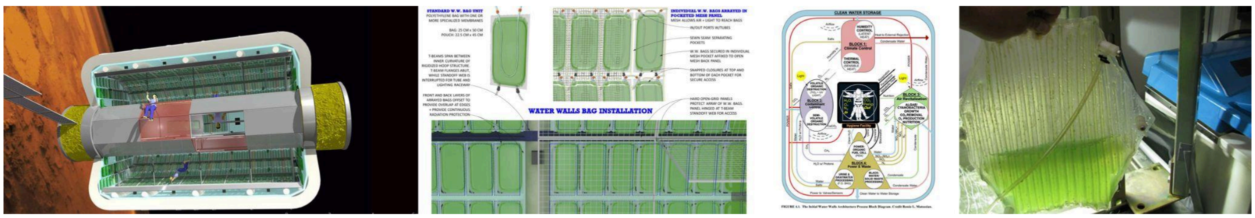

Figure 51. Image of Water Walls concept proposed by Cohen et al. From left to right: Illustration of station using "Water Walls" from Cohen et al. 2012 NIAC Phase I, prototype of the system, diagram showing the life support systems, and prototype for hexagonal unit. Taken from NIAC final report .[51]

Figure 52 images a future iteration of the design for the IBS where the Cohen and Rothschild NIACs are combined. Here the waterwall concept could be used as a closed loop process to create oxygen, scrub, CO_2, and process human waste all while producing biomass to make more bioterials, food or organic compost for plants as time goes on. The scenario envisions that the bioreactors become integrated into the design which on a barren planet or Moon may induce the aforementioned biophilic response that helps people cope with isolation and mitigate stress. In addition to oxygen and biomass, microorganisms could be prioritized for creating hydrogen that could be used to power fuel cells and for radiation shielding.[52]

[51] Cohen, Marc & Flynn, Michael & Matossian, Renee. (2013). Water Walls Life Support Architecture. 43rd International Conference on Environmental Systems. 10.2514/6.2013-3517.

[52] Vardar-Schara G, Maeda T, Wood TK. Metabolically engineered bacteria for producing hydrogen via fermentation. Microb Biotechnol. 2008 Mar;1(2):107-25. doi: 10.1111/j.1751-7915.2007.00009.x. PMID: 21261829; PMCID: PMC3864445.

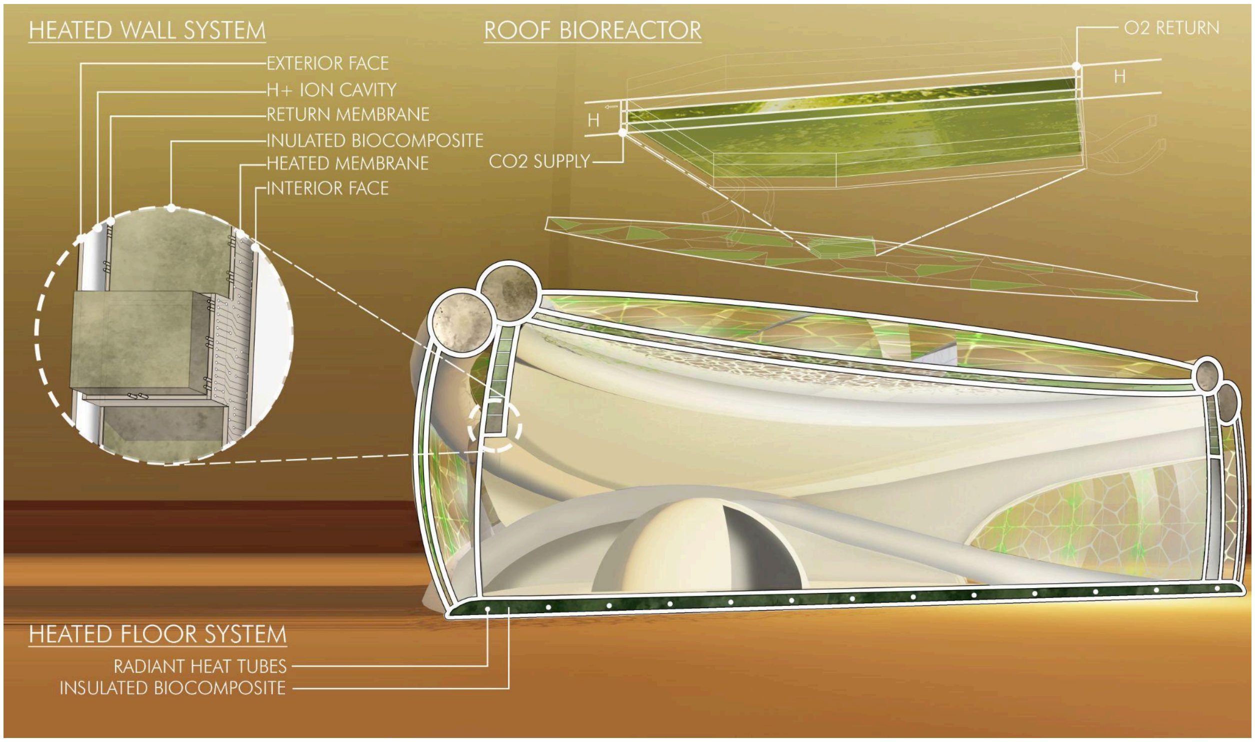

Figure 52. A future interaction incorporating our mycotecture project with the Water Walls concept. Future iterations of this design could incorporate life-support systems as the fenestration that protects from radiation while scrubbing CO_2 and waste, and creating biomass.

MASS Estimates: Table III is a comparison is a comparison between this NIAC BioHab and the Human Dwelling Unit- Deep Space Habitat (HDU-DSH) which was a multi-center NASA design for an outpost that has been tested by D-RATS.[53] Table IV is a more detailed breakdown estimate for the NIAC mycotecture off planet concept.

	NIAC BioHAB		HDU-DSH		reference
					NASA
Uplift Mass	10.00	tons	31.00	tons	Griffin et al
In Situ Mass	120.00	tons	-	tons	
Final Mass	120.00	tons	31.00	tons	Griffin et al

[53] Kennedy, Kris. HDU Deep Space Habitat (DSH) Overview. International Conference on Environmental Systems, Portland, Oregon, July 2011 AIAA abstract control ID #1021654. https://ntrs.nasa.gov/api/citations/20110008769/downloads/20110008769.pdf

Livable Area	169.00	m^2	46.00	m^2	NASA
Area/Uplift Mass	16.90	m^2/t	1.48	m^2/t	
Livable Volume	460.00	m^3	148.00	m^3	NASA
Volume/Uplift Mass	46.00	m^3/t	4.77	m^3/t	
Occupants	3.00	ppl	4.00	ppl	NASA
Occ/m2	0.02	p/m^2	0.09	p/m^2	
Occ/Uplift Mass	0.30	p/$	0.13	p/$	
Uplift Cost (@$1m/t)	10,000,000	$	31,000,000	$	Estimated
Uplift cost per Area	59,171.60	$/m^2$	673,913.04	$/m^2$	
BIOHAB Benefits	More than 10x the area for uplift cost				
	More than 4x area per occupant				
	Private "hygienic" areas / bathrooms				

Table III. Comparison of mass trade-offs between our habitat design (BIOHAB) and Human Dwelling Unit- Deep Space Habitat (HDU-DSH).

Up-Mass Estimates:	HM500D #2 IBS			*In Situ*-Mass Estimates	HM500D#2 IBS	
Fabrics / Inflatables	6	tons		Water	119	tons
Reactors	2	tons		Oxygen (from water)	1	tons
Piping/conduits	1.3	tons		TOTAL	120	tons
Organisms	0.04	tons				
Hydrogels /nutrients	0.04	tons				
Enclosure	0.12	tons				
Airlocks/ ports	0.5	tons				
TOTAL	10	tons				

Table IV. Estimates of upmass requirements and in situ *requirements for our habitat design.*

4.6 Mission architecture.

4.6.1 Hadley Max 500-Day Design Reference Mission (DRM) to the Apollo 15 Hadley-Apennine Region: Phase I-Application Of Science Goals And Objectives To Planning Long Duration Exploration Architecture [54]

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Abstract: We call on Apollo Lunar Exploration Program experience and scientific results, and human/robotic exploration concepts, to design a 500-day (Mars-like duration) Design Reference Mission (DRM) to the Hadley-Apennine region of the Moon. We specifically use the lunar exploration DRM 1) to identify the architectural and technological requirements and challenges for a successful long-duration mission, 2) to study the application to the Artemis South Circumpolar Environment (ACE):, and 3) to help inform us of feed-forward insights for a comparable mission to Mars.

1. Introduction and Background:
Among the six successful Apollo Lunar Exploration Program landed missions, Apollo 15 was the first Lewis and Clark-like "Scientific Expedition to the Moon" [1]. Experience with Apollo 11, 12 and 14 walking traverses provided fundamental scientific results, demonstrating pinpoint landing techniques, and increasing stay-time, EVA durations, and mobility assistance (such as the Mobile Equipment Transporter, MET, on Apollo 14), but also clearly demonstrated the need for increased numbers of EVAs and mobility in order to reach multiple and more distant scientific objectives [2]. The Apollo 15 mission [1] carried the first Lunar Roving Vehicle (LRV) that enabled Astronauts Dave Scott and Jim Irwin to reach distant objectives and plan traverse out to a radial distance of 7 km [3] (Fig. 1a-b), the maximum distance permitted by the need for them to walk back if the rover failed (the 'walkback' constraint).

The initial Standup EVA (SEVA), was inserted as a result of Commander Scott's desire to get an initial overview of the terrain (due to the fact that only 20 m resolution images were available for pre-mission planning; Fig. 1c) [4]. The following three periods of EVA visited four of the five major mission objectives (lunar maria, lunar highlands (Hadley Delta), Hadley Rille, secondary crater cluster, and North Complex) (Fig. 1d). The Apollo 15 mission [1,5,6] completed 19.7 hours of surface exploration, deployed a complex set of scientific instruments, drilled a 2.4 m drill core, traversed 27.9 km of the lunar surface, and returned 77 kg of samples [7], 56% more than the previous Apollo 14 mission. The results of this very successful exploration mission [8] addressed many fundamental scientific questions [1,7], and an entire science conference dedicated to Apollo 15 mission results was held in Houston. As is usually the case in science and exploration, the findings from Apollo 15 [1,7] led to a whole new set of questions, and

[54] NOTE: section 4.6.1 is the draft of a manuscript. Thus the figure numbers and references are for a stand-alone work. Regular page numbering and footnoted references begin again in section 4.7.

posed an additional set of exploration destinations in the larger area of the Hadley-Apennine landing site (Fig. 2).
In the years subsequent to the Apollo Lunar Exploration Program, orbital remote sensing missions (such as Lunar Prospector, Clementine, Lunar Reconnaissance Orbiter, Chandrayaan-1) permitted the extension of the Apollo and Luna Mission sample return results to the entire Moon, and a much more refined vision of the Moon as a planetary body emerged [9]. Concurrently, robotic exploration of Mars with landers, orbiters and rovers was providing a view of a planet that appeared to have been Earth-like in its earliest history, but had then evolved to an extremely cold hyperarid polar desert that we see today [10]. Could Mars have harbored life in its earlier "warm and wet" climate history? These emerging results obviously kindled an interest in NASA in the human exploration of Mars. Several committees were formed and engineering studies were undertaken to assess scientific goals and objectives and mission architecture. The following type of questions were posed to these groups: What will be the state of robotic Mars exploration and Mars knowledge in 2030? What are the key science questions that humans can address that will not be addressed by robotic missions by 2030? What is the best way to deploy/utilize humans on Mars? What is the role of IT/robotics alongside humans?

In a 2007 study [11], one of the first issues that arose in reference to the mission architecture was the question of duration of the Mars mission surface stay time for the astronauts. Did scientists want to go and return in one access opportunity, during which the approximate stay time would be ~30 days, or stay on Mars and return at the following access opportunity, during which the approximate stay time would be ~500 days?
The skepticism of the engineers ("What would you be doing on Mars for 500 days???") was met with euphoria from the geologists and other scientists ("Wow! Let us show you what we could do!"). The ensuing reports [11] outlined the results of these studies, identifying 40 candidate Mars human exploration sites (Fig. 3a), designed around MEPAG Goal III: 'Determine the Evolution of the Surface and Interior', and showing Design Reference Missions (DRMs) for scientific points of interest and traverses that would enable the astronauts to reach them (Fig. 3b). Subsequent studies periodically revisited these issues [12,13], most recently, the MEPAG Human Exploration of Mars Science Objectives (HMSOTT) report [14].

In response to the challenges from the NASA engineers in 2007 ("What would you be doing on Mars for 500 days???"), we reasoned that presenting a more familiar and previously successful mission scenario, but expanded to a 500-day duration, would help both the engineers and scientists develop a design reference mission (DRM) that would mutually educate, as well as identify the key mission parameters and technology requirements for a 500-day mission to Mars. In addition, the several attempts at sustained human lunar exploration (SEI, Constellation) underlined the need for similar thinking approaches for the Moon. And in 2017, the announcement of the NASA Artemis Moon to Mars initiative underlined the importance of such an approach.

Thus was born the 500-day Human Exploration Mission back to the Hadley-Apennine region (Fig. 2), the landing site region of the Apollo 15 first scientific expedition to the Moon. Named the "Hadley Max" mission by Apollo 15 Commander Dave Scott, this Design Reference Mission (DRM) concept called on utilizing the Apollo mission planning and execution experience of two of the co-authors (Scott and Head), mission operations LRV design and thermal control planning and execution experience (Creel), crew training and operations

experience (Eppler), Myco-Architecture as in situ building materials (Rothschild) [15], in situ construction methodologies [25] and the enthusiasm and creativity of Brown University geology, physics, engineering and computer science students (a team that has grown over the years as the Hadley Max DRM concept has evolved).
The major goals and objectives of the "Hadley Max" 500-Day Hadley-Apennine Design Reference Mission (DRM) project were to identify the key mission elements and requirements in the following areas: 1) Background and motivation ; 2) Scientific Goals and Objectives; 3) Implications for Mission Design and Architecture; 4) Traverse Design & Implementation, 5) Implications for Habitat Requirements-Role of Myco-Architecture; and 6) Implications for Habitat Design and Construction, with areas 2-7 described below.

2. Science Goals and Objectives
We supplemented the initial five major Apollo 15 scientific goals and objectives (Imbrium impact basin; Sinuous rille origin; Mare history; Ejecta from distant craters; Regolith history), with several additional objectives related to questions raised by Apollo 15 crew exploration/observations, analysis of the Apollo 15 sample suite, and subsequent mapping [1, 7, 17, 18].

1) <u>Lunar Maria Lava Flow Emplacement</u>: Prior to Apollo 15, it was thought that Mare Imbrium lavas might be the rapid response of Imbrium basin mantle uplift and resulting massive pressure-release melting, and thus have formed nearly contemporaneously with Imbrium; however Apollo 15 Imbrium ejecta (~3.9 Ga) and mare basalt radiometric dates (3.3 Ga) showed that they were ~600 Ma apart, disproving this hypothesis. The mare basalt dates did reveal evidence for two different flow sequences emplaced a few tens of Ma apart, and somewhat different in composition (quartz-normative and olivine-normative). Visual descriptions and high-resolution images by D. Scott revealed distinctive layering in the W Hadley rille wall, and portions of these layers were sampled in blocky probable outcrops along the E rille rim. Irwin noted marginal steps along the base of Mount Hadley, suggesting topographic decrease of the mare surface after initial flooding due to lava drainage or and/or solidification. Scott and Irwin discovered clods of green glass beads, an entirely unexpected finding that suggested dense clouds of fine liquid droplets from either pyroclastic or impact crater events, apparently temporally unassociated with the mare basalts, and later shown to be of pyroclastic origin and contain unexpected amounts of H_2O. Intrigued by the question of volatiles in the basalts, D. Scott observed a lone, very vesicular rock (15016) on the maria and made an unscheduled stop to sample it for assessment of volatiles in the lab. Additional crew observations and sampling of highly vesicular mare rocks added new insights about the role of magmatic volatiles in mare basalts and pyroclastic eruptions. But what was the origin of the green glass beads, shown to have originated ~400 km deep in the mantle, their geological context and sequence and relation to the vesicular basalts, and how were they related to the Hadley Rille vent dozens of km to the south (Fig. 2), and to the origin of the rille itself? Apollo 12 had sampled an unusual KREEP-rich breccia (12013) of uncertain provenance and perhaps related to a Procellarum-KREEP Terrane (PKT) crustal province on the NW nearside. Apollo 15 discovered KREEP basalts (15382, 15386) with a crystallization age of 3.9 Ga, indistinguishable for the age of the Imbrium basin, reopening the question of whether the Imbrium impact event induced basaltic volcanism, perhaps contaminated by passing through PKT crust.

These observations and results raised a series of outstanding scientific questions: Does the layering and vertical structure observed in the walls of Hadley Rille correspond to the two sampled lava flows? What is the vertical structure of the two lava flows (vesicle distribution, cooling behavior and history, mineralogical segregation, etc.) and the nature of their interface (thickness of regolith between them, atop)? Which of these lava flows are associated with the origin of Hadley Rille and in what manner? Do these two flows show evidence of differences in magnetization and field orientation during this critical period? Is there evidence for additional flows (and pre-mare units) deeper in Hadley Rille? What is the regional distribution of the green glass, its associations with the vesicular basalts, and its distribution relative to the Hadley Rille vent? Are either basalt group petrogenetically correlated with the green glass beads? What is the origin and provenance of the KREEP basalts, where do they outcrop, are they related to basalts of different ages outside the Apollo 15 sampling area [4], are they related to the high-albedo smooth plains in the Archimedes area (Apennine Bench Fm.), and are they extrusive basalts or ponded Imbrium basin impact melt? What is the full range of basalt compositions and ages in the SE Imbrium basin [4] and where and how does Hadley Rille fit into the picture? What is the origin and significance of the marginal terraces observed by Irwin? What is the origin of the several irregularly shaped craters in the Apollo 15 landing region? Do these represent additional vents for lavas seen at Apollo 15?

2) <u>Nature and Origin of Hadley Rille</u>: Early hypotheses ranged from iced-over aqueous fluvial channel erosion to lava-related (open lava flow median channel, highly turbulent thermal erosion, collapsed subsurface lava tubes). Astronaut observations and samples significantly improved our understanding, supporting a lava-related origin, but the actual rille origin remains enigmatic, due primarily to its great length (>135 km) and lack of access to its source vent to the SSW. The broad, low-elevation rim described by Irwin, and the W rille wall stratigraphy documented by Scott, are consistent with an open lava channel and/or thermal erosion, but very narrow portions of the rille elsewhere suggest a collapsed lava tube. Later global documentation of sinuous rilles shows that Hadley rille is anomalously long compared to the global population, and quantitative modeling suggest that the shorter rilles formed by turbulent lava flow and thermal erosion of the substrate, but the formation of the significantly longer, deeper and wider Hadley Rille remains enigmatic.

This raised a series of outstanding questions: What is the nature of the two Hadley Rille source vents, are they surrounded by pyroclastic deposits (the green glass?), and what is their relationship to the rille? Do they represent separate eruptions or two phases of a single eruption? What are the proximal to distal characteristics of the rille (rim deposits, width, depth, wall-rock stratigraphy, evidence for roof narrowing and collapse)? How does the morphology and structure of the rille differ between straight segments and bends in the rille? What is the thickness of the lava and is pre-lava substrate (Apennine Bench Fm?) exposed? What is the petrogenetic relationship of any circum-vent pyroclastics and adjacent lava flows? Could the marginal terraces observed by Irwin be related to flooding of the valley and drainage of lava into Palus Putredinis? What is the nature and origin of the Schaber Hills (North Complex): volcanic construct or highland lava-covered kipuka?

3) <u>Imbrium Impact Basin</u>: Apollo 14 was targeted to the ejecta deposit of the Imbrium Basin (Fra Mauro Fm.) and Apollo 15 to the rim of the Imbrium Basin (Apennine Mts.) to sample the deepest ejecta (deep, slow-cooling magma ocean anorthosites), date the Imbrium event,

understand the highlands diversity in the ejecta, and search for possible mantle material. At Spur Crater on EVA 2, Scott spotted a perched rock brightly glinting in the sunlight, and stated "Houston, I think we found what we came for!", recognizing from a distance that this glint meant large plagioclase feldspar crystals/twinning, a phenomenon he knew meant deep-seated, slowly cooling highland crust. This sample (15415), dubbed the "Genesis Rock" by the media, did indeed reveal much about the nature and cooling history of the lunar highlands crust, with an age of 4.1 Ga, probably much older but partially reset by the Imbrium event. Other highland norite/spinel troctolite samples (15445) had ages of 4.28-4.46 Ga and are thought to represent the actual solidification age of the deeper magma ocean. Scott and Irwin observed enigmatic 100 m-scale inclined layering in the Apennine Mts. from afar (Silver Spur), well outside the traverse range.

This raised a series of outstanding questions: What is diversity of highland samples excavated and exposed by the Imbrium impact? What is the full range of rocks in the highland crust and how do they relate to different models of magma ocean formation and cooling? How does the sample petrology change with elevation, possibly related to target stratigraphy? Did the Imbrium impact event sample: mantle material; the KREEP-rich residual layer at the base of the magma ocean; the source regions of pre-Imbrian magmatism (the Mg-suite); ejecta from the adjacent, earlier Serenitatis basin (Silver Spur)? Are there deposits of pure Imbrium impact melt ponded in the Apennine Mts. summit lows? How do such new results compare to highlands sample collected at other sites? A distinctive highland meter-scale wrinkled texture is observed in orbital images (elephant-hide terrain-EHT) and may explain some of the walking and LRV traverse difficulties in the highlands; what is the origin of the EHT and how can this new understanding be used to increase human and robotic mobility in the highlands?

4) <u>Provenance and Age of Secondary Craters and Clusters</u>: Impact craters excavate material from depth and redistribute it laterally in ejecta deposits, the most distant of which are rays and secondary craters and clusters. Thus, samples of such material provide clues as to distant substrate geology and stratigraphy, and can assist in dating the parent impact crater (ages reset by the event). One of the Apollo 15 traverse targets was the crater cluster S of the landing site, attributed to either Aristillus or Autolycus, two large craters, 100-200 km N of the site (Fig. 4a,b). Dune, one of the prominent craters in the South Cluster, was sampled at Station 4, EVA 2, including a prominent regolith breccia boulder with multiple basaltic clasts (15498). The 2.1 Ga age of some samples have been attributed to an Autolycus source, but later analyses show some ambiguity due to the overprint of younger Aristillus ejecta [4]; Spur crater KREEP basalts may be delivered from the Th-rich Aristillus crater.

Among the outstanding questions were: What is the variety of ages in foreign materials delivered to the study region from distal crater substrates, and what are their cosmic ray exposure age in relation to their parent crater AMA? How can these data be integrated to provide a stratigraphy for the broader region surrounding the exploration area?
5) <u>Regolith Stratigraphy</u>: One of the most important questions addressed by Apollo 15 was the vertical stratigraphy of the lunar regolith, its thickness, lateral variability, and implications for processes of mechanical breakdown of the bedrock regolith protolith [35] and chemical alteration induced by impact melting of the transition from bedrock to thick regolith. Scott and Irwin drilled and extracted a 2.4 m core that revealed 42 major textural units and showed conclusively that the regolith was not composed of a homogeneous mixture of impact disrupted materials, but instead was mostly derived from overlapping ejecta layers from nearby

craters. Additional drive tube samples were collected along the traverses, and some showed mixing of highland and mare crater ejecta layers. Important informant was gained on the overall changes in regolith development with depth, and the role of lateral mixing near the mare-highland boundary, as well as the role of regolith mass wasting down into the rille. Among the outstanding questions were: How does regolith stratigraphy vary over a much larger area and are there any regional layers that might act as a chronologic datum? What is the thickness and age of regolith layers on top of buried flows in Hadley rille wall? Can core stratigraphy quantify lateral mixing degree and processes along the mare-highland boundary? How does regolith stratigraphy differ distal to secondary craters and clusters and what is the proportion of ejecta from the primary and locally excavated material?

In the following sections, we use these expanded scientific goals and objectives to outline specific mission desired regions of scientific interest (ROSI) (Fig. 4a) that are designed to address them; we then utilize the ROSI locations to assess implications for the broad architecture of the Hadley Max 500-day Mission.

3. Mission Architecture Definition:
We use the expanded broad scientific goals and objectives derived from Apollo 15 mission results [19] described above, and recent regional geologic mapping [17], as a basis to outline specific Hadley Max mission [21] desired regions of scientific interest (ROSI) (Fig. 4)and more detailed traverse goals and objectives [20]. Here we utilize the distribution of ROSI locations (red dots in Fig. 4) to assess implications for the broad architecture of the Hadley Max 500-day Mission [22] (exploration range, mobility requirements, crew size, number of bases, number of EVAs, upmass and downmass requirements, human-robotic partnership requirements, habitat requirements, etc.).

1. Operational Access Requirements: Landing Sites: On the basis of extending the major goals to a broader exploration and sampling region [20] consistent with current NASA science goals and objectives [23], we locate the primary landing site at the initial Apollo 15 landing site. The 100-200 km radius of operations defined by the distance from the A15 site to the farthest ROSI [21], and uncertainties in the ability to cross Hadley Rille, dictates that two separate landing site/base of operations are required. To optimize the scientific return, we place the second landing site/base of operations to the W of Hadley Rille in mare unit Im3 (Fig. 4).

2. Crew Size, Space Suit and Mobility Requirements: Crew Size: On the basis of the scientific requirement for two landing site bases, and the contingency of crew EVA rescue, we assume 4 crew/base, also an important element of scientific goal division of labor (e.g., one crew rille, one crew highlands) and the possibility of simultaneous EVAs. For the full Hadley Max mission this equals 8 crew on the surface, 4 at each base. Suit Requirements: Minimum is Apollo suit capabilities, assisted by enabling technologies in consumables and mobility, in order to extend traverse time, optimize highland traverses, and expand EVA efficiencies. Mobility Requirements: Minimum is Apollo LRV design and capability.; two rovers/landing site (4 total). Enabling technologies include increased efficiency in slope trafficability to ensure exploration of the rille and highlands, ability to carry four astronauts (rescue), ability to survive lunar night ('rover garage' at base), design lifetime >>500 days.

3. Human-Robotic Mission Types and Relationships: Human Mission Types: These include 8-hour EVAs, 10 km radius of operations, with the possible extension to 14 km using the 7 km circumference "outpost" capability [34] (Fig. 4). Robotic Mission Types: It is clear from the Human EVA 10 km radius of operations and the maximum traversable slope constraints (~20° degrees) that a series of parallel robotic missions will be required to meet the scientific objectives, particularly those in the highlands, and at radial distances beyond 10 km from the landing site, and to ensure Human EVA scouting, interpolation and extrapolation [23]. Human-Robotic Mission Relationships: On the basis of Apollo mission planning and astronaut operational experience, we advise against an Astronaut-tended robotic geologic 'field assistant' as an inefficient use of crew exploration time on the surface. Instead, we strongly urge the development of parallel human-robotic partnerships, generally simultaneous operations to enable precursor, scouting, interpolation, extrapolation, and post-mission exploration activities [23].

4. Definition of Required Habitats, Enclosures and Related Architectural Elements: 1. *Landing Pads (LP)*: These are necessary for both Human and Robotic missions and are like helicopter pads; flat, devoid of soil backwash contaminant, retroreflector for guidance. 2. *Initial Base Structure (IBS)*: Living and working habitat; follows the initial stages where there is a landing module (LM). 3. *Evolutionary Base Structure (EBS)*: Larger scale, separation of work/living activities; increased in situ science activities; IBS evolves to dust mitigation structure. 4. *Outposts: Remote Science Bases (RSB)*: Modeled after IBS, but located >10 km radius from Landing Site. Require up to ~5 RSBs for in depth, in situ science activities. Increase number in order of science priority. 5. *'Pony Express' Stations (PEX)*: These are the lunar 'pup tents' [34] that will be precursors to the Remote Science Bases (RSB), and then Earth-day sleep-stations on the way to the final Remote Science Bases (RSB). Sample storage stations, geophysical stations; can be resupplied/samples collected by CLPS missions. 6. *Robotic Rover Requirements*: a) LRV garage at base for surviving lunar night, re-outfitting; b) Robotic LRV 'pup tents' for surviving lunar night, caching samples. 7. *Application to the Artemis Circumpolar Environment (ACE)*: How do we optimize these basic requirements and DRM concepts for the harsh conditions of the South Circumpolar Region, and the lunar farside? 8. *Assessing Feed-Forward to Mars Exploration*: How does the Mars environment modulate and modify these DRM strategies and architectural elements?

5. Identification of Required Key Enabling Technologies and Operational Concepts: a) *Upmass Requirements*: The multiple base/outpost (RSB)/pup-tent habitat requirements and their necessary range of complexity and ability to survive lunar day/night cycles, as well as robotic LRV remote servicing stations, places huge mass requirements for delivery of construction materials to the Moon. In order to help alleviate this "upmass roadblock", we pursue two promising technologies: 1) Myco-Architecture [15,24], where building materials can be "grown in situ" in order to significantly minimize upmass penalties, and 2) Inflatable Structural Elements [25], in which low-volume, low-mass inflatables can be combined with Myco-Architecture to produce a wide range of enclosures in situ. b) *Human-Robotic Partnerships*: The great distances required to reach all ROSI, the increase in area as a function of radius from the base (increasing the need for scouting, interpolation and extrapolation), the steep slopes within the rille and on the highlands, as well as the presence of the Elephant-Hide Terrain (EHT), and the trafficability on these slopes, all dictate a requirement for a robotic LRV (RLRV) operating independently of the human traverses and controlled from the base or the ground. RLRV design and technology

challenges include ability to traverse slopes approaching 30°, an advanced suite of remote sensing instruments, constant navigation imaging, near real-time communications with the ground, the ability to collect, document and store individual rock and soil samples, remote operations from base and ground, enclosures (RLRV garages) for lunar night, servicing and sample storage, and a design lifetime >>500 days. c) *Supply-Resupply Technology and Infrastructure Requirements*: Despite alleviation of upmass construction requirements through Myco-Architecture and Inflatables, significant supply (and resupply) (S/RS) requirements are dictated by the widespread and long-duration exploration strategy. Many dozens of human and robotic S/RS missions to diverse locations, delivering different payloads, and ensuring crew cycling and sample return, are required by the 500-day DRM architecture. Optimal resupply mission require landing, offloading cargo, and onloading crew, rock/soil samples and other materials for return to Earth. d) *Mission Operations and Feed-Forward to M*ars: Lunar communications latency (~2.5 sec) presented no difficulties during Apollo, but Mars latency (5-20 min) precludes useful direct communications with ground during exploration. In addition, after a few day exploring the lunar surface, astronauts will have by far the best situational awareness and thus be capable of real-time planning and execution of traverses, the goals and objectives of which are planned pre-EVA in consultation with the base/ground. Research into optimal operational frameworks in which Moon-Earth traverse briefings/debriefings/planning take place between EVAs, and the highly trained crews are left to execute the pre-planned traverses according to their superior, in situ, situational awareness. Such operational frameworks will be required for Mars exploration. Ground will more likely focus on continuous, parallel operations of the RLRV, and integrating these results into the inter-EVA debriefings and planning sessions.

<u>Synthesis</u>: These Architectural Definition concepts and requirements can now be used to explore low-upmass in situ building materials [15,24] and inflatable architectural elements [25] for further conceptualization and design of the Hadley Max 500 day DRM (see sections 5 and 6, below).

4. Traverse Design And Implementation:
We now utilize the results and guidelines above to explore traverse design and implementation. *Mission Planning and Operations Guidelines*: During a lunar day (~30 Earth days), we assume 15 consecutive Earth days during Lunar Night (dedicated base laboratory and traverse debriefing-planning activities), followed by 15 consecutive Earth days in Lunar Day (dedicated to EVA operations). In order to optimize human performance, we assume a 6-3-6 Earth day duty cycle, with 3-day 'weekends'. For daily duty cycles, we assume 8-hour sleep periods, and 16 hour work-rest periods. We further assume 8 hour EVAs (further adjusted for changing lighting geometry), and a 10 km radius of operations (walk-back constraint) from the base. Hadley Max Robotic LRV (RLRV) traverses will be designed following the DRM Astronaut traverses with the goal of complementing these with scouting, interpolation, and extrapolation RLRV campaigns. *Landing Sites*: On the basis of the desire to first build on the Apollo 15 crew observations and analysis of the returned samples, and secondly to extend the major goals to a broader exploration and sampling region [17], we chose the Apollo 15 landing site as the prime landing site (Fig. 5a,c). Using the 100-200 km radius of operations defined by the distance from the A15 site to the farthest ROSI (Fig. 5a), we chose the second landing site/base of operations to the W of Hadley Rille in order to access all ROSI and explore the full range of mission scientific goals and objectives. EVA Radius of Operations: This was defined by the 'walkback

distance' (~7 km) during Apollo, the maximum distance that the astronauts could walk back to the LM in the case of LRV mobility failure (in turn constrained by consumable supply and astronaut metabolic rates). For Hadley Max, we assume an improved suit capability to deliver a 10 km radius of operations, but identify this as a key enabling technology requirement. We also introduce and explore the concept of doubling the radial distance of operations by having "Human Outposts" at key locations within the 10-20 km radial circumference from each site, installations that would permit outpost pressurization, human overnight stays and suit consumable recharge, all requiring key technology and design developments. We utilize this, and related concepts (e.g., 'pup' tents, simultaneous and parallel robotic operations, etc.) developed in the Mission Architecture contribution [22]. *EVA Station Duration*: During Apollo 15-17, the average station duration was 38.7 min, largely defined by the multiple objectives and their intervening separation distances. The longest duration stations were A15-EVA 2-Station 6/6a (highlands boulder, steep slope; 1 hr 20'), A16-EVA 3-Station 13 (Shadow Rock; 1 hr 19') and A17-EVA 3-Station 6 (large boulder; 1 hr 14'). Due to the improved scientific understanding and more focused questions for Hadley Max, we assume a typical station duration of 1 hour. *EVA Duration Duty Cycles*: Typical A15-17 EVA durations were ~7 hours each, a number constrained by human physiology/diurnal cycles. We assume modest improvements in suit efficiency and mobility speed and adopt an EVA duration of 8 hours for Hadley Max. *Station Duration*: A15-17 on-station times averaged 38.7' and we adopt 40' for a typical station for Hadley Max. *Stations per EVA*: Assuming 8 hour EVAs, we adopt a planning number of 5 stations/EVA (the A15-17 average for 3 EVAs was ~12 stations, 4/EVA). *Drive Times Between Stations and Average LRV speeds*: Average A15-17 drive times between stations were ~17' and average LRV speeds ~7.3 km/hr: given increased efficiency in terrain knowledge, route planning algorithms, and improved LRV design, we adopt 15' and 10 km/hr for Hadley Max. *EVA Planning Strategy*: On the basis of the above considerations, for Hadley Max average traverse planning guidelines, we assume 5 stations/EVA (5 hours), ~15' travel between stations (1.5 hours) and 1.5 hours for flexibility, for a total of 8 hours. During Apollo, due to the walkback constraint and consumable consumption, EVAs were designed to visit the most distant station first, and then work back toward the LM. We adopt a similar strategy for Hadley Max.

Ability to Traverse Slopes: A major scientific goal is to explore the lunar highlands and the Hadley Rille floor and wall. Experience with Apollo 15 shows that traversing steeper slopes at the base of Hadley Delta (EVA2-S2) (Fig. 5b,c) had a major effect on LRV mobility (wheel slippage, etc.) and Astronaut mobility and metabolic rate. On the basis of data from Apollo and Lunokhod [26] we adopt a maximum traversable slope of 20°, and increase non-mare station times by ~20%. We identify improved human and robotic rover capability on slopes and improved astronaut slope exploration and sampling strategies, as necessary key enabling technologies. One of the major unknowns in traversing and sampling the highlands is the origin of the "elephant-hide terrain", a wrinkled, terrain-parallel morphological texture associated with highland slopes; [27] found that the majority of slopes steeper than 6°–8° in their analysis are covered with EHT. Ridge separation distances are estimated at meters-scale and heights <~ a meter, but the origin of the ridges, their grain-size, and mobility characteristics are unknown. Thus, understanding the EHT is one of the highest operational and scientific priorities in highlands exploration.

*Improved Data for Human and Robotic Traverse Planning and Analy*sis: Apollo 15 site selection and traverse planning were accomplished utilizing 20 m-resolution LO-V images available at the time [19]. The NASA Lunar Reconnaissance Orbiter Mission has operated in lunar orbit for the last 14 years and has provided extremely high resolution images, altimetry, stereo photogrammetry, thermal inertia, water detection and radar data [28]. These fundamental data sets can be readily utilized to produce very high resolution image, topography, roughness, blockiness, slope and evolving lighting conditions maps that are essential ingredients to determining detailed science objectives, station locations, and traverse planning routes for optimal EVA planning. For example, Fig. 6a shows a slope map for one section of Hadley Rille and adjacent highlands, and Fig. 6b shows a slope map for defining human-robotic traverse access from the 20° slope constraint, data essential for human/robotic traverse design.
In addition, the advent of sophisticated mission and traverse planning software that can ingest and maintain cognizance of these multiple spatial data sets in real-time has revolutionized both pre-mission traverse design and planning, and real-time traverse assessment and contingency planning. For example, recent developments in mathematical frameworks for reasoning under uncertainty (Partially Observable Markov Decision Processes; POMDPs) have been applied to automated decision support frameworks for planetary exploration. Such applications include SHERPA (System Health Enabled Real-time Planning Adviser) [29] that is designed to take different sources of uncertainty into account when generating decision recommendations for traverse planning and real-time operations. We are currently exploring a range of recently described algorithms for optimizing traverse planning. In the next stages of the Hadley Max DRM project, we plan to test and assess some of these for optimizing human-robotic performance and science return.

5. Reducing Upmass Demands With *In Sit*u Myco-Architecture:
Here we address one of the most significant problems for long-duration and sustained human presence on the Moon and concurrent scientific exploration success: the Key Enabling Technology to alleviate the huge and continuous upmass requirements necessary to support the base and exploration infrastructure [22]. In order to help alleviate this "upmass roadblock", we have pursued two promising technologies: 1) *Myco-Architecture* [15,30-32], where building materials can be "grown in situ" in order to significantly minimize upmass penalties, and 2) *Inflatable Structural Elements* [25], in which low-volume, low-mass inflatables can be combined with Myco-Architecture to produce a wide range of enclosures in situ. Here we outline the evolution of our progress on "Myco-Architecture" and future goals and objectives.
In section 4 (Table 1) we defined and described *Required Habitats, Enclosures and Related Architectural Elements*. Here we investigate elements 1-6, and explore how producing construction materials in situ on the Moon can help alleviate the upmass problem. We plan to treat 7 and 8 in future analyses.

Background and Approach: Transporting materials beyond Earth, such as spacecraft, Astronauts, and construction materials, is limited by mass constraints. Yet long-term residence, operation and scientific exploration on the lunar surface requires an extensive infrastructure, a significant upmass, and a major large-mass component of this is in habitats, designed to protect crew and equipment from radiation, extreme temperatures and micrometeorite bombardment. There is a significant mismatch between habitat requirements at destination and what can realistically be transported there. Infrastructure for human survival is not automatically "user ready" on the Moon. Habitats could be built with locally sourced regolith

or ice materials by In situ Resource Utilization (ISRU), but in the end, even this requires significant upmass. To alleviate this problem, we have been exploring technologies [15, 30-32] that are self-replicating and self-repairing, to assess their utility in circumventing the upmass problem. Life meets these technological criteria for space utilization, and in addition can be reprogrammed through synthetic biology. In this quest, we look to exploit the genetic hardware store inherent in our vast biodiversity, moving capabilities from familiar forms such as trees for wood, to a more tractable space-faring chassis such as yeast or bacteria.
Strategy and Concepts: A critical aspect of human space exploration and eventual settlement is the ability to construct habitats while minimizing payload mass launched from Earth. To respond to this challenge, and as a continuation of our research program initiated under the auspices of the "Myco-architecture Off Planet" NASA NIAC Team, we have explored the use of fungal biocomposites, for example Bio-Bricks, (Fig. 7) for growing extra-terrestrial structures and building materials, directly at the destination, significantly lowering the mass of structural materials transported from Earth and minimizing the need for high mass robotic operations and infrastructure preparations. Currently, the idea of working with living biological organisms, and the phenomenon of growth itself, is of increasing interest in architecture and space applications. Here, we describe the use of mycelium-based composites as an alternative, biological approach for constructing regenerative and adaptive buildings for extraterrestrial habitats. These composites are fire-resistant and insulating, and do not consist of volatile organic compounds from petrochemical products. These can be used independently or in conjunction with regolith, and could employ the living biological growth in a controlled environment for the process of material fabrication, assembly, maintenance, and repair, providing structures resilient to extra-terrestrial hazards. We explored avenues to make this biological approach feasible, providing new, growing materials for designing and building sustainable habitats for long-duration space missions.

Our research has explored the potential and challenges of using mycelium-based biocomposites for space applications. The approach of using biological growth for the off-Earth construction, similarly to other researched ISRU-based approaches, is designed to lower the mass of materials needed to be transported from Earth. In addition, it focuses on lowering the energetic costs of the construction of in situ habitats, such as the work required to assemble the habitat. In the long-term, using biological materials and growth as a construction method, opens up the potential of ELMs (Engineered materials composed of Living cells that form or assemble the Material itself or modulate the functional performance of the material in some manner) [33]) to potentially provide supplementary capabilities, such as sensing and responding to environmental stimuli, self-healing, etc. Such developments could make the habitats even more flexible and reliable. The further development of research on ELMs and mycelium-biocomposites will allow for advancements in the field of biotechnology and habitat construction. These concepts employ living biological growth in a controlled environment for the process of material fabrication, assembly, and maintenance. Positive attributes of these approaches and techniques include the modest upmass requirements of a few spores, nutrition for mycelial growth, and a growth framework, along with the potential to reproduce using in situ resources, the ability to grow to accommodate on-site terrain, and the potential additional control provided by the tunability of the materials. We see myriad possibilities for mycotecture utilization off planet. Because the research is still in an early stage, one of our major goals once the enabling technologies are identified, is to use the Hadley Max 500-day DRM Architecture to develop a technology roadmap and recommendations for further development.

6. Reducing Upmass Demands Utilizing *In Situ* Inflatable Structures:
The required structures and architectural elements described in Section 3 (Table 1) are the basis for using the Myco-Architecture [24] concept to explore reducing upmass demands utilizing *in situ inflatable structures*.

Architectural designs and deployable in situ construction methodologies: In concert with the development of Myco-Architectural biological material, architectural designs and deployable in situ construction methodologies were developed at redhouse studio, including plans, 3D models, section details, and animations of various designs and building processes. After assessing many ways to deploy bio-composites off planet (including lightweight formwork, masonry and additive manufacturing), redhouse arrived at a 'sealed bag' deployment (Fig. 8) as the best option to control the environment for growth, develop the shape of the shelter, and protect the lunar environments from potential contamination. The sealed bag concept will allow the bio-composites to self-assemble in multiple layers of membranes that can provide redundant protection, channel nutrients, and create warm habitable spaces within the framework. This can be achieved at many scales and could be utilized as a platform technology for building any mission structure or object (Fig. 8-10).

Evolution of Architectural Concepts: The design concept started as deployable habitat shell that would grow like a living organism at destination with the aid of in situ resources. This would be less energy intensive and leave a smaller planetary footprint then mining or melting surface material. Intense team study and analysis has enabled the initial concept to grow and evolve new multi-functional facets. We found that the biological functions that enable growth of the materials also provide such benefits as oxygen production and may also be used to generate heat and electricity. Thus, this biomimetic and bio-utilitarian option provides potential options to very high up-mass costs of prefabricated structures that come fully outfitted, and other construction materials. Detailed architectural and design analyses suggested that necessary attributes, such as plumbing lines, stovetops, and floormats, can be folded into the form, plugged-in ready to go, and the floors, walls and windows can be grown in place so that the in situ grown building is comparable to a high-mass Earth-fabricated, and then delivered to the site, structure. In order to accomplish this, however, the challenge is in the packing of the habitat shell into off-planet deliverable cargo geometry constraints. We found that many of the domestic utilities, scientific equipment, furnishings, and fixtures can be built directly into the expandable shell. More detailed assessments showed that such self-contained modules can be wrapped into the larger structure and secondarily deployed once robotic-enabled construction of the shell is finished.

Materialization: The process of making fungal composites includes growing filamentous saprophytic fungi on biomass substrates that can become fused at a cellular level. Our team has demonstrated composites that have structural characteristics superior to wood framing, thermal resistance characteristics superior to fiber-glass batt insulation, and fire resistance equivalent to type-X gypsum board, that is, construction industry standards, all comparable or superior to other ISRU suggested materials.

Construction Methods: Building Envelope: The building envelope grows as three pneumatic rings provide initial structure and the circulatory system then delivers nutrients to the building

membrane cells. An intersection of three rings inflates to set the structural form. This can be delivered by compressed gasses and/or water, or from compressed canisters embedded within the rings. Rovers can be used to deliver the in situ resources, or the mechanisms could be embedded within the folded skin. The rings will later be filled bio-composites, but as air filled tubes they initially and immediately serve as scaffolding to let the micro-organism begin permanent construction. The building's circulatory system feeds the membrane cells to grow the bio-composite structure. Cyanobacteria embedded within the cells are fed water, nitrogen, carbon dioxide, and other nutrients sourced in situ. Heat is supplied to the cells creating the right conditions for growth. The organisms grow, releasing oxygen that is stored within a special bladder. Once the cyanobacteria have reached a critical biomass, the nutrient rich substrate is dehydrated to a level that would support myceliation by saprophytic fungi. Oxygen is then released back into the cells and the fungi can feed on the oxygenated algal biomass. The fungi branch between the algal cells and begin to devour them by secreting enzymes and converting the external dissolved cellulosic material into chitin within the fungal cell walls. The algal biomass becomes fused with the mycelium at a cellular level and is heated and compressed by the heat-traced pneumatic membranes. The multilayer system allows for redundant protection and separation of materials in various states of matter. The hydrogen produced allows fenestration while providing radiation protection.
Synthesis: In situ inflatable structures clearly permit significant reductions in upmass penalties, and also offer many other ancillary benefits, such as radiation protection. They also offer significant reduction in environmental impacts of on-site construction from local material. We are continuing to explore innovative ways in which a) in situ Myco-Architecture, combined with b) inflatable structure concepts, can optimize the scientific goals and objectives of long-term missions to the Moon and Mars, as illustrated by the Hadley Max 500-day DRM [17, 19-22, 24].

7. Architectural Implications, Outstanding Problems, Next Steps and Feed-Forward to Mars:
The results of Phase 1 of our Hadley Max 500-day Design Reference Mission (DRM) have served to illustrate the fundamental scientific goals and objectives, the distribution of Regions of Interest (ROA) at which they can be accomplished, and the nature and distribution of the architectural elements (Table 1) that are necessary in order to support this extended and extensive scientific exploration. We have further identified two promising technologies and approaches (Myco-Architecture and In-Situ Inflatable Structures) to addressing the significant upmass challenges facing any long-term missions to the Moon and Mars.
But how can we bring together these results (Table 1) to better define and clarify whether the Myco-Architecture/In-Situ Inflatable Structures approach can successfully provide the architectural elements that are necessary to support the long-term, long-duration scientific exploration of the Moon and Mars?

Phase 2 of the Hadley Max 500-day Design Reference Mission (DRM) will address this question. During Phase 1 we learned many lessons and encountered some problems associated with defining more specifically the architectural elements for which the Myco-Architecture/In-Situ Inflatable Structures approach is critical. Among these were:

1. <u>Trafficability and Traverse Design and Implementation</u>: We found, on the basis of the experience of the Apollo astronauts, that slopes in excess of ~15 degrees were very difficult to traverse in both walking and LRV modes. We also found that many fundamental scientific objectives were in areas with slopes in excess of 15 degrees, from which we concluded that

both human and robotic rover exploration would be necessary for mission success. In the next phase, we will compile multiple sets of human and robotic rover traverses designed to address the basic scientific objectives, and from this, we will clarify the number, locations, and types of architectural elements (Table 1) required for mission success. We will also 1) explore further the use of Shape from Shading (SfS) techniques [36] for the production of high resolution topo/slope maps to improve our traverse planning, and 2) explore new algorithms to assist in traverse design and implementation [e.g., 29].

2. Environmental Conditions: What are the *environmental conditions* under which humans can effectively work and live, and how does this affect the scale, nature and robustness of the architectural elements (Table 1). For example, how do the locations of the elements, and the duration of crew staytimes, influence the requirements for enclosure design, and micrometeorite/thermal/cosmic ray protection? These data will be important for further definition and requirements for the Myco-Architecture/In-Situ Inflatable Structures approach. The same factors apply to the robotic rover support and caching/servicing enclosures (Table 1, Item 6). Traverses designed I Phase 2 will help to define the number and mass of samples collected, the distances between stations, the ancillary requirements for mission support (landing pads for robotic sample return, instrument resupply/servicing, etc.).

3. Resupply Frequency: On the basis of our results in Phase 1 and 2, what is the resupply frequency for the Myco-Architecture/In-Situ Inflatable Structures approach, and how do these estimates feed back into the design and implementation of the approach in order to minimize upmass.

4. Application to the South Circumpolar Region of the Moon: The Artemis campaign is designed to provide long-term bases in the South Circumpolar region of the Moon. During our Hadley Max DRM Phase 2, we will assess how the very different geology of this region [37-39] and the extremely rigorous environmental conditions (illumination geometry and temperature variations) will to a first order, influence the requirements of the Myco-Architecture/In-Situ Inflatable Structures approach there.

5. Feed-Forward to Mars: As part of our Hadley Max DRM Phase 2, we will also assess the feed-forward of such mission designs and architectural elements to similar long-duration missions to Mars. In contrast to the Moon, the Mars environment is characterized by a more Earth-like length of day, access to water ice, but an atmosphere that is characterized by often high velocity winds. We will call on our extensive experience in exploring the Antarctic Dry Valleys [e.g., 40-41] to assess how the architectural elements will differ in design and structure, as a way to assess the full range of requirements for the successful use of the Myco-Architecture/In-Situ Inflatable Structures approach technology.

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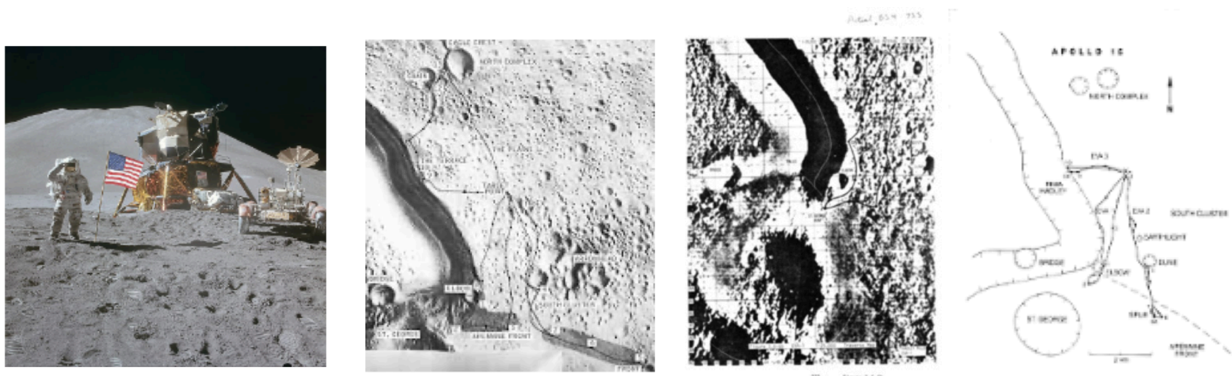


(A) (B) (C) (D)

Fig. 1: (a) LMP Jim Irwin, the Lunar Module (LM) and the deployed LRV; Mt Hadley in the background. (b) The nominal preplanned traverses with the LRV. (c) Apollo 15 onboard traverse map with Lunar Orbiter 20 m resolution images used as the highest resolution base planning data. (d) Completed Apollo 15 Traverses.

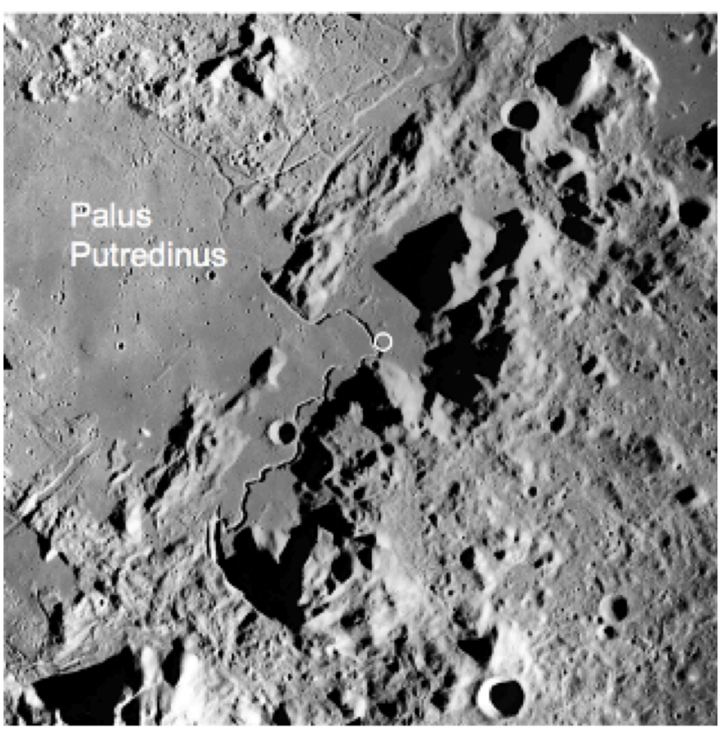
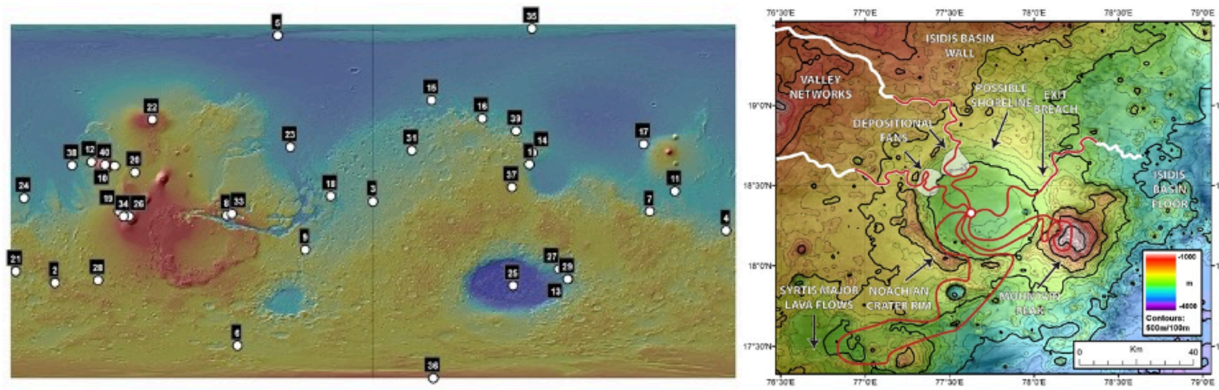
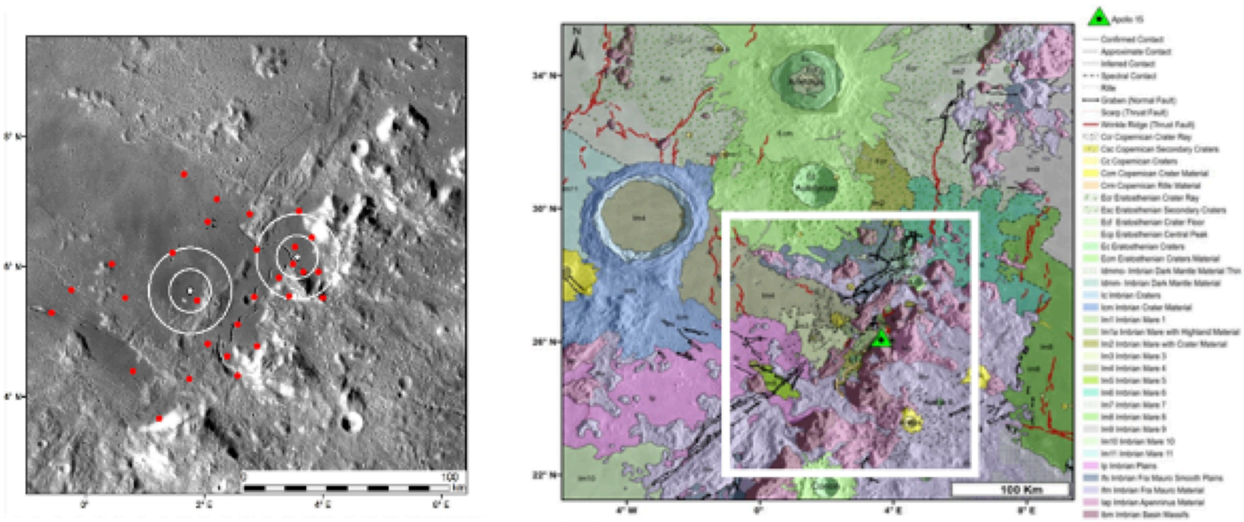


Fig. 2. The broader Hadley-Apennine region showing multiple points of geological interest beyond the 7 km exploration radius around the Apollo 15 landing site (white circle) (see Fig. 1b, d).
(a) (b)



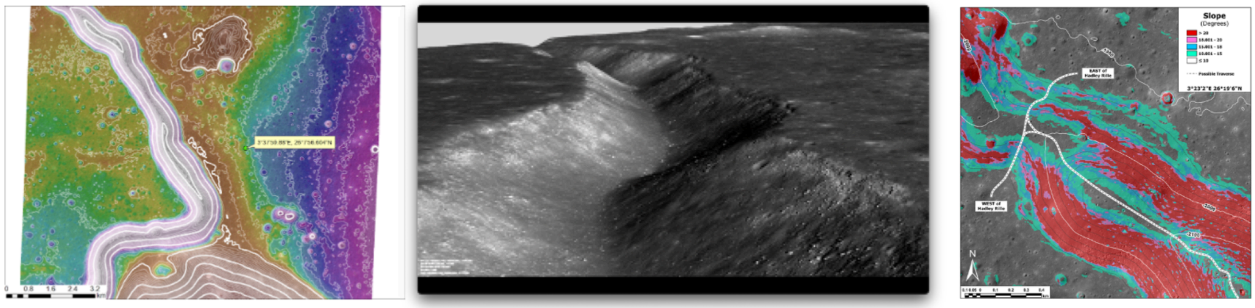
(A) (B)

Fig. 3 (a): Forty scientific human exploration landing sites identified on Mars. (b). Example DRM and traverses at Jezero Crater (from [12]).
(a) (b)



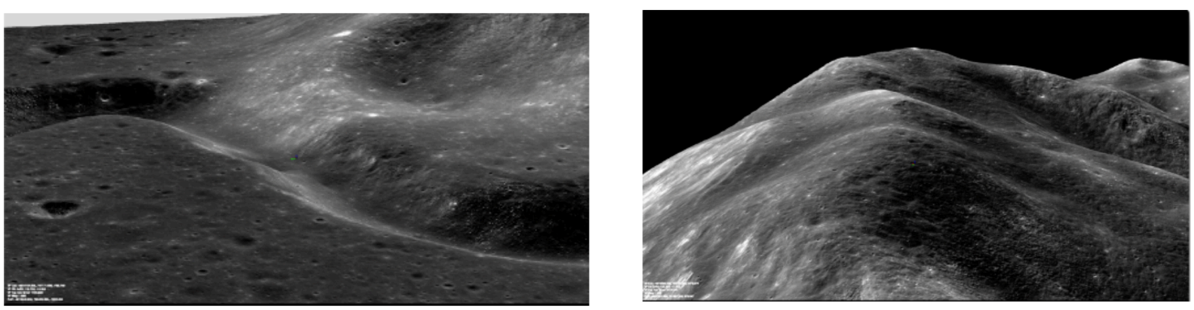
(A) (B)

Fig. 4. (a) Hadley Max region; circles show 2 base sites and 10 and 20 km radius around each. Red dots; selected ROSI [4]. (b) Recent geological map [2]; box shows left image (LROC WAC) location.



(A) (B) (C)

Fig. 5. In Figure 4a, the Hadley Max exploration region showing two base locations surrounded by 5 & 10 km radius circles with dots representing selected ROSI locations, represents the scientific input into human/robotic traverse design. For traverse design, and to distinguish where robotic rovers are required for extended exploration, we highlight in (a) a topographic contour map for a section of Hadley Rille and the rille-mare-highlands area near the A15 landing site (green dot) (thick lines 100 m, thin 10 m). b) Perspective view of Hadley Rille, facing approximately NNW at a height of ~800 m above ground. c) Slope map for a portion of Hadley Rille, showing potential entry points for human and robotic traverses from both the East and West to explore the rille wall and floor.



(A) (B)

Fig 6. (a) Perspective view of a section of Hadley Rille and St. George crater and adjacent highlands (base of Hadley Delta) showing the lower slopes of Hadley Delta explored by Apollo 15 Astronauts Scott and Irwin. Perspective view facing approximately ESE at a height ~1.2 km above ground. (b) Perspective view of a section of the summit region of the Hadley Delta highlands in an area only accessible by robotic rovers due to the 20° slope constraint. View facing approximately SSE at a height of ~3.5 km above ground.

(A) (B)

Fig. 7. (a): Bio-Brick made of Myco-Architecture Materials (Courtesy Chris Maurer): This brick is a composite of wood and fungal mycelium. Fungi break down biochemicals like cellulose converting them into their own chitin-rich biomass. By growing mycelium on plant fodder they fuse at a cellular level allowing us to utilize the best characteristics of their respective Kingdoms - Plantae and Fungi. We are currently developing methods to grow these multi-kingdom composites off-planet to save transport cost, reduce energy demands, and utilize bio-performative aspects such as radiosynthesis, that may one day convert space travel's biggest liability, ionizing radiation, into a resource for material production. Bio-Brick dimension is 17 x 12.5 x 5.5 cm. (b) J. Head and NASA Administrator Bill Nelson examine one of our Bio-Bricks at Brown University. Photo by RI Senator Jack Reed.

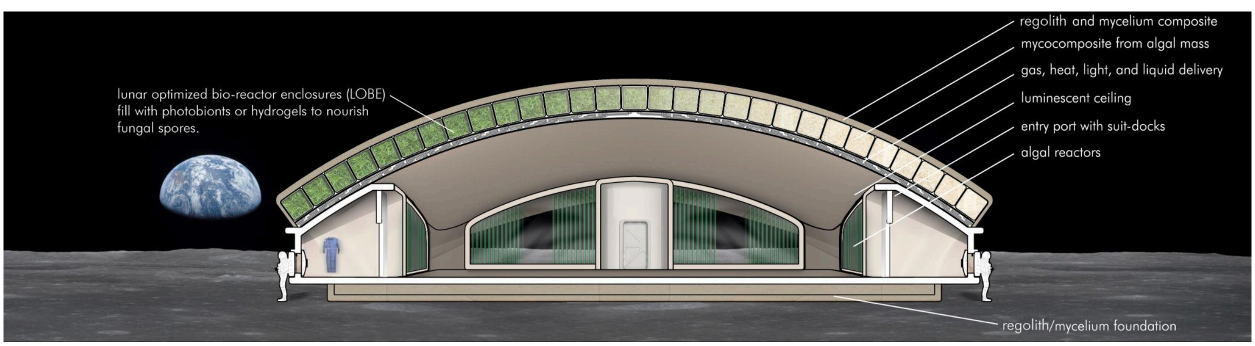


Fig. 8: Section through prototypical habitat showing lunar optimized bioreactor enclosures (LOBEs) from left to right first growing photosynthetic organisms that are then converted into a composite bioterial by the growth of fungal mycelium.

Figure 9: Stills from animation showing the "growth" of a prototypical building.

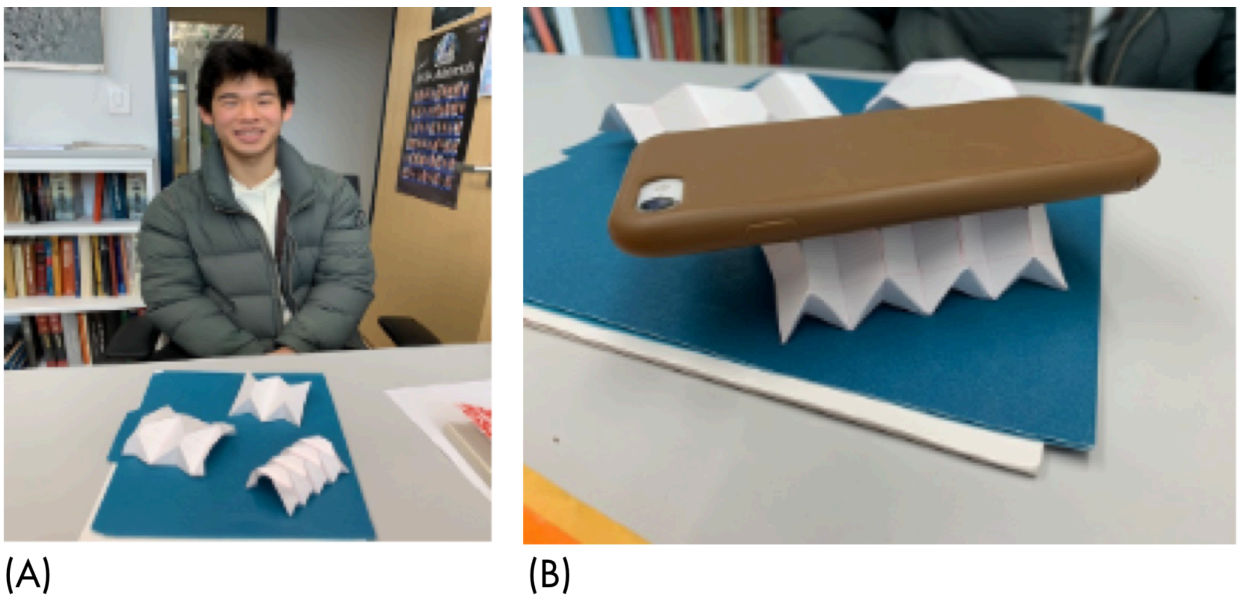


Fig. 10. a. Brown Undergraduate Christian Wu and his "Flexible Origami" inflatable bag cargo storage solutions. Right. Strength of "Flexible Origami" (I-Phone atop structure).

Table 1: Hadley Max design reference mission baseline required architectural elements:
1. Landing Pads (LP): Helo pad-like (flat, devoid of soil backwash contaminant). Both Human and Robotic missions.
2. Initial Base Structure (IBS): Living and working habitat; follows initial landing module (LM) stages.
3. Evolutionary Base Structure (EBS): Larger scale, separation of work/living activities; increased in situ science activities; IBS evolves to dust mitigation structure.
4. Outposts: Remote Science Bases (RSB): Modeled after IBS, but located >10 km radius from Landing Site. Require up to ~5 RSBs for in depth, in situ science activities. Increase number in order of science priority.
5. 'Pony Express' Stations (PEX): These are the lunar 'pup tents' [34]that will be precursors to the Remote Science Bases (RSB); also serve as Earth-day sleep-stations on the way to the final Remote Science Bases (RSB). Sample storage stations, geophysical stations; can be resupplied/samples collected by CLPS missions.

6. Robotic Rover Requirements: a) LRV garage at base for surviving lunar night, re-outfitting; b) Robotic LRV 'pup tents' for surviving lunar night, caching samples.

4.6.2 LPSC abstracts submitted 2024

Hadley Max 500-Day Design Reference Mission (DRM To The Apollo 15 Hadley-Apennine Region: Application Of Science Goals And Objectives To Planning Long Duration Exploration Architecture See Appendix 11.3.

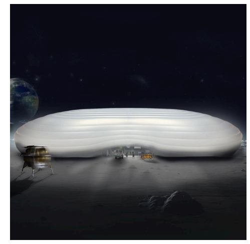
4.7 Identification of enabling technologies, and production of a technology roadmap and recommendations for further development

The technology road map from the end of Phase II to the first Martian self-growing habitat will move through many steps and programs but is within sight. The team has built many models, developed novel materials, planned mission architectures, and built the Earth's first structural mycelium habitat, through the spin-off technology at MycoHab.

Next steps involve continuing to develop ever larger and more sophisticated prototypes, advancing material formation, and testing integrated models in various environments. We have identified two major goals en route to realizing our goal of structures on the Moon and Mars. The first is to integrate our materials in LEO on a space station, and second test the prototypes on the surface of the Moon through the CLPS program. Table V describes major milestones. We recognize there will be many sub-tasks on the critical path not mentioned below.

Table V. Technology roadmap.

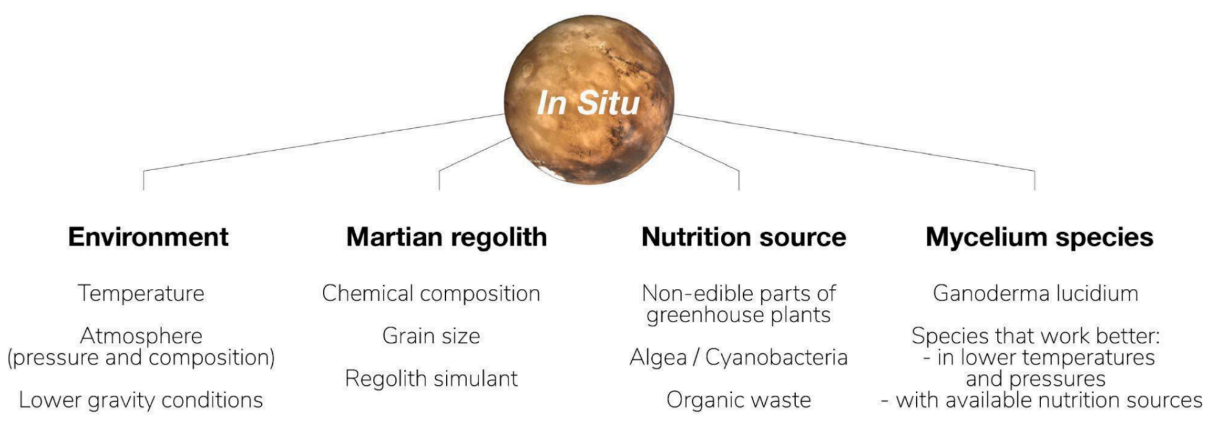
Program	Step	Description
Post NIAC II	0	Continue developing and testing grow in place (GIP) technologies. The team will continue to grow bio-reactivatable aerogels and test their thermal insulation and radiation attenuation characteristics. Tests are ongoing with McMaster Planetary Simulator using UVA,B, and C and extreme temperatures. With our colleagues at Melatech LLC we will be able to test materials on Brookhaven National Laboratory's galactic cosmic ray simulator. Continue developing wild-type fungal strains and accompanying living consortia for material purposes.
NIAC Phase III	1	Develop prototype for Starlab® Space Station interior: The prototype will be a mixture of living and inert materials. The inert materials will be bio-manufactured by our team on Earth and will be placed in conjunction with GIP modules that form materials to finish the wall in LEO. Develop bioengineering capabilities for target fungi and accompanying living consortia: transformation, synthetic biology, laboratory automation and artificial intelligence tools. 

	2	Develop Prototype for testing model of grow-in-place architecture for CLPS mission. One route is through the NASA PRISM program. Alternatively, we can focus on the needs of the providers themselves. Astrobotic Technology® is in need of a rover garage which fits with our building types 6a and 6b of the Hadley Max 500 day design reference manual (HM 500D DRM) for LRV stations. The model will be enclosed with a rack of the CLPS with 2 way communication for assisting and documenting the growth of the model building.Use developed bioengineering tools to improve survivability and growth rate of target fungi under environmental conditions. Use bioengineering tools to functionalize mycelium materials with added capabilities, such as melanization, mineralization, remediation of toxic compounds, biomining and self-healing. Develop composite materials composed of engineered consortia of living organisms for added functionality, such as growing a lunar launch pad using lunar regolith. 
Program TBD	3	Develop full size prototypes of the HM 500D DRM on Earth for testing in various conditions including vacuum and extreme temperatures like that of the Space Environments Center at the Neil Armstrong Test Facility in Sandusky, Ohio. Test newly developed living composite materials alongside full size prototypes.
Program TBD	4	Develop and test lunar landing pad as part of HM 500D DRM 1.0LP on lunar surface.
Program TBD	5	Develop and test remaining archetypes as part of HM 500D DRM including 2.0 Initial base Structure on lunar surface.
Program TBD	6	Occupy, monitor, and evaluate deployed archetypes.
Program TBD	7	Optimize architecture, materials, and processes for martian environment and test in martian simulated environments on Earth (Armstrong Test Facility or others)
Program TBD	8	Test archetypes on martian surface.
Program TBD	9	Occupy, monitor, and evaluate deployed archetypes.

Next steps for the development of the regolith-mycelium composites: The extremity of the targeted environment, Mars - the environmental conditions (temperature, atmospheric pressure, composition, lower gravity), the chemical composition of the regolith, and the variety (or lack of variety) of possible nutrition sources provide a major challenge to the study. The initial experiments with mycelium and sand proved that mycelium can successfully hold sand particles together and act as a reinforcement system to create structural elements. However, to fully adapt this approach to martian conditions, other environmental aspects need to be considered, like minimizing water content, and oxygen levels.

Some of the tests were with sand and some with regolith simulant. In the next steps, a chemically and geologically credible regolith simulant should be used to test the impact of its grain size and chemical composition on the creation of biocomposites. It needs to be established whether the processing is required to use martian regolith for composite creation, and if so, what kind of processing is needed and what kind of infrastructure could provide that. Addressing these questions would allow for a clearer understanding of the potential for using mycelium together with a martian regolith as a building material for future missions to Mars.

Another important aspect that needs to be considered is the source of nutrients. Biological matter providing the mycelium with nutrients and therefore energy to grow should be replaced with potential greenhouse plants and/or algae or cyanobacteria. However, a nutrient dense food source, such as used in a laboratory setting, might ultimately prove to have lower upmass and derisk the mission with water obtained at destination. Changing the source of nutrients would affect the growth of mycelium therefore every step and new variable needs to be tested and carefully monitored. All of the mentioned factors - environment, regolith, and nutrition source will have a direct impact on the behavior of the chosen mycelium, therefore, in future research, different mycelium species and strains should also be tested. Research on using algae as a nutrition source for specific mycelium species is already being conducted in collaboration with this study .[55] However, to advance this approach as a construction system for Mars, all studies need to come together [Fig. 53].



[55] Fuentes Musitu, N., 2011. "Myco-algae composites for space architecture: strain engineering of mycelium grown in cyanobacterial substrates," DTU Department of Biotechnology and Biomedicine Masters Thesis..

Figure 53. Considerations associated with the in situ *production of the mycelium-based regolith composites on Mars - a far more extreme environment than Earth.*

The next steps, leading to the development of in situ fabrication and construction roadmap, would also include solutions for the regolith collection and processing (mixing with biomass and mycelium) the mold fabrication together with molding and de-molding process, the assembly process (dropping the elements) and the development of the "growing chamber", providing the right conditions for mycelium growth. The growing chamber would also hold the planetary protection role; working with biological materials is crucial to ensure that life and other forms of contamination are not transferred from Earth to Mars.[56]

Ultimately, key factors such as mass, volume, and power will determine the success of the construction system. Therefore further research needs to be carried out, to answer unresolved issues. For example, it would be important to determine whether sufficient nutrients can be provided to sustain mycelium growth using greenhouse byproducts. The amount of material that needs to be transported from Earth and the amount of structure that can be constructed using the proposed mycelium-based system will also be explored. Therefore, a comparison will be made between the proposed system and traditional habitat construction approaches in terms of these metrics to determine its feasibility and potential advantages.

4.7 Terrestrial applications

This NIAC has led to many terrestrial applications and terrestrial analogs. A common question we hear about our work is, "if we can grow buildings off-planet, why can't we grow them on earth?" And we respond by saying thanks to this NIAC funded research, we now can. The first structural mycelium building was completed in December 2023 in Windhoek, Namibia bragging a lineage to NASA's NIAC program.

4.7.1 MycoHab

Figure 54. Mycohab

Co-I Maurer has founded a company in Africa called MycoHab that is currently using mycotectural processes to convert an encroaching species of bush that is causing desertification in Namibia into food and housing made of carbon sequestering "mycoblocks" (Fig. 55). The team there has built the world's first structural mycelium building in Windhoek, Namibia. At one point the company had experimented with inflatable formwork like that in this NIAC, but has ultimately come away from that opting for more traditional building methods. The process works by extracting the deleterious bush, grinding it into sawdust, placing the ground up biomass in autoclavable bags, pasteurizing the bags, inoculating the pasteurized

[56] Kminek, G., Conley, C., Hipkin, V., Yano, H. 2017. "COSPAR's Planetary Protection Policy," Committee on Space Research.

substrate with mycelium (Pleurotus ostreatus), and incubating for 30 days. After 30 days the bags are fruiting mushrooms and the mushrooms are harvested and taken to market. The resulting waste material is composite of mycelium and sawdust bound tightly by the hyphae. The composite is loaded into a press and compacted into blocks using standard thermos-mechanical densification methods. In 2023 MycoHab completed the Earth's first structural mycelium habitat in Windhoek, Namibia.

Figure 55. Mushrooms are grown on waste encroacher bush and the by-product of the cultivation (mycelium composite) is pressed into blocks that rival concrete strength and store organic carbon.

4.8.2 Every day aerogel products.

In an effort to expand the research on mycelium infused hydrogels and aerogels, the team has submitted a proposal to the National Science Foundation's Convergence Accelerator. The Team lead by Co-I Senesky aims to develop terrestrial applications for the bio-gels we are developing and testing in this NIAC and take that aspect of the design much further (Fig. 56).

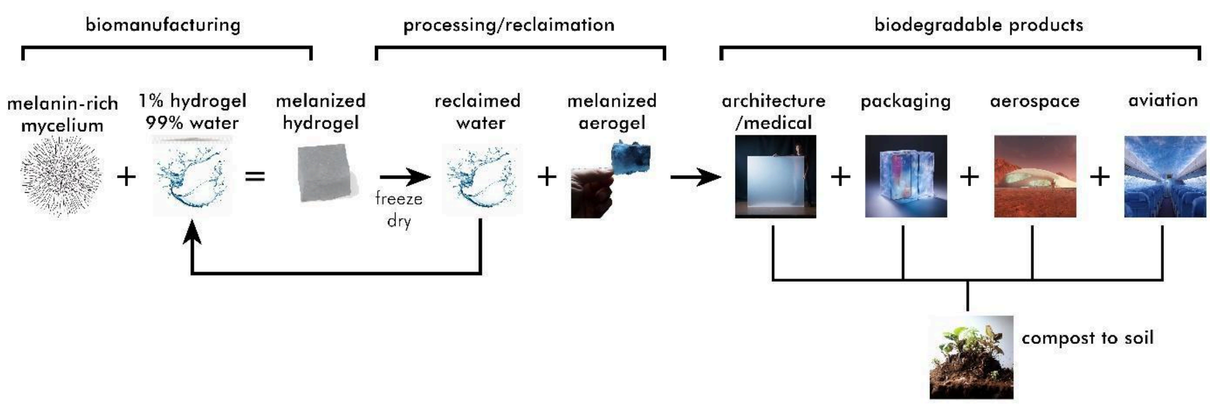


Figure 56. The above image shows the basic bio-manufacturing process and possibilities for everyday products made from melanized aerogels. The fungi grow naturally on nutrient rich hydrogels and are converted into aerogels by freeze-drying. An added benefit is that the water could be reclaimed in the manufacturing process.

This work will focus on simultaneously achieving shell-like protection, ultra-lightweight properties, and zero waste production. Mycelium biocomposites are a unique class of materials where a three-dimensional filamentous network is formed with fungal mycelium and a biogenic substrate. These biocomposites have generated excitement due to its lightweight structure, self-assembly, and thermal insulation, as well as biodegradable properties. Yet, difficulties in realizing large-scale manufacturability of mycelium biocomposites have hindered mass adoption into target applications such as architecture, aerospace, and packaging. This NSF work will explore mycelium aerogel synthesis to enable lightweight structures. In addition, melanin deposits will be integrated into the mycelium aerogel matrix to provide environmental protection (thermal and radiation). Furthermore, the role of zero-waste synthesis parameters such as drying techniques, biodegradable feedstock, and reuse of reclaimed water will be examined. We will use bio-inspired approaches to achieve bio-performative everyday materials with unprecedented weight, strength, and environmental impact, as well as develop ethics-based curriculum.

4.8.3 Biocycler

Co-I Maurer founded a company called Biocycler that is developing a process of remediating and recycling organic construction and demolition (C&D) waste. The process works similar to Mycohab but instead of turning bush into food and buildings, biocycler turns old and potentially dangerous buildings into new and healthy ones (Fig. 57).

Figure 57.*Overview of the Biocycler process. The biocycler uses fungal bio-manufacturing to recycle and remediate construction and demolition waste.*

The process works by utilizing the metabolic processes of the fungi that reconstitute the hammermilled and pasteurized C&D waste. The fungi excrete enzymes that break down petrochemicals in the debris like asphalt, bitumen, and acrylics. Further research is being done to understand if biocycler can sequester and remediate heavy metals. This work is in conjunction with researchers at Case Western Reserve University, and Johns Hopkins University.

4.8.4 The Chill

As mentioned in the prototyping section of this document, part of our investigation into developing inflatable forms at various scales could lead to another terrestrial application. The Chill is a concept for temporary urban summer cooling stations that are highly insulative and 100% biodegradable (Fig. 58). The system works by inflating an air supported bioreactor for growing mycelium composites as an insulating shell. The cooling action could be standard air conditioning or as proposed here deploying an einstein-szilard refrigerator system for cooling water into ice at the center of the Chill. According to the USEPA 1300 heat-related deaths have been reported in recent years and the trend is upward.

Figure 58. *Concept for THE CHILL. A biodegradable bio-insulation is made in between reusable inflatable layers to make temporary cooling stations in urban centers for people at risk of heat related illnesses or death. The inflatable-mycomaterial concept was directly derived from this NIAC study.*

4.8.5 Mycotecture for tableware and food

At the three Michelin star Azurmendi restaurant in Larrabetzu, Spain, a pioneer in sustainability, a novel approach was taken to harness the biotechnological capabilities of *Aspergillus oryzae.* This project aimed at transforming coffee waste into valuable dishware prototypes, embodying the principles of a circular economy. The innovative process began with blending coffee grounds with sugar to create a nutrient-rich substrate for the fungus. This mixture was then sterilized and shaped into molds, followed by inoculation with *Aspergillus oryzae* spores. The setup was maintained at a constant 25°C, allowing the fungus to proliferate and form a dense network of hyphae over 48 hours, which conferred strength and stability to the molded forms. Finalizing the process, the structures were exposed to a high temperature of 180 °C for 5 minutes to cease fungal growth, culminating in the creation of durable, sustainable dishware prototypes (Fig. 59) designed to accompany coffee, thereby closing the sustainability loop at Azurmendi.

*Figure 59. From A*spergillus oryzae *agar inoculum to coffee ground wasted, used by Massa to produce a mycelium cup*

At the same time, fungal mycelium holds significant importance in food culture across various cultures, such as the development of Oncom in West Java, Indonesia, which is made from the filamentous fungus *Rhizopus oligosporus*. This fungus is noted for its high protein content, rapid growth, and low resource requirements, making it a subject of interest for testing at institutions like Mondragon University and the Basque Culinary Centre as a sustainable alternative protein source. In this project, the growth of this fungus on hydrogel surfaces is being explored to develop potential structures for edible purposes (Fig. 60). This innovative approach aims to leverage the unique properties of *Rhizopus oligosporus*, exploring its potential to contribute to sustainable food systems through the creation of new, edible structures.

Figure 60. Rhizopus oligosporus; *Edible Tissue*

4.9 Programmatic deliverables

The symposia posters from 2021 and 2022 are provided in Appendix 11.4.
The continuation review was held September 13, 2022 at redhouse studios in Cleveland, Ohio.
The Outside Reviewers were Jenn Macalady [PSU] and Quincy Bean [MSFC])

Morning presentations in redhouse office building.

9:30 -9:45 am - Chris welcome, Lynn intro

9:45 - 11 - The big picture
1. Architectural work (Chris, Monika, Martyn)
2. Inflatables (Dave, Chris) - includes virtual tour of Moonprint
3. Sand Work (Monika)
4. Technical Roadmap (Nicolas, Lynn)

10:45 - 11 Mission Context (Jim)

11:15-12:15 - How does this work?
1. Fungal work (Chris, Monika, Nicolas, Lynn?)
2. Mechanical testing (Debbie, Kat)
3. Intro to the McMaster simulator tests (Lynn)
4. MISSE (Kim deGroh,, Radames Cordero)

12:15-12:30 Earth based applications: Azurmundi (Eneko(s), Chris)

12:30-1:30 break for lunch and go downstairs where my chef friend who is very clever with fungi has agreed to prepare an astromycological themed menu.

1:30 caravan to Chris workshop (10 minute drive)
1:45-2:30 tour of redhouse (fungi growing on the inflatable and other fungi/inflatable assemblies. Nicolas algal station)
2:30-3 Program feedback - Feedback from Program; how to move forward
3 pm McMaster simulator=Virtual tour of simulator.

5 Management Approach including Team

Project management. PI Lynn Rothschild, a pioneer in using synthetic biology for space, is responsible for the success of the project. She oversaw the project, coordinated the team, and supervised the bioengineering work in her lab and provided a resource for the architects and mission design teams. Coordination was facilitated with biweekly virtual team meetings. All team members will continue to contribute to future papers, presentations and outreach as needed. All team members contributed to this final report.

Prototyping. Rothschild's lab at ARC and Co-I Christopher Mauer (below) will develop prototypes including scaffolding and hydrogels, and coordinate members of the team as appropriate (e.g., architects, *B. subtilis,* mission architecture for parameters). Rothschild's facilities include renovated lab space with equipment for synthetic, molecular and microbiology. Rolando Perez (Co-I), mycologist and radiation expert, conducted the biological work in Rothschild's lab and helped with grant deliverables. Eneko Axpe (Collaborator), a postdoc at Stanford & NASA specializing in nanotechnologies, will devise and provide nutrient hydrogels. Students were engaged in Rothschild's lab (see section 9). Other students will be under non-US team members at no cost to the proposal. Prof. Christopher Workman, Technical University of Denmark, is an expert in synthetic biology, systems and computational biology. He provided a masters student to assist on the project.d

Architecture Co-I Christopher Maurer is an architect and professor of architecture who designs and manages many innovative projects in limited resource environments. He will manage architecture, engineering, graphic representation, and form prototyping. Additional inputs and applications will be done by Prof. Martyn Dade-Robertson, who has a history in the area from his "Living Soils" project. Dade-Robertson is a Professor of Emerging Technology at Newcastle University with expertise in Architectural Design and Synthetic Biology. He developed prototype materials which integrate multiple functions with active environmental responses. Architect Monika Brandić Lipińska worked on the project in Rothschild's lab for her MS from the ISU, continuing during her Ph.D. under Dade-Robertson, with a focus on sand/mycotecture biocomposites.. Inflatables were produced by David Cadogan, President of Moonprint Solutions, and his team.

Mission Architecture was led by Distinguished Professor, James Head, Brown University, with the support of Brown students working under him. Head has expertise in planetary science and human flight from the Apollo missions on. The work was conducted at the Brown Center for Computation & Visualization and included input from David Scott, Commander of Apollo 15.

Exposure of prototype materials to extraterrestrial conditions took place in the state-of-the-art planetary simulator at McMaster University (Canada) by Ph.D. candidate Hannah Krivik, under the direction of Prof. Maikel Rheinstädter, a biophysicist.

Materials testing Mechanical characterization and thermal decomposition analysis of mycelia & prototypes.were conducted in the Soft & Hybrid Materials Facility at Stanford[57] under Prof. Debbie Senesky's direction by graduate student Katheryn Kornegay. SMF is a research facility for analysis and characterization of synthetic polymers, soft materials and polymer devices.

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[57] https://snsf.stanford.edu/equipment/smf/index.html

Co-I Jessica Snyder, a mechanical engineer with bioprinting expertise, provided expertise in materials testing and scaffolding..

Enhancements to fungal mycelia. Use of *B. subtilis* to supplement fungal mycelia was to be under the direction of Prof. Anil Wipat (Collaborator), a specialist in bioengineering B. subtilis to make useful products. Unfortunately he retired unexpectedly. Dr. Rolando Perez has begun that work. Rothschild, Perez, Workman and Maurer ideated other enhancements.

6 Journal Publications

To date we have one peer-reviewed paper published (Lipińska et al., 2022). Several manuscripts are in preparation including the mission architecture (draft manuscript in section 4.5.1).

Brandić Lipińska, M., Maurer, C., Morrow, R., Dade-Robertson, M., Senesky, Magdalini Theodoridou, D., Zhang, M. and Rothschild, L. 2022. Biological Growth as an Alternative Approach to On and Off-Earth Construction. *Frontiers in Built Environment/* PUBLISHED 19 September 2022 doi.org/10.3389/fbuil.2022.965145

7 Conferences

- Genetics Society of America 2022 (March 2022)
  - Invited presentation, Systems biology and biomaterials session. Mycotecture off planet: fungi as a building material on the Moon and Mars. Lynn Rothschild. Presentation 243.

- MIT Bioengineering Department (April 2022)
  - Lynn Rothschild gave annual invited graduate student lecture

- American Chemical Society, San Diego (April 2022)
  - Lynn Rothschild gave invited symposium talk

- Fungal Biomaterials and Biofabrication Penn State) (May 2022)
  - Lynn Rothschild gave invited keynote talk

- DTU BIOSUSTAIN, Novo Nordisk Foundation Center for Biosustainability, Copenhagen (May 2022)
  - Lynn Rothschild gave invited departmental seminar

- Applied Synthetic Biology in Europe, Edinburgh (November 2022)
  - Lynn Rothschild gave invited keynote talk

- Association for Computer Aided Design in Architecture ACADIA 2023 Conference, Denver, CO (26-28 October 2023)
  - Paper presented: Brandić Lipińska, Monika, Martyn Dade-Robertson, Meng Zhang. (2023) Space Architecture, Biotechnology, and Parametric Processes: Component Design through Assembly, Growth, and Fabrication Parameters in an Iterative Feedback Loop, Proceedings from ACADIA 2023, 26-28 October, Denver, USA.

- Biocene 2023, Ohio Aerospace Institute, Cleveland, OH (18-20 October 2023)

- Monika was a speaker and panelist at the Building Better session, talking about the Myco-Architecture project

- Lunar and Planetary Science Conference (March 2023)
  - Poster presented: L. J. Rothschild, C. Maurer, J. W. Head, M.B. Lipińska4, D. Senesky, K. Kornegay, M. C. Rheinstädter, M. Dade-Robertson, N. F. Musitu, C. Workman, E. Axpe4, and D. Cadogan. Poster 2983. https://www.hou.usra.edu/meetings/lpsc2023/pdf/2983.pdf

- BioFutures Symposium, Northumbria University, UK (July 2023)
  - Invited keynote, Lynn Rothschild

- 8th Interstellar Research Group meeting, Montreal (July 2023)
  - Invited Keynote Presentation, (synthetic) biology + mycotecture as enabling technologies for space exploration. Lynn Rothschild

- International Conference on Environmental Systems (ICES), Calgary, Canada (16-20 July 2023)
  - Paper presented: Brandić Lipińska, Monika, Martyn Dade-Robertson, Meng Zhang, Lynn J. Rothschild. (2023) Drop the Base: Biological, ISRU- Based Aleatory Construction System for Martian Habitats. 52nd International Conference on Environmental Systems (ICES), 16-20 July, Calgary, Canada.

- 26TH North American Mushroom Conference 2024/ 20th Congress International Society for Mushroom Science, Las Vegas (February 2024)
  - Invited Keynote Presentation, (synthetic) biology + mycotecture as enabling technologies for space exploration. Lynn Rothschild

- Lunar and Planetary Science Conference 2024 (March 2024)
  - Posters presented: see section 4.5 of this report

- Genetics Society of America (March 2024)
  - Presentation, Characterizing the effects of simulated space environmental conditions on the biological and mechanical properties of fungal composite biomaterials. Rolando Cruz Perez and Lynn Rothschild

8 Other Presentations

- Imperial Lates, Imperial College London, 7 December 2023
  - Monika was a panelist at the Zero Pressure podcast, talking about the Myco-Architecture project. It was hosted by Britain's first astronaut Helen Sharman.

- Moving to Mars (M2M) workshop, November 2022
  - Monika was an invited speaker at the Moving to Mars (M2M) workshop, co-organized by the European Space Agency (ESA) and the Mars Society of Canada, talking about the Myco-Architecture project.

- Human Spaceship - Upon the Moon Exhibition, Vane Gallery, Gateshead, United Kingdom, October 2022
  - Monika was an Invited speaker at the Human Spaceship - Upon the Moon Exhibition, talking about the Myco-Architecture project.

- Erasmus Tech Summit "World in 2024", Rotterdam, Netherlands, June 2022
  - Monika was a keynote speaker at the Erasmus Tech Summit "World in 2024" hosted by the Erasmus Tech Community, talking about the Myco-Architecture project.

- Design with the Living Symposium, Design Museum, London, November 2021
  - Monika was an invited Speaker, talking about the Myco-Architecture project.

9 Student involvement

Monika Brandić Lipińska, Ph.D. Student of Newcastle University, team organization and prototypes solidifying sand substrate, biowelding

Katheryn Korngay, Ph.D. Student, Stanford University, mechanical testing of samples

Hannah Krivic, Ph.D. Student, McMaster University, testing of samples in Planetary Simulator

Alessandra Massa (Ph.D. Student, Basque Culinary Institute, working under Eneko Axpe and Lynn Rothschild)

Nicolas Fuentes Musitu, “Myco-Algae Composites for Space Architecture: Strain Engineering of Mycelium grown in Cyanobacterial Substrates” DTU Masters Thesis, participated in-person at midterm review (September)

Sujith Pakala, Undergraduate Brown University, summer 2023 working under Dr. Rolando Cruz Perez in the Rothschild lab, NASA Ames Research Center

Victoria Porto, Undergraduate Stanford University, working with Kat on mechanical testing

Christian Wu, Undergraduate Brown University, working under Prof. Jim Head on mission architecture

David Fryd, Computer Science, Undergraduate, Brown University, working under Prof. Jim Head on mission architecture

WaTae Mickey, Geology-Engineering-Computer Science, Undergraduate, Brown University, working under Prof. Jim Head on mission architecture

Michael Daniti, Engineering/Physics, Graduate, Brown University, working under Prof. Jim Head on mission architecture

Benjamin Boatwright, Graduate Student/Postdoc, Geological Sciences, Brown University, working under Prof. Jim Head on mission architecture

10 Press

2021

2022 - Rothschild
Interviews
Podcast, Yale iGEM team (July 8)
Orange Radio 94.0 (Vienna) Spaceuriosity (April 14)
Madeleine Gregory, reporter, Discover Magazine, article on mycotecture (March 31)
Interview with Muriel Valin, journalist for Epsiloon (French Science Magazine) about mycotecture (Jan 24)

TV
NHK, Japan's Public TV, Documentary "Power of Microbes" re Synthetic Biology, Myco-architecture (June 30)

2023
LPSC

2024
Keynote, Mushroom Science (Feb, Las Vegas)
LPSC (6 abstracts)
Fungal Genetics 24 (March, Asilomar, 1 poster, 1 talk)
TEDx NIAC

11. Appendices

11.1 Abbreviations and acronyms

BEC: bioreactor enclosure cell
BioHab: Another name for our "mycotecture off planet" habitat
Bioterials: a biomaterial made with life; introduced here to distinguish it from a more common definition of "biomaterial" as "a substance that has been engineered to interact with biological systems for a medical purpose, either a therapeutic (treat, augment, repair, or replace a tissue function of the body) or a diagnostic one."[58] The words "biomaterials" and "fungal biocomposites" are used interchangeably here.
Biowelding: a process in which the natural bonding properties of mycelium create strong, cohesive joints between two or more pieces of mycelium-based materials. See, for example, 4.2
CBD: chitin binding domain, a portion of a protein that binds the polymer chitin
ETFE: Ethylene Tetrafluoroethylene is a melt processable, fluorine-based plastic designed to have high corrosion resistance and strength over a wide temperature range. The crystallinity of ETFE ranges from 40-60%.
Fenestration: the arrangement of windows and doors on the elevations of a building
FFE: furniture, fixtures, and equipment
GIP: Grow in Place concept that utilizes simple circulatory systems to nourish simulated LOBEs (SLOBEs)
IBS: The Initial base structure
LOBE: Lunar Optimized Bioreactor Enclosures
MEP: Mechanical, Electrical, Plumbing
Mycomaterial: material made with fungi
PDA: potato dextrose agar, the most common medium for growing fungi
PDY: potato, dextrose, yeast medium, another common fungal growth medium
SLOBEs: Simulated Lunar Optimized Bioreactor Enclosures
TPU: Thermoplastic polyurethane is any of a class of polyurethane plastics with many properties, including elasticity, transparency, and resistance to oil, grease, and abrasion. Technically, they are thermoplastic elastomers consisting of linear segmented block copolymers composed of hard and soft segments.

11.2 Fungi used in these studies

Aspergillus oryzae is a culinary fungus that has had some genetic engineering tools developed for use in food and materials.
*Aspergillus nige*r has long been used for industrial production and benefits from a wide array of genetic engineering tools and techniques.*Ganoderma lucidum* is a medicinal mushroom that has also been used for mycelium materials.
Neurospora crassa is a model fungus that has long been used for genetic studies and benefits from genetic engineering tools. Recently, it has also garnered increased interest as an alternative protein source.
Pleurotus ostreatus is a culinary mushroom that has also been used for mycelium materials.

[58] Wikapedia, accessed 14 March 2024;
https://en.wikipedia.org/wiki/Biomaterial#:~:text=A%20biomaterial%20is%20a%20substance,body)%20or%20a%20diagnostic%20one.

11.2 Abstract from LPSC 2022.

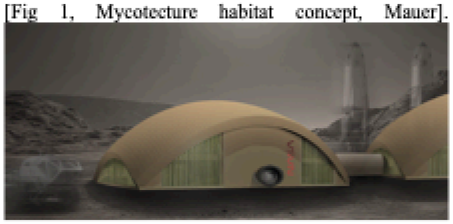
53rd Lunar and Planetary Science Conference (2022) 2544.pdf

MYCOTECTURE OFF PLANET: FUNGI AS A BUILDING MATERIAL ON THE MOON AND MARS. L. J. Rothschild[1], C. Maurer[2], M.B. Lipińska[3], D. Senesky[4], I. Paulino-Lima[5], J. Snyder[5], M. Dade-Robertson[4], A. Wipat[4], M. C. Rheinstädter[6], E. Axpe[4], C. Workman[7], D. Cadogan[8] and J. W. Head[9]. [1]NASA Ames Research Center, Moffett Field, CA, 94035, USA, Lynn.J.Rothschild@nasa.gov, [2]redhouse studio, Cleveland, OH 44113, USA, Newcastle University, Newcastle upon Tyne NE1 7RU, UK, [4]Stanford University, Stanford, CA 94305, USA, [5]Blue Marble Space Institute of Science at NASA Ames Research Center, Moffett Field, CA, 94035, USA, [6]McMaster University, Hamilton, ON L8S 4M1 CANADA, [7]DTU, Kongens Lyngy, DENMARK, [8]Moonprint Solutions, Dover DE, 19901, USA, [9]Brown University, Providence, RI, 02912, USA.

Introduction: A turtle carries its own habitat. While reliable, it costs energy and is not easily adapted for the environment. NASA makes the same trade-off when it transports habitats and other structures needed to lunar and planetary surfaces. Astronauts stayed on the lunar surface for up to 75 h (Apollo 17), so the lunar module (LM) could double as a habitat. An example of the "build it on Earth, launch it into space" approach is the Habitat Demonstration Unit (HDU) Deep Space Habitat, developed by the Habitat Systems Project (NASA AES). The hardware consists of a composite fiberglass resin-infused shell attached to eight steel ribs, providing living and working space for a crew of four. Even with the use of advanced materials, it weighs >14,000 kg (~ 466 kg/m^2 living space), leading to high launch costs. Upmass and resupply will result in reduced surface operations, greater mission risk, loss of productivity and psychological stress.

In contrast, a bird builds its home at destination using sustainable manufacturing and *in situ* materials. In this vein, NASA's Centennial Challenges program ran a 3D printed Habitat design challenge for the Moon, Mars and beyond **[1]**. Top designs used ISRU, focusing on agglutinated regolith or frozen water. Requirements included a vapor barrier, and a robotic infrastructure for preparing the site, gathering regolith and building. While regolith and ice have advantages as building materials and are compatible add-ons to our concept, regolith has disadvantages including rigidity, poor thermal insulation, massive energy demand, potential mineral/chemical toxicity and incompatibilities, and a dedicated infrastructure required for production of both.

In a NASA Innovative Advanced Concepts (NIAC) Phase 1 study, we introduced the use of structures grown by fungal mycelial biocomposites at destination [Fig 1, Mycotecture habitat concept, Mauer].



Mycelial materials are known thermal insulators, fire resistant, and unlike plastics and glues, do not outgas. They are more flexible and ductile than regolith alone. The density and material properties are tuned during production. The material could be used dry, wet, frozen with water or as part of a self-produced biocomposite which would allow such enhancements as radiation protection and a vapor seal. Even better, it is self-replicating so the habitat could be extended at a future date, and thus also be self-repairing. Some form of this material could be used for a habitat at destination, furniture, storage, additional buildings, and the shell of multiple rovers. As a standalone material or in conjunction with agglutinated or sintered regolith, a mycotectural building envelope could significantly reduce the energy required for building because in the presence of food stock and water it would grow itself. After the arrival of humans, additional structures could be grown with feedstock of mission-produced organic waste streams including inedible plant or soil components, or human waste. When protected, the mycomaterials can have a long life, but at the end of its life cycle the material could be become fertilizer for mission farming or production of new mycomaterials.

Radiation has been considered a "show stopper" for human missions, but some black fungi not only survive, but may thrive in space radiation **[2]**. We could supplement our mycomaterials with either genetic engineering of the mycelia to bind materials such as metals as we did in Phase 1, or with bacteria with which they would form a mutualistic relationship. These bacteria could supplement the structural integrity of the mycotectural envelope through bio-mineralization, polymer production or filament formation. Alternatively, they could act as an intelligent input (biosensor) in the mycomaterial synthesis process detecting pressure and flaws in the mycotectual structural integrity by measuring mechanical strength, and reporting anomalies through color change or fluorescence. Autotrophs could provide to, and receive from, the fungal mycelia, essential metabolites to speed up the growth of the structure. These bacteria would be a flight-proven spore former *Bacillus subtilis*, whose remarkable space-compatible abilities were proven during the nearly six year LDEF mission **[3]** and during the PowerCell mission on EuCROPIS (Rothschild, PI).

If we succeed in developing a biocomposite material that can grow itself, we will provide NASA with a radically new, cheaper, faster, more flexible, and lighter material for habitats for extended duration lunar and Mars missions, as well as furniture and other structures. While our habitat shell is designed to be inert, we can envision its extension into a living state participating actively in waste recycling, oxygen production, and detoxification similar to a living roof. The long-term goals of this concept would be to create a living shell that functions beyond structure and warmth, where the organisms can be manipulated to perform tasks like: self-healing, humidity regulation, energy production, nutrient production, and bio-luminescence. Such living architecture was demonstrated by a five-story Bio Intelligent Quotient building in Hamburg, Germany **[4]** showing that this approach can scale.

The use of mycotecture on Earth, its variable density, lack of outgassing, ability to tune the material, construct it through multiple routes including ones with little to no on-site infrastructure, suggest that the concept is credible for building structures off planet. However, unknowns remain that are not readily determined, thus is intended to begin to address these with experiments and paper studies. Mycelia products use two main approaches: either the mycelium series to agglutinate materials such as wood chips, or it is grown to feature the mycelium itself as the product, resulting in an imitation leather. Both could be useful to NASA. In Phase 1 we imagined a co-culture of cyanobacteria and fungi in a bag. Here we introduce a novel third approach where the mycelia grow to fill a plastic shell containing a lightweight, compressible, porous scaffold, coated with a nutrient-rich hydrogel, possibly embedded with *B. subtilis* spores, and seeded with mycelia. The structure will be deployed by the expansion of the scaffold, and the heating to 30 °C during the beginning of the lunar night. Ideally the structure will be complete during this time allowing the heat of the lunar day to bake the structure in place. If the growth cannot be completed during lunar night, cooling will be needed for thermal stability during the lunar day until the structure is ready for baking.

Benefits to NASA and the broader aerospace community. There are numerous possibilities for off-planet building with mycotecture. Features include the modest upmass requirements of a few organisms, scaffold, hydrogel feedstock and plastic enclosure, their future potential to reproduce with *in situ* resources, the ability to grow to accommodate on-site terrain, and the tunability and multi-functionality of the materials. Other ISRU building proposals suggest agglutination of regolith, which could be done by fungal mycelia. In Phase 1 we demonstrated growth of mycelium on regolith simulant with added nutrients. Other benefits of mycotecture for NASA include production of furniture and fabrics on site, to water purification. Several of the fungi used have other uses. *Aspergillus oryzae* is used to make soy sauce, miso, sake and rice vinegar, and is used in biotechnology for protein production. We are assessing the potential of mycotecture to aid in building solid substrate at destination for landing or launch.

Terrestrial spin-offs: The building processes to be developed in Phase 2 may have profound effect on the building industry. This is responsive to the UN Sustainable Development Goals 9, 11, 12 **[5].** Currently the building industry is responsible for 40% of Earth's carbon emissions. The concept of a rapidly deployable, self-building self-healing structure potentially with embedded biosensors and eco-friendly in that it would be biodegradable and emit no toxic volatiles, is appealing. The commercial sector is exploring insulation and packing materials, but with the addition of our new feedstocks, scaffold and embedded sensing capabilities, they could be more useful. The techniques can be used to build quickly deployable, warm safe shelters with little carbon footprint to house the hundreds of millions of refugees anticipated by midcentury. With the right material properties, new applications include lightweight protective gear, buoys and custom grown shoes in areas where access to clothing that fits may be limited. We are currently exploring the use of mycotecture to increase sustainability in the restaurant, Azurmundi.

Acknowledgments: We are indebted to two iGEM teams in the development of this work, Stanford-Brown 2018 (Rothschild's lab) and DTU 2018 team (Workman's lab), and Rothschild and Head's groups for mentoring (https://2018.igem.org/Team:Stanford-Brown-RISD and http://2018.igem.org/Team:DTU-Denmark). We gratefully acknowledge support from the NIAC program for Phase I and II, and STMD, Rhode Island and California Space Grant Consortium, and NASA Ames for support for the iGEM teams. This research is partially funded by Northern Bridge Consortium as part of the Hub for Biotechnology in the Built Environment (HBBE), and by the research group Bio-Futures for Transplanetary Habitats.

References:[1] https://www.nasa.gov/directorates/spacetech/centennial_challenges/3DPHab/about.html [2] Dadachova, E., & Casadevall, A. (2008) *Curr Opinion Microbiol, 11(6)*, 525–531. [3] Horneck, G. (1993) *Orig Life Evol Biosph 23(1)*, 37-52. [4] Wallis, D. (2013) *The New York Times*, *http://www.nyti.ms/11Ceu9J* [5]. *https://sustainabledevelopment.un.org/?menu=1300*

11.3 Abstract from LPSC 2023

MYCOTECTURE OFF PLANET: FUNGI AS A BUILDING MATERIAL ON THE MOON AND MARS.

L. J. Rothschild[1], C. Maurer[2], J. W. Head[3], M.B. Lipińska[4], D. Senesky[5], K. Kornegay[5], M. C. Rheinstädter[6], M. Dade-Robertson[4], N. F. Musitu[7], C. Workman[7], E. Axpe[4], and D. Cadogan[8]. [1]NASA Ames Research Center, Moffett Field, CA, 94035, USA, Lynn.J.Rothschild@nasa.gov, [2]redhouse studio, Cleveland, OH 44113, USA, [3]Brown University, Providence, RI, 02912, USA, [4]Newcastle University, Newcastle upon Tyne NE1 7RU, UK, [5]Stanford University, Stanford, CA 94305, USA [6]McMaster University, Hamilton, ON L8S 4M1 CANADA, [7]DTU, Kongens Lyngy, DENMARK, [8]Moonprint Solutions, Dover DE, 19901, USA.

Introduction: A turtle carries its own habitat. While reliable, it costs energy and is not adaptable to the environment. NASA makes the same trade-off when it transports habitats and other structures needed to destination. Astronauts stayed on the lunar surface for up to 75 h (Apollo 17), with the lunar module doubling as a habitat. An example of the "build it on Earth, launch it into space" approach is the Habitat Demonstration Unit (HDU) Deep Space Habitat, developed by the Habitat Systems Project (NASA AES). It has a composite fiberglass resin-infused shell attached to eight steel ribs, providing living and working space for four. Even with advanced materials, it weighs >14,000 kg (~466 kg/m^2 living space), leading to high launch costs. Upmass and resupply will result in reduced surface operations, greater mission risk, loss of productivity and psychological stress.

In contrast, a bird builds its home at destination using sustainable manufacturing and in situ materials. In this vein, NASA's Centennial Challenges program ran a 3D printed Habitat design challenge for the Moon, Mars and beyond [1]. Top designs used ISRU, focusing on agglutinated regolith or frozen water. Requirements included a vapor barrier, and a robotic infrastructure for preparing the site, gathering regolith and building. While regolith and ice have advantages as building materials and are compatible add-ons to our concept, regolith alone has disadvantages including rigidity, poor thermal insulation, potential mineral/chemical toxicity and incompatibilities, and a dedicated infrastructure required for production of both [2].

Vision: In a NASA Innovative Advanced Concepts (NIAC) Phase 1 study, we introduced the concept of structures grown by fungal mycelial at destination (Fig 1). Mycelial materials are thermal insulators, fire resistant, and unlike plastics and glues, do not outgas. They are more flexible and ductile than regolith alone. The density and material properties are tuned during production. The material could be used dry, wet, frozen with water or as part of a self-produced biocomposite which would allow such enhancements as radiation protection and a vapor seal. Even better, it is self-replicating so the habitat could be extended at a future date, and thus also be self-repairing. Some form of this material could be used for a habitat at destination, furniture, storage, additional buildings, and the shell of multiple rovers. As a standalone material or in conjunction with agglutinated or sintered regolith, a mycotectural building envelope could significantly reduce the energy required for building because in the presence of food stock and water it would grow itself. After the arrival of humans, additional structures could be grown with feedstock of mission-produced organic waste streams including inedible plant or soil components, or human waste. When protected, the mycomaterials can have a long life, but at the end of its life cycle the material could be become fertilizer for mission farming or production of new mycomaterials.

Figure 1. Mycotecture habitat on Mars (redhouse).

Radiation has been considered a "showstopper" for human missions, but some black fungi not only survive, but may thrive in space radiation [3]. We could supplement our mycomaterials with either bioengineering of the mycelia to bind materials such as metals as we did in Phase 1, or with bacteria with which they would form a mutualistic relationship. These bacteria could supplement the structural integrity of the mycotectural envelope through bio-mineralization, polymer production or filament formation. Alternatively, they could act as an intelligent input (biosensor) in the mycomaterial synthesis process detecting pressure and flaws in the mycotectual structural integrity by measuring mechanical strength, and reporting anomalies through color change or fluorescence. Autotrophs could provide to, and receive from, the fungal mycelia, essential metabolites to speed up the growth of the structure.

Mission architecture: (1) Before launch, a plastic inflatable is seeded with dried fungal mycelia and algae or cyanobacteria and deflated for launch. The shell allows for liquid water and meets planetary protection protocols for Mars. A concentrated nutrient solution will de-risk the mission. (2) At destination, the inflatable is deployed to the surface, water brought from Earth or collected at destination is added to the inflatable to activate the algae and fungi. (3) The algae grow during daylight, converting sunlight, CO_2 and water into biomass as well as producing O_2 which inflates the structure. The fungi use the O_2 and biomass to grow, providing structural integrity. The structure will be heated to allow growth during the lunar night. Ideally the structure will be complete during this time allowing the heat of the lunar day to bake the structure adding rigidity. If the growth cannot be completed during lunar night, cooling will be needed for thermal stability during the day until the structure is grown.

Progress to date:

Inflatable. Moonprint solutions built a 4x4 m inflatable prototype (Fig 2). The internal dropstitching allows the inflatable to maintain shape as well as providing scaffolding for the fungi to grow on.

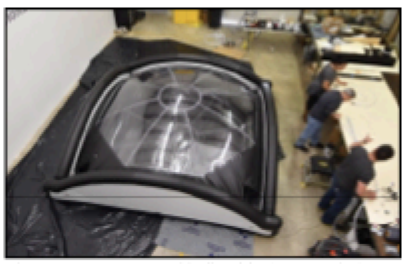


Figure 2. Prototype of inflatable (Moonprint).

Architectural. A 4x4 m prototype has been grown separate from the inflatable. In addition, the use of "sand towers" or "sand bricks", and concept that can be extended to use on lunar or Martian regolith, has been tested. Cyanobacterial/fungal biocomposites were made and tested for their mechanical properties (Fig. 3).

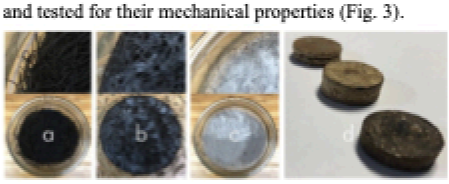


Figure 3. Bio-composites grown by redhouse. a) Dried *Nostoc flagelliforme* (diazotrophic cyanobacterium) in a sterilized glass jar. b) hydrated *N. flagelliforme* inoculated with *A. oryzae* liquid culture. c) fully colonized composite. d) compressed composites that are structural and insulative. Note: similar samples by redhouse achieved 30 MPa compression strengths.

Materials testing. Materials testing was begun on fungal samples and biocomposites grown in the lab, as well as those exposed to the planetary simulator at McMaster University.

Benefits to NASA and the broader aerospace community: If we succeed in developing a biocomposite material that can grow itself, we will provide NASA with a radically new, cheaper, faster, more flexible, and lighter material for habitats for extended duration lunar and Mars missions, as well as furniture and other structures. While our habitat shell is designed to be inert, we can envision its extension into a living state participating actively in waste recycling, oxygen production, and detoxification similar to a living roof. Such living architecture was demonstrated by a five-story Bio Intelligent Quotient building in Germany **[4]** showing that this approach can scale. Other ISRU building proposals suggest agglutination of regolith, which could be done by fungal mycelia. In Phase 1 we demonstrated growth of mycelium on regolith simulant with added nutrients.

Terrestrial spin-offs: The building processes to be developed in Phase 2 may have profound effect on the building industry. This is responsive to the UN Sustainable Development Goals 9, 11, 12 **[5].** The building industry is responsible for 40% of Earth's carbon emissions. The concept of a rapidly deployable, self-building self-healing structure potentially with embedded biosensors and is biodegradable and emits no toxic volatiles, is appealing. The commercial sector is exploring insulation and packing materials, but with the addition of our new feedstocks, scaffold, and embedded sensing capabilities, they could be more useful. We are currently exploring the use of mycotecture to increase sustainability in the award-winning Basque restaurant, Azurmundi.

Acknowledgments: We grateful for support from NASA's NIAC program. This research is also funded by Northern Bridge Consortium as part of the Hub for Biotechnology in the Built Environment (HBBE), and by the group Bio-Futures for Transplanetary Habitats.

References:[1] *https://www.nasa.gov/directorates/spacetech/centennial_challenges/3DPHab/about.html* [2] Lipińska, M., Maurer, C., Morrow, R., Dade-Robertson, M., Senesky, Magdalini Theodoridou, D., Zhang, M. & Rothschild, L. (2022) *Front Built Environ* doi.org/10.3389/fbuil.2022.965145 [3] Dadachova, E., & Casadevall, A. (2008) *Curr Opinion Microbiol, 11(6),* 525–531. [4] Wallis, D. (2013) *The New York Times*, *http://www.nyti.ms/11Ceu9J* [5]. *https://sustainabledevelopment.un.org/?menu=1300*

11.4 Abstracts from LPSC 2024 (6 total)

Hadley Max 500-Day Design Reference Mission (DRM) To The Apollo 15 Hadley-Apennine Region: Application Of Science Goals And Objectives To Planning Long Duration Exploration Architecture (1. Background)

Hadley Max 500-Day Design Reference Mission (DRM) To The Apollo 15 Hadley-Apennine Region: (2. Science Goals And Objectives).

Hadley Max 500-Day Design Reference Mission (DRM) To The Apollo 15 Hadley-Apennine Region: (3. Mission Architecture Definition).

Hadley Max 500-Day Design Reference Mission (DRM) To The Apollo 15 Hadley-Apennine Region: (4. Traverse Design And Implementation).

Hadley Max 500-Day Design Reference Mission (DRM) To The Apollo 15 Hadley-Apennine Region: (5. Reducing Upmass Demands With In Situ Myco-Architecture).

Hadley Max 500-Day Design Reference Mission (DRM) To The Apollo 15 Hadley-Apennine Region: (6. Reducing Upmass Demands Utilizing In Situ Inflatable Structures)

HADLEY MAX 500-DAY DESIGN REFERENCE MISSION (DRM) TO THE APOLLO 15 HADLEY-APENNINE REGION: APPLICATION OF SCIENCE GOALS AND OBJECTIVES TO PLANNING LONG DURATION EXPLORATION ARCHITECTURE (1. BACKGROUND). M. Daniti[1], J. Head[1], D. R. Scott[1], B. Boatwright[1], L. Rothschild[2], C. Maurer[3], D. Eppler[4], R. Creel[5], R. Martin[2], W. Mickey[1], D. Fryd[1], C. Wu[1]. [1]Brown University, Providence RI, [2]NASA Ames Research Center, Mountain View CA, [3]redhouse studio, Cleveland OH, [4]San Antonio Mountain Consulting, Houston TX, [5]Huntsville, AL (NASA MSFC Ret.) (james_head@brown.edu).

Introduction: Among the six successful Apollo Lunar Exploration Program landed missions, Apollo 15 was the first Lewis and Clark-like "Scientific Expedition to the Moon" [1]. Experience with Apollo 11, 12 and 14 walking traverses provided fundamental scientific results, demonstrating pinpoint landing techniques, and increasing stay-time, EVA durations, and mobility assistance (such as the Mobile Equipment Transporter, MET, on Apollo 14), but also clearly demonstrated the need for increased numbers of EVAs and mobility in order to reach multiple and more distant scientific objectives [2]. The Apollo 15 mission [1] carried the first Lunar Roving Vehicle (LRV) that enabled Astronauts Dave Scott and

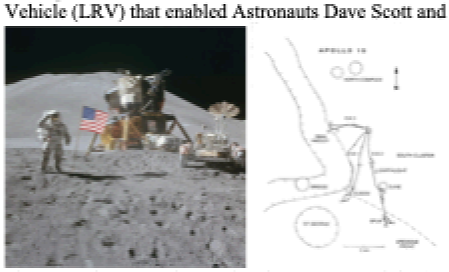


Fig. 1: Left: LMP Jim Irwin, the Lunar Module (LM) and the deployed LRV; Mt Hadley in the background. Right: Completed Apollo 15 Traverses.

Jim Irwin to reach distant objectives and traverse out to a radial distance of 7 km [3] (Fig. 1), the maximum distance permitted by the need for them to walk back if the rover failed (the 'walkback' constraint).

The initial Standup EVA (SEVA), was inserted as a result of Commander Scott's desire to get an initial overview of the terrain (due to the fact that only 20 m resolution images were available for pre-mission planning) [4]. The following three periods of EVA visited four of the five major mission objectives (lunar maria, lunar highlands (Hadley Delta), Hadley Rille, secondary crater cluster, and North Complex). The Apollo 15 mission [1,5,6] completed 19.7 hours of surface exploration, deployed a complex set of scientific instruments, drilled a 2.4 m drill core, traversed 27.9 km of the lunar surface, and returned 77 kg of samples [7], 56% more than the previous Apollo 14 mission. The results of this very successful exploration mission [8] addressed many fundamental scientific questions [1,7], and an entire science conference dedicated to Apollo 15 mission results was held in Houston. As is usually the case in science and exploration, the findings from Apollo 15 [1,7] led to a whole new set of questions, and posed an additional set of exploration destinations in the larger area of the Hadley-Apennine landing site (Fig. 2).

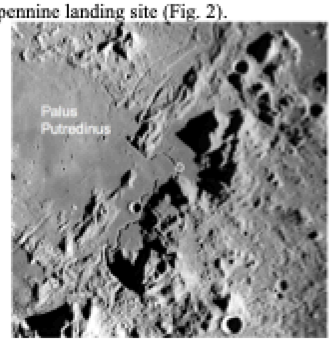


Fig. 2. The broader Hadley-Apennine region showing multiple points of geological interest beyond the 7 km exploration radius around the Apollo 15 landing site (white circle) (Fig. 1, right).

In the years subsequent to the Apollo Lunar Exploration Program, orbital remote sensing missions (such as Lunar Prospector, Clementine, Lunar Reconnaissance Orbiter, Chandrayaan-1) permitted the extension of the Apollo and Luna Mission sample return results to the entire Moon, and a much more refined vision of the Moon as a planetary body emerged [9]. Concurrently, robotic exploration of Mars with landers, orbiters and rovers was providing a view of a planet that appeared to have been Earth-like in its earliest history, but had then evolved to an extremely cold hyperarid polar desert that we see today [10]. Could Mars have harbored life in its earlier "warm and wet" climate history? These emerging results obviously kindled an interest in NASA in the human exploration of Mars. Several committees were formed and engineering studies were undertaken to assess scientific goals and objectives and mission architecture. The following type of questions were posed to these groups: What will be the state of robotic Mars exploration and Mars knowledge in 2030? What are the key science questions that humans can address that will not be addressed by robotic missions by 2030? What is the best way to deploy/utilize humans on Mars? What is the role of IT/robotics alongside humans?

In a 2007 study [11], one of the first issues that arose in reference to the mission architecture was the question of duration of the Mars mission surface stay time for the astronauts. Did scientists want to go and return in one access opportunity, during which the approximate stay time would be ~30 days, or stay on Mars and return at the following access opportunity, during which the approximate stay time would be ~500 days?

The skepticism of the engineers ("What would you be doing on Mars for 500 days???") was met with euphoria from the geologists and other scientists ("Wow! Let us show you what we could do!"). The ensuing reports [11] outlined the results of these studies, identifying 40 candidate Mars human exploration sites Fig. 3, top), designed around MEPAG Goal III: 'Determine the Evolution of the Surface and Interior', and showing Design Reference Missions (DRMs) for scientific points of interest and traverses that would enable the astronauts to reach them (Fig. 3, bottom).

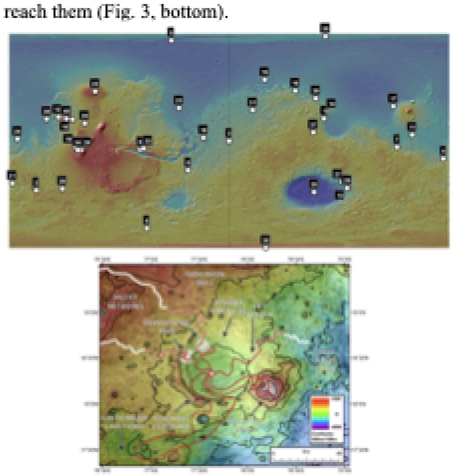


Fig. 3. Top: Forty scientific human exploration landing sites identified on Mars. Bottom: Example DRM and traverses at Jezero Crater (from [12]).

Subsequent studies periodically revisited these issues [12,13], most recently, the MEPAG Human Exploration of Mars Science Objectives (HMSOTT) report [14].

In response to the challenges from the NASA engineers in 2007 ("What would you be doing on Mars for 500 days???"), we reasoned that presenting a more familiar and previously successful mission scenario, but expanded to a 500-day duration, would help both the engineers and scientists develop a design reference mission (DRM) that would mutually educate, as well as identify the key mission parameters and technology requirements for a 500-day mission to Mars. In addition, the several attempts at sustained human lunar exploration (SEI, Constellation) underlined the need for similar thinking approaches for the Moon. And in 2017, the announcement of the NASA Artemis Moon to Mars initiative linked the importance of such an approach.

Thus was born the 500-day Human Exploration Mission back to the Hadley-Apennine region (Fig. 2), the landing site region of the Apollo 15 first scientific expedition to the Moon. Named the "Hadley Max" mission by Apollo 15 Commander Dave Scott, this Design Reference Mission (DRM) concept called on utilizing the Apollo mission planning and execution experience of two of the co-authors (Scott and Head), mission operations LRV design and thermal control planning and execution experience (Creel), crew training and operations experience (Eppler), Myco-Architecture as *in situ* building materials (Rothschild) [15], and the enthusiasm and creativity of Brown University geology, physics, engineering and computer science students (a team that has grown over the years as the Hadley Max DRM concept has evolved).

The major goals and objectives of the "Hadley Max" 500-Day Hadley-Apennine Design Reference Mission (DRM) project were to identify the key mission elements and requirements in the following areas: 1) Background and motivation (this abstract); 2) Scientific Goals and Objectives; 3) Implications for Mission Design and Architecture; 4) Traverse Design & Implementation, 5) Implications for Habitat Requirements-Role of Myco-Architecture; and 6) Implications for Habitat Design and Construction, each treated in separate LPSC 55 contributions.

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HADLEY MAX 500-DAY DESIGN REFERENCE MISSION (DRM) TO THE APOLLO 15 HADLEY-APENNINE REGION: (2. SCIENCE GOALS AND OBJECTIVES). W. Mickey[1], J. W. Head[1], D. R. Scott[1], B. Boatwright[1], L. Rothschild[2], C. Maurer[3], D. Eppler[4], R. Creel[5], R. Martin[2], D. Fryd[1], M. Daniti[1], C. Wu[1], C. van der Bogert[6], H. Hiesinger[6], W. Iqbal[6]. [1]Brown University, Providence RI, [2]NASA Ames Research Center, Mountain View CA, [3]redhouse studio, Cleveland OH, [4]San Antonio Mountain Consulting, Houston TX, [5]Huntsville, AL (NASA MSFC Ret.) [6]Universität of Münster, Münster Germany. (james_head@brown.edu).

Science Goals and Objectives: We supplemented the initial five major Apollo 15 scientific goals and objectives (Imbrium impact basin; Sinuous rille origin; Mare history; Ejecta from distant craters; Regolith history), with several additional objectives related to questions raised by Apollo 15 crew exploration/observations, analysis of the Apollo 15 sample suite, and subsequent mapping [1-4].

1) Lunar Maria Lava Flow Emplacement: Prior to Apollo 15, it was thought that Mare Imbrium lavas might stem from the rapid response of Imbrium basin mantle uplift and resulting massive pressure-release melting, and thus have formed nearly contemporaneously with Imbrium. However, Apollo 15 Imbrium ejecta and mare basalt radioisotopic dates (3.3 Ga) showed that they were ~600 Ma apart, disproving this hypothesis. The mare basalt dates did reveal evidence for two different flow sequences emplaced a few tens of Ma apart, somewhat different in composition (quartz-normative & olivine-normative). Visual descriptions and high-resolution images by D. Scott revealed distinctive layering in the W rille wall, and portions of these layers were sampled in blocky probable outcrops along the E rille rim. Irwin noted marginal steps along the base of Mount Hadley, suggesting topographic decrease of the mare surface after initial flooding due to lava drainage or and/or solidification. Scott and Irwin unexpectedly discovered clods of green glass beads, an entirely unexpected finding that suggested the formation of dense clouds of fine liquid droplets from either pyroclastic or impact crater events. These were apparently temporally unassociated with the mare basalts, and were later shown to be of pyroclastic origin and contain unexpected amounts of H_2O, originating ~400 km deep in the mantle. Intrigued by the question of volatiles in the basalts, D. Scott observed a lone, very vesicular rock (15016) on the maria and made an unscheduled stop to sample it for assessment of volatiles in the lab. Additional crew observations and sampling of highly vesicular mare rocks added new insights about the role of magmatic volatiles in mare basalts and pyroclastic eruptions. But the geological context, including the sequence and relation of the enigmatic green beads to the vesicular basalts, and their relation to the origin of the rille and the Hadley Rille vent dozens of km to the south, remain unresolved. Apollo 12 had sampled an unusual KREEP-rich breccia (12013) of uncertain provenance and perhaps related to a Procellarum-KREEP Terrane (PKT) crustal province on the NW nearside. Apollo 15 discovered KREEP basalts (15382, 15386) with a crystallization age of 3.9 Ga, indistinguishable from the age of the Imbrium basin, reopening the question of whether the Imbrium impact event induced basaltic volcanism, perhaps contaminated by passing through PKT crust. ***Outstanding Questions*:** Does the layering and vertical structure observed in the walls of Hadley Rille correspond to the two sampled lava flows? What is the vertical structure of the two lava flows (vesicle distribution, cooling behavior and history, mineralogical segregation, etc.) and the nature of their interface (thickness of regolith between them, atop)? Which of these lava flows are associated with the formation of Hadley Rille and in what manner? Do these two flows show evidence of differences in magnetization and field orientation during this critical period? Is there evidence for additional flows (and pre-mare units) deeper in Hadley Rille? What is the regional distribution of the green glass, its associations with the vesicular basalts, and its distribution relative to the Hadley Rille vent? Are either basalt group petrogenetically correlated with the green glass beads? What is the origin and provenance of the KREEP basalts, where do they outcrop, are they related to basalts of different ages outside the Apollo 15 sampling area [4], are they related to the high-albedo smooth plains in the Archimedes area (Apennine Bench Fm.), and are they extrusive basalts or ponded Imbrium basin impact melt? What is full range of basalt compositions and ages in the SE Imbrium basin [4] and where and how does Hadley Rille fit? What is the origin and significance of the marginal terraces observed by Irwin? What is the origin of the several irregularly shaped craters in the Apollo 15 landing region? Do these represent additional vents for lavas seen at Apollo 15?

2) Nature and Origin of Hadley Rille: Early hypotheses ranged from iced-over aqueous fluvial channel erosion to lava-related (open lava flow median channel, highly turbulent thermal erosion, collapsed subsurface lava tubes). Astronaut observations and samples significantly improved our understanding, supporting a lava-related origin, but the actual rille origin remains enigmatic, due primarily to its great length (>135 km) and lack of access to its source vent to the SSW. The broad, low-elevation rim described by Irwin, and the W rille wall stratigraphy documented by Scott, are consistent with an open lava channel and/or thermal erosion, but other very narrow portions of the rille are indicative of a collapsed lave tube. Later global documentation of sinuous rilles shows that Hadley rille is anomalously long compared to the global population. While quantitative modeling suggests that the shorter rilles formed by turbulent lava flow and thermal erosion of the substrate, the formation of the significantly longer, deeper and wider Hadley Rille remains enigmatic. ***Outstanding Questions*:** What is the nature of the two Hadley Rille source vents, are they surrounded by pyroclastic deposits (e.g., green glass?), and what is their relationship to the rille? Do they represent separate eruptions or two phases of a single eruption? What are the proximal to distal characteristics of the rille (rim deposits, width, depth, wall-rock stratigraphy, evidence for roof narrowing and collapse)? How does the morphology and structure of the rille differ between straight segments and bends? What is the thickness of the lava and is pre-lava substrate (e.g., Apennine Bench Fm.) exposed? What is the petrogenetic relationship of any circum-vent pyroclastics and adjacent lava flows? Could the marginal terraces observed by Irwin be related to flooding of the valley and drainage of lava into Palus Putredinis? What is the nature and origin of the Schaber Hills (North Complex): volcanic construct or highland lava-covered kipuka?

3) Imbrium Impact Basin: Apollo 14 was targeted to the ejecta deposit of the Imbrium Basin (Fra Mauro Fm.) and Apollo 15 to the rim of the Imbrium Basin (Apennine Mts.) to sample the deepest ejecta (deep, slow-cooling magma ocean anorthosites), date the Imbrium event, understand the highlands diversity in the ejecta, and search for possible mantle material. At Spur Crater on EVA 2, Scott spotted a perched rock brightly glinting in the sunlight, and stated "Houston, I think we found what we came for!", recognizing from a distance that this glint meant large plagioclase feldspar crystals/twinning, a phenomenon he knew meant deep-seated, slowly cooling highland crust. This sample (15415), dubbed the "Genesis Rock" by the media, did indeed reveal much about the nature and cooling history of the lunar highlands crust, with an age of 4.1 Ga, and may have been older but partially reset by the Imbrium event. Other highland norite/spinel troctolite samples (15445) had ages of 4.28-4.46 Ga and are thought to represent the actual solidification age of the deeper magma ocean. Scott and Irwin observed enigmatic 100 m-scale inclined layering in the Apennine Mts. from afar (Silver Spur), well outside the traverse range. ***Outstanding Questions***: What is diversity of highland samples excavated and exposed by the Imbrium impact? What is the full range of rocks in the highland crust and how do they relate to different models of magma ocean formation and cooling? How does the sample petrology change with elevation, possibly related to target stratigraphy? Did the Imbrium impact event sample: mantle material; the KREEP-rich residual layer at the base of the magma ocean; the source regions of pre-Imbrian magmatism (the Mg-suite); ejecta from the adjacent, earlier Serenitatis basin (Silver Spur)? Are there deposits of pure Imbrium impact melt ponded in the Apennine Mts. summit lows? How do such new results compare to highlands sample collected at other sites? A distinctive highland meter-scale wrinkled texture is observed in orbital images (elephant-hide terrain, or EHT) and may explain some of the walking and LRV traverse difficulties in the highlands; what is the origin of the EHT and how can this new understanding be used to increase human and robotic mobility in the highlands?

4) Provenance and Age of Secondary Craters and Clusters: Impact craters excavate material from depth and redistribute it laterally in ejecta deposits, the most distant of which are rays and secondary craters and clusters. Thus, samples of such material provide clues as to distant substrate geology and stratigraphy, and can assist in dating the parent impact crater (ages reset by the event). One of the Apollo 15 traverse targets was the crater cluster S of the landing site, attributed to either Aristillus or Autolycus, two large craters, 100-200 km N of the site. Dune, one of the prominent craters in the South Cluster, was sampled at Station 4, EVA 2, including a prominent regolith breccia boulder with multiple basaltic clasts (15498). The 2.1 Ga age of some samples have been attributed to an Autolycus source, but later analyses show some ambiguity due to the overprint of younger Aristillus ejecta [4]; Spur crater KREEP basalts may be delivered from the Th-rich Aristillus crater. Outstanding Questions: What is the variety of ages in foreign materials delivered to the study region from distal crater substrates, and what are their cosmic ray exposure age in relation to their parent crater AMA? How can these data be integrated to provide a stratigraphy for the broader region surrounding the exploration area?

5) Regolith Stratigraphy: One of the most important questions addressed by Apollo 15 was the vertical stratigraphy of the lunar regolith, its thickness, lateral variability, and implications for processes of mechanical breakdown of the bedrock regolith protolith and chemical alteration induced by impact melting of the transition from bedrock to thick regolith. Scott and Irwin drilled and extracted a 2.4 m core that revealed 42 major textural units and showed conclusively that the regolith was not composed of a homogeneous mixture of impact disrupted materials, but instead was mostly derived from overlapping ejecta layers from nearby craters. Additional drive tube samples were collected along the traverses, and some showed mixing of highland and mare crater ejecta layers. Important informatin was gained on the overall changes in regolith development with depth, and the role of lateral mixing near the mare-highland boundary, as well as the role of regolith mass wasting down into the rille. ***Outstanding Questions***: How does regolith stratigraphy vary over a much larger area and are there any regional layers that might act as a chronologic datum? What is the thickness and age of regolith layers on top of buried flows in Hadley rille wall? Can core stratigraphy quantify lateral mixing degree and processes along the mare-highland boundary? How does regolith stratigraphy differ distal to secondary craters and clusters and what is the proportion of ejecta from the primary and locally excavated material?

Mission Architecture Defined by Science Requirements: In separate contributions, we use these expanded scientific goals and objectives to outline specific mission regions of scientific interest (ROSI) and then utilize the ROSI locations to asses implications for the broad architecture of the Hadley Max 500-day Mission.

References: 1) A15 PSR (1973) NASA SP-289. 2) A15 PET (1972) *Science 175*. 3) Jolliff et al. (2006) *RMG 60*. 4) Iqbal et al. (2021) LPSC 52 #1917. **Acknowledgement:** We gratefully acknowledge support from the NASA NIAC Program and the Brown University UTRA program.

HADLEY MAX 500-DAY DESIGN REFERENCE MISSION (DRM) TO THE APOLLO 15 HADLEY-APENNINE REGION: (3. MISSION ARCHITECTURE DEFINITION). D. Fryd[1], J. Head[1], D. R. Scott[1], B. Boatwright[1], L. Rothschild[2], C. Maurer[3], D. Eppler[4], R. Creel[5], R. Martin[2], W. Mickey[1], M. Daniti[1], C. Wu[1]. [1]Brown University, Providence RI, [2]NASA Ames Research Center, Mountain View CA, [3]redhouse studio, Cleveland OH, [4]San Antonio Mountain Consulting, Houston TX, [5]Huntsville, AL (NASA MSFC Ret.) (james_head@brown.edu).

Mission Architecture Defined by Science Requirements: Regions of scientific interest (ROSI) for the Hadley Max mission [1] are derived from Apollo 15 mission results [2] and recent regional geologic mapping [3]. From this, we synthesize more detailed traverse goals and objectives [1,5]. Here, we utilize the distribution of ROSI to assess implications for the broad architecture of the Hadley Max 500-day Mission (exploration range, mobility requirements, crew size, number of bases, number of EVAs, upmass and downmass requirements, human-robotic partnership requirements, habitat requirements, etc.).

1. Operational Access Requirements: Landing Sites: On the basis of attaining a broader exploration and sampling region [1,5] consistent with current NASA science goals and objectives [6], we select the original Apollo 15 landing site as the primary landing site. The 10-20 km radius of operations necessitated by the distance from the A15 site to the farthest ROSI [1], and uncertainties in the ability to cross Hadley Rille, dictate that two separate landing sites/bases of operations are required. To optimize the scientific return, we place the second landing site/base of operations to the W of Hadley Rille in mare unit Im3 (Fig. 1).

2. Crew Size, Space Suit and Mobility Requirements: Crew Size: We assume 4 crew per base in order to accommodate contingencies (i.e. EVA rescue), allow for the possibility of simultaneous EVAs, and to properly allow for the division of labor among scientific goals (e.g., one crew rille, one crew highlands). For the full Hadley Max mission with both landing sites, this equals 8 crew on the surface, 4 at each base. **Suit Requirements:** Minimum is Apollo suit capabilities, assisted by enabling technologies in consumables and mobility, in order to extend traverse time, optimize highland traverses, and expand EVA efficiencies. **Mobility Requirements:** Minimum is Apollo LRV design and capabilities. Two rovers/landing site (4 total). Enabling technologies include increased efficiency in slope trafficability to ensure exploration of the rille and highlands, ability to carry four astronauts (rescue), ability to survive lunar night ('rover garage' at base), design lifetime >>500 days.

3. Human-Robotic Mission Types and Relationships: Human Mission Types: These include 8-hour EVAs, 10 km radius of operations, with the possible extension to 14 km using the 7 km circumference "outpost" capability (Fig. 1). **Robotic Mission Types:** It is clear from the Human EVA 10 km radius of operations and the maximum traversable slope constraints (~20° degrees) that a series of parallel robotic missions will be required to meet the scientific objectives, particularly those in the highlands, and at radial distances beyond 10 km from the landing site, and to ensure Human EVA scouting, interpolation and extrapolation [4]. **Human-Robotic Mission Relationships:** On the basis of Apollo mission planning and astronaut operational experience, we advise against Astronaut-tended robotic 'field assistants' as an inefficient use of crew exploration time on the surface. Instead, we strongly urge the development of independent robotic devices to enable parallel human-robotic partnerships. This allows for generally simultaneous operations, enabling precursor, scouting, interpolation, extrapolation, and post-mission exploration activities [6].

4. Definition of Required Habitats, Enclosures and Related Architectural Elements: 1. Landing Pads (LP): For both Human and Robotic missions; like helo pads; flat, devoid of soil backwash contaminant, retroreflector for guidance. **2. Initial Base Structure (IBS):** Living and working habitat; follows the initial stages where there is a landing module (LM). **3. Evolutionary Base Structure (EBS):** Larger scale, separation of work and living activities; increased *in situ* science activities; IBS evolves to dust mitigation structure. **4. Outposts: Remote Science Bases (RSB):** Modeled after IBS, but located >10 km radius from Landing Site. Require up to ~5 RSBs for in depth, *in situ* science activities. Increase number in order of science priority. **5. 'Pony Express' Stations (PEX):** These are lunar 'pup tents' that will be precursors to the Remote Science Bases (RSB), and then Earth-day sleep-stations on the way to the final Remote Science Bases (RSB). Sample storage stations, geophysical stations; can be resupplied/samples collected by CLPS missions. **6. Robotic Rover Requirements:** a) LRV garage at base for surviving lunar night, re-outfitting; b) Robotic LRV 'pup tents' for surviving lunar night, caching samples.

Application to the Artemis Circumpolar Environment (ACE): How do we optimize these basic requirements and DRM concepts for the harsh conditions of the South Circumpolar Region, and the lunar farside? ***8. Assessing Feed-Forward to Mars Exploration:*** How does the Mars environment modulate and modify these DRM strategies and architectural elements?

5. Identification of Required Key Enabling Technologies and Operational Concepts: a) Upmass Requirements: The multiple base/outpost (RSB)/pup-tent habitat requirements and their necessary range of complexity and ability to survive lunar day/night cycles, as well as robotic LRV remote servicing stations, places *huge* mass requirements for delivery of construction materials to the Moon. In order to help alleviate this "upmass roadblock", we pursue two promising technologies: 1) Myco-Architecture

[7,8], where building materials can be "grown *in situ*" in order to significantly minimize upmass penalties, and 2) Inflatable Structural Elements [9], in which low-volume, low-mass inflatables can be combined with Myco-Architecture to produce a wide range of enclosures *in situ*. **b) Human-Robotic Partnerships:** Various mission requirements dictate the need for a robotic LRV (RLRV) controlled from the base or the ground (independent of human traverses). These mission requirements include the great distances required to reach all ROSI, the increase in area as a function of radius from the base (increasing the need for scouting, interpolation and extrapolation), the steep slopes within the rille and on the highlands, as well as the presence of the Elephant-Hide Terrain (EHT), and the trafficability on these slopes. RLRV design and technology challenges include ability to traverse slopes approaching 30°, an advanced suite of remote sensing instruments, constant navigation imaging, near real-time communications with the ground, the ability to collect, document and store individual rock and soil samples, remote operations from base and ground, enclosures (RLRV garages) for lunar night, servicing and sample storage, and a design lifetime >>500 days. **c) Supply-Resupply Technology and Infrastructure Requirements:** Despite alleviation of upmass construction requirements through Myco-Architecture and Inflatables, significant supply (and resupply) (S/RS) requirements are dictated by the widespread and long-duration exploration strategy. Many dozens of human and robotic S/RS missions to diverse locations, delivering different payloads, and ensuring crew cycling, are required by the 500-day DRM architecture. Optimal resupply mission require landing, offloading cargo, and onloading crew, rock/soil samples, and other materials for return to Earth. **d) Mission Operations and Feed-Forward to Mars:** Lunar communications latency (~2.5 sec) presented no difficulties during Apollo, but Mars latency (5-20 min) precludes useful direct communications with the ground during exploration. In addition, after a few days exploring the lunar surface, astronauts will have superior situational awareness (compared to pre-mission planners) and thus be capable of real-time planning and execution of traverses (the goals and objectives of which are planned pre-EVA in consultation with the base/ground). Additional research into optimal operational frameworks for planning, briefing, and de-briefing traverses is necessary due to latency restrictions. We advise that collaborative planning take place *between* EVAs, and that the highly trained crews are left to execute the pre-planned traverses according to their enhanced, in situ, situational awareness. With the development of mission-planning software, crews will be able to directly access and leverage these decision support tools unencumbered by communication latency with the ground. Such operational frameworks will be *required* for Mars exploration. Ground will more likely focus on continuous, parallel operations of the RLRV, and integrating these results into the inter-EVA debriefings and planning sessions.

Synthesis: These Architectural Definition concepts and requirements are now used to explore low-upmass *in situ* building materials [7,8] and inflatable architectural elements [9] for further conceptualization and design of the Hadley Max 500 day DRM.

References: 1. Mickey et al. (2024) LPSC 55 #1638. 2. NASA SP-289 (1973). 3. Iqbal et al. (2021) LPSC 52 #1917. 4. Eppler et al. (2024) LPSC 55. 5. Daniti et al. (2024) LPSC 55 #1667. 6. NASA M2M Strategy and Objective Development. 7. Brandić Lipińska et al. (2022), Front. Built Environ. 8. 8. Rothschild et al. (2024) LPSC 55 #1576. 9. Maurer et al. (2024) LPSC 55 #1587. **Acknowledgement:** We gratefully acknowledge support from the NASA NIAC Program and the Brown University UTRA program.

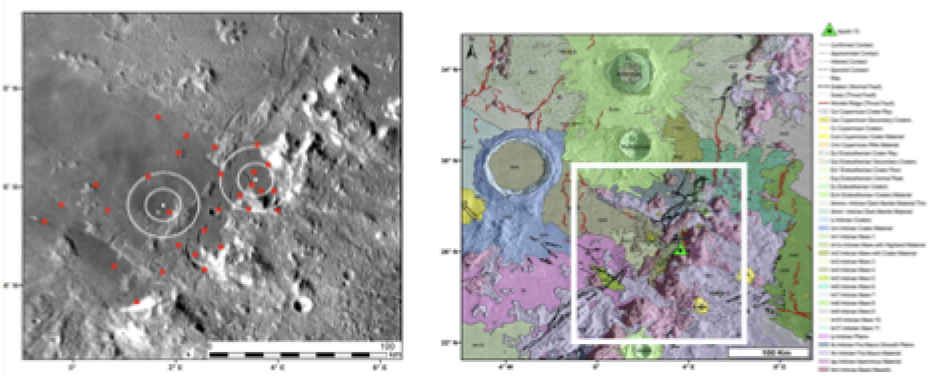

Fig. 1. Left: Hadley Max region; circles show 2 base sites and 10 and 20 km radius around each. Red dots; selected ROSI [4]. Right: Recent geological map [3]; white box shows left image (LROC WAC) location.

HADLEY MAX 500-DAY DESIGN REFERENCE MISSION (DRM) TO THE APOLLO 15 HADLEY-APENNINE REGION: (4. TRAVERSE DESIGN AND IMPLEMENTATION). D. Eppler[1], J. Head[2], D. R. Scott[2], B. Boatwright[2], L. Rothschild[3], C. Maurer[4], R. Creel[5], R. Martin[3], W. Mickey[2], D. Fryd[2], M. Daniti[2], C. Wu[2]. [1]The Aerospace Corporation, Houston TX , [2]Brown University, Providence RI, [3]NASA Ames Research Center, Mountain View CA, [4]redhouse studio, Cleveland OH, [5]Huntsville, AL (NASA MSFC Ret.) (dean.b.eppler@sanantoniomountainconsulting.com).

Traverse Design and Implementation: Beginning with the Hadley Max 500-day Design Reference Mission (DRM) conceptual background [1], we proceeded to utilize Apollo 15 mission scientific goals and objectives, together with expanded broad scientific goals and objectives derived from Apollo 15 mission results and recent regional geologic mapping [2], as a basis to identify the resulting Regions of Scientific Interest (ROSI) for the Hadley Max DRM [3], and used these scientific requirements to define the Mission Architecture (exploration range, mobility requirements, crew size, number of bases, number of EVAs, human-robotic partnership requirements, habitat requirements, etc.) [4], in preparation for more detailed mission design and traverse planning activities, discussed here.

Mission Planning and Operations Guidelines: During a lunar day (~30 Earth days), we assume 15 consecutive Earth days during Lunar Night (dedicated base laboratory and traverse debriefing-planning activities), followed by 15 consecutive Earth days in Lunar Day (dedicated to EVA operations). In order to optimize human performance, we assume a 6-3-6 Earth day duty cycle, with 3-day 'weekends'. For daily duty cycles, we assume 8-hour sleep periods, and 16 hour work-rest periods. We further assume 8 hour EVAs (further adjusted for changing lighting geometry), and a 10 km radius of operations (walk-back constraint) from the base. Hadley Max Robotic LRV (RLRV) traverses will be designed following the DRM Astronaut traverses with the goal of complementing these with scouting, interpolation, and extrapolation RLRV campaigns. ***Landing Sites*:** On the basis of the desire to first build on the Apollo 15 crew observations and analysis of the returned samples, and secondly to extend the major goals to a broader exploration and sampling region [2], we chose the Apollo 15 landing site as the prime landing site (Fig. 1a,c). Using the 100-200 km radius of operations defined by the distance from the A15 site to the farthest ROSI (Fig. 1a), we chose the second landing site/base of operations to the W of Hadley Rille in order to access all ROSI and explore the full range of mission scientific goals and objectives. ***EVA Radius of Operations*:** This was defined by the 'walk-back distance' (~7 km) during Apollo, the maximum distance that the astronauts could walk back to the LM in the case of LRV mobility failure (in turn constrained by consumable supply and astronaut metabolic rates). For Hadley Max, we assume an improved suit capability to deliver a 10 km radius of operations, but identify this as a key enabling technology requirement. We also introduce and explore the concept of doubling the radial distance of operations by having "Human Outposts" at key locations within the 10-20 km radial circumference from each site, installations that would permit outpost pressurization, human overnight stays and suit consumable recharge, all requiring key technology and design developments. We utilize this, and related concepts (e.g., 'pup' tents, simultaneous and parallel robotic operations, etc.) developed in the Mission Architecture contribution [4]. ***EVA Station Duration***: During Apollo 15-17, the average station duration was 38.7 min, largely defined by the multiple objectives and their intervening separation distances. The longest duration stations were A15-EVA 2-Station 6/6a (highlands boulder, steep slope; 1 hr 20'), A16-EVA 3-Station 13 (Shadow Rock; 1 hr 19') and A17-EVA 3-Station 6 (large boulder; 1 hr 14'). Due to the improved scientific understanding and more focused questions for Hadley Max, we assume a typical station duration of 1 hour. ***EVA Duration Duty Cycles*:** Typical A15-17 EVA durations were ~7 hours each, a number constrained by human physiology/diurnal cycles. We assume modest improvements in suit efficiency and mobility speed and adopt an EVA duration of 8 hours for Hadley Max. ***Station Duration*:** A15-17 on-station times averaged 38.7' and we adopt 40' for a typical station for Hadley Max. ***Stations per EVA*:** Assuming 8 hour EVAs, we adopt a planning number of 5 stations/EVA (the A15-17 average for 3 EVAs was ~12 stations, 4/EVA). ***Drive Times Between Stations and Average LRV speeds*:** Average A15-17 drive times between stations were ~17' and average LRV speeds ~7.3 km/hr: given increased efficiency in terrain knowledge, route planning algorithms, and improved LRV design, we adopt 15' and 10 km/hr for Hadley Max. ***EVA Planning Strategy*:** On the basis of the above considerations, for Hadley Max average traverse planning guidelines, we assume 5 stations/EVA (5 hours), ~15' travel between stations (1.5 hours) and 1.5 hours for flexibility, for a total of 8 hours. During Apollo, due to the walkback constraint and consumable consumption, EVAs were designed to visit the most distance station first, and then work back toward the LM. We adopt a similar strategy for Hadley Max.

Ability to Traverse Slopes: A major scientific goal is to explore the lunar highlands and the Hadley Rille floor and wall. Experience with Apollo 15 shows that traversing steeper slopes at the base of Hadley Delta (EVA2-S2) (Fig. 1b,c) had a major effect on LRV mobility (wheel slippage, etc.) and Astronaut mobility and metabolic rate. On the basis of data from Apollo and Lunokhod [5] we adopt a maximum traversable slope of 20°, and increase non-mare station times by ~20%. We identify improved human and robotic rover capability on slopes and improved astronaut slope exploration and sampling strategies, as necessary key enabling technologies. One of the major unknowns in traversing and sampling the highlands is the origin of the "elephant-hide terrain", a wrinkled, terrain-parallel morphological texture associated with highland slopes; [6] found that the majority of slopes steeper than 6°–8° in their analysis are covered with EHT. Ridge separation distances are estimated at meters-scale and heights <~ a meter, but the origin of the ridges, their grain-size, and mobility characteristics are unknown. Thus, understanding the EHT is one of the highest operational and scientific priorities in highlands exploration.

Improved Data for Human and Robotic Traverse Planning and Analysis: Apollo 15 site selection and traverse planning were accomplished utilizing 20 m-resolution LO-V images available at the time [1]. The NASA Lunar Reconnaissance Orbiter Mission has operated in lunar orbit for the last 14 years and has provided extremely high resolution images, altimetry, stereo photogrammetry, thermal inertia, water detection and radar data [7]. These fundamental data sets can be readily utilized to produce very high resolution image, topography, roughness, blockiness, slope and evolving lighting conditions maps that are essential ingredients to determining detailed science objectives, station locations, and traverse planning routes for optimal EVA planning. For example, Fig. 2a shows a slope map for one section of Hadley Rille and adjacent highlands, and Fig. 2b shows a slope map for defining human-robotic traverse access from the 20° slope constraint, data essential for human/robotic traverse design.

In addition, the advent of sophisticated mission and traverse planning software that can ingest and maintain cognizance of these multiple spatial data sets in real-time has revolutionized both pre-mission traverse design and planning, and real-time traverse assessment and contingency planning. For example, recent developments in mathematical frameworks for reasoning under uncertainty (Partially Observable Markov Decision Processes; POMDPs) have been applied to automated decision support frameworks for planetary exploration. Such applications include SHERPA (System Health Enabled Real-time Planning Adviser) [8] that is designed to take different sources of uncertainty into account when generating decision recommendations for traverse planning and real-time operations. We are currently exploring a range of recently described algorithms for optimizing traverse planning. In the next stages of the Hadley Max DRM project, we plan to test and assess some of these for optimizing human-robotic performance and science return.

References: 1. Daniti et al. (2024) LPSC 55 #1667. 2. Iqbal et al. (2021) LPSC 52 #1917. 3. Mickey et al. (2024) LPSC 55 #1638. 4. Fryd et al. (2024) LPSC 55 #1887. 5. Basilevsky et al. (2019) *SSRes 53*, 383. 6. Kreslavsky et al. (2021) LPSC 52, #1826. 7. Chin et al. (2007) *SSRev 129*, 391. 8. Baliban et al. (2018) *AIAA SciTech Forum*, 10.2514/6.2018-1150. **Acknowledgement:** We gratefully acknowledge support from the NASA NIAC Program and the Brown University UTRA program.

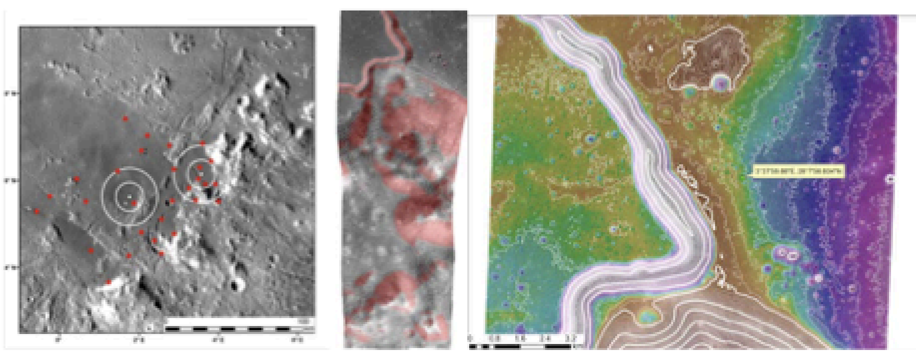

Fig. 1. (a) Left: Hadley Max exploration region showing two base locations surrounded by 5 & 10 km radius circles. Dots represent selected ROSI locations, the scientific input into human/robotic traverse design. (b) Middle: Slope map for a section of the Hadley Max A15 base location, showing the 20° slope constraint (red) on human and robotic rover access. (c) Right: Topographic contour map (thick lines 100 m, thin 10 m) of the rille-mare-highlands area near the A15 site Hadley Max base (green dot).

HADLEY MAX 500-DAY DESIGN REFERENCE MISSION (DRM) TO THE APOLLO 15 HADLEY-APENNINE REGION: (5. REDUCING UPMASS DEMANDS WITH *IN SITU* MYCO-ARCHITECTURE).

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Working Toward a Solution to the Upmass Problem: We began with the Hadley Max 500-day Design Reference Mission (DRM) conceptual background [1], and proceeded to call on Apollo 15 (A15) mission scientific goals and objectives, combined with expanded scientific goals and objectives derived from A15 mission results and recent regional geologic mapping [2]. We then identified the Regions of Scientific Interest (ROSI) for the Hadley Max DRM [3], and the used these scientific requirements to define the Mission Architecture [4], and more detailed Hadley Max mission design and traverse planning activities [5]. Here we address one of the most significant problems for long-duration and sustained human presence on the Moon and concurrent scientific exploration success: the Key Enabling Technology to alleviate the huge and continuous upmass requirements necessary to support the base and exploration infrastructure [4]. In order to help alleviate this "upmass roadblock", we have pursued two promising technologies: 1) Myco-Architecture [6-9], where building materials can be "grown *in situ*" in order to significantly minimize upmass penalties, and 2) Inflatable Structural Elements [10], in which low-volume, low-mass inflatables can be combined with Myco-Architecture to produce a wide range of enclosures *in situ*. Here we outline the evolution of our progress on "Myco-Architecture" and future goals and objectives.

Definition of Required Habitats, Enclosures and Related Architectural Elements: As a baseline for required architectural elements, we called on the Hadley Max DRM Architecture [4] and Traverse Planning [5] studies that produced these baseline elements. ***1. Landing Pads (LP)***: For both Human and Robotic missions; like helo pads, flat, devoid of soil backwash contaminant. ***2. Initial Base Structure (IBS)***: Living and working habitat; follows the initial stages where there is a landing module (LM). ***3. Evolutionary Base Structure (EBS)***: Larger scale, separation of work/living activities; increased *in situ* science activities; IBS evolves to dust mitigation structure. ***4. Outposts: Remote Science Bases (RSB)***: Modeled after IBS, but located >10 km radius from Landing Site. Require up to ~5 RSBs for in depth, *in situ* science activities. Increase number in order of science priority. ***5. 'Pony Express' Stations (PEX)***: These are the lunar 'pup tents' that will be precursors to the Remote Science Bases (RSB), and then Earth-day sleep-stations on the way to the final Remote Science Bases (RSB). Sample storage stations, geophysical stations; can be resupplied/samples collected by CLPS missions. ***6. Robotic Rover Requirements***: a) LRV garage at base for surviving lunar night, re-outfitting; b) Robotic LRV 'pup tents' for surviving lunar night, caching samples. ***7. Application to the Artemis Circumpolar Environment (ACE)***: How do we optimize these basic requirements and DRM concepts for the harsh conditions of the South Circumpolar Region. ***8. Assessing Feed-Forward to Mars Exploration***: How does the Mars environment modulate and modify these DRM strategies and architectural elements? Here we investigate elements 1-6, and explore how producing construction materials *in situ* on the Moon can help alleviate the upmass problem. We plan to treat 7 and 8 in future analyses.

Background and Approach: Transporting materials beyond Earth, such as spacecraft, Astronauts, and construction materials, is limited by mass constraints. Yet long-term residence, operation and scientific exploration on the lunar surface requires an extensive infrastructure, a significant upmass, and a major large-mass component of this is in habitats, designed to protect crew and equipment from radiation, extreme temperatures and micrometeorite bombardment. There is a significant mismatch between habitat requirements at destination and what can realistically transported there. Infrastructure for human survival is not automatically "user ready" on the Moon. Habitats could be built with locally sourced regolith or ice materials by in *In Situ* Resource Utilization (ISRU), but in the end, even this requires significant upmass. To alleviate this problem, we have been exploring technologies [6-9] that are self-replicating and self-repairing, to assess their utility in circumventing the upmass problem. Life meets these technological criteria for space utilization and in addition can be reprogrammed through synthetic biology. In this quest, we look to exploit the genetic hardware store inherent in our vast biodiversity, moving capabilities from familiar forms such as trees for wood, to a more tractable space-faring chassis such as yeast or bacteria.

Strategy and Concepts: A critical aspect of human space exploration and eventual settlement is the ability to construct habitats while minimizing payload mass launched from Earth. To respond to this challenge, and as a continuation of our research program initiated under the auspices of the "Myco-architecture Off Planet" NASA NIAC Team, we have explored the use of fungal biocomposites, for example Bio-Bricks, (Fig. 1) for growing extra-terrestrial structures and building materials, directly at the destination, significantly lowering the mass of structural materials transported from Earth and minimizing the need for high mass robotic operations and infrastructure preparations. Currently, the idea of working with living biological organisms, and the phenomenon of growth itself, is of increasing interest in architecture and space applications. Here, we describe the use of mycelium-based composites as an alternative, biological approach for constructing regenerative and adaptive buildings for extraterrestrial habitats. These composites, are fire-resistant and insulating, and do not consist of volatile organic compounds from petrochemical products. These can be used independently or in conjunction with regolith, and could employ the living biological growth in a controlled environment for the process of material fabrication, assembly, maintenance, and repair, providing structures resilient to extra-terrestrial hazards. We explored avenues to make this biological approach feasible, providing new, growing materials for designing and building sustainable habitats for long-duration space missions.

Our research has explored the potential and challenges of using mycelium-based biocomposites for space applications. The approach of using biological growth for the off-Earth construction, similarly to other researched ISRU-based approaches, is designed to lower the mass of materials needed to be transported from Earth. In addition, it focuses on lowering the energetic costs of the construction of in situ habitats, such as the work required to assemble the habitat. In the long-term, using biological materials and growth as a construction method, opens up the potential of ELMs (Engineered materials composed of Living cells that form or assemble the Material itself or modulate the functional performance of the material in some manner) [11].) to potentially provide supplementary capabilities, such as sensing and responding to environmental stimuli, self-healing, etc. Such developments could make the habitats even more flexible and reliable. The further development of research on ELMs and mycelium-biocomposites will allow for advancements in the field of biotechnology and habitat construction. These concepts employ living biological growth in a controlled environment for the process of material fabrication, assembly, and maintenance. Positive attributes of these approaches and techniques include the modest upmass requirements of a few spores, nutrition for mycelial growth, and a growth framework, along with the potential to reproduce using in situ resources, the ability to grow to accommodate on-site terrain, and the potential additional control provided by the tunability of the materials. We see myriad possibilities for mycotecture utilization off planet. Because the research is still in an early stage, one of our major goals once the enabling technologies are identified, is to use the Hadley Max 500-day DRM Architecture to develop a technology roadmap and recommendations for further development.

References: 1. Daniti et al. (2024) LPSC 55. 2. Iqbal et al. (2021) LPSC 52 #1917. 3. Mickey et al. (2024) LPSC 55. 4. Fryd et al. (2024) LPSC 55. 5. Fryd et al. (2024) LPSC 55/ Eppler et al. (2024) LPSC 55. 6. Rothschild et al. (2017) Astrobio. Sci. Conf. #3720. 7. Rothschild et al. (2022) LPSC 53 #2983. 8. Rothschild et al. (2023) LPSC 54 #2544. 9. Brandić Lipińska et al. (2022), Front. Built Environ. 8. 10. Maurer et al. (2024) LPSC 55. 11. Nguyen et al. (2018) Adv. Mat. 30, 1704847.
Acknowledgement: We gratefully acknowledge support from the NASA NIAC Program and the Brown University UTRA program.

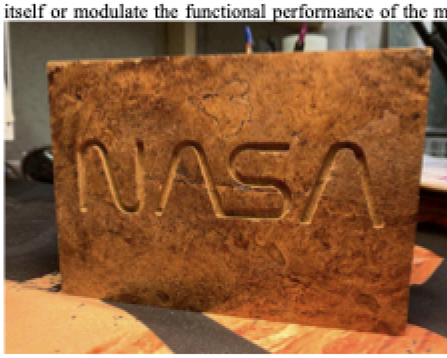

Fig. 1. Left: Bio-Brick made of Myco-Architecture Materials (Courtesy Chris Maurer): This brick is a composite of wood and fungal mycelium. Fungi break down biochemicals like cellulose converting them into their own chitin-rich biomass. By growing mycelium on plant fodder they fuse at a cellular level allowing us to utilize the best characteristics of their respective Kingdoms - Plantae and Fungi. We are currently developing methods to grow these multi-kingdom composites off-planet to save transport cost, reduce energy demands, and utilize bio-performative aspects such as radiosynthesis, that may one day convert space travel's biggest liability, ionizing radiation, into a resource for material production. Bio-Brick dimension is 17 x 12.5 x 5.5 cm. Right: J. Head and NASA Administrator Bill Nelson examine one of our Bio-Bricks at Brown University. Photo by RI Senator Jack Reed.

HADLEY MAX 500-DAY DESIGN REFERENCE MISSION (DRM) TO THE APOLLO 15 HADLEY-APENNINE REGION: (6. REDUCING UPMASS DEMANDS UTILIZING *IN SITU* INFLATABLE STRUCTURES). C. Maurer[1], J. Head[2], D. R. Scott[2], B. Boatwright[2], L. Rothschild[3], D. Eppler[4], R. Creel[5], R. Martin[3], W. Mickey[2], D. Fryd[2], M. Daniti[12], C. Wu[2]. [1]redhouse studio, Cleveland OH, [2]Brown University, Providence RI, [3]NASA Ames Research Center, Mountain View CA, [4]San Antonio Mountain Consulting, Houston TX, [5]Huntsville, AL (NASA MSFC Ret.) (james_head@brown.edu).

Introduction: Beginning with the Hadley Max 500-day Design Reference Mission (DRM) conceptual background [1], we proceeded to utilize Apollo 15 mission scientific goals and objectives, together with expanded broad scientific goals and objectives derived from Apollo 15 mission results and recent regional geologic mapping [2], as a basis to identify the resulting Regions of Scientific Interest (ROSI) for the Hadley Max DRM [3], and used these scientific requirements to define the Mission Architecture [4], in preparation for more detailed mission design and traverse planning activities [5]. We then turned to addressing the major upmass challenges revealed by any long-term residence and sustained presence on the Moon, first assessing reduction in upmass demands by employing in situ Myco-Architecture [6], and here exploring reducing upmass demands utilizing in situ inflatable structures.

Definition of Required Habitats, Enclosures and Related Architectural Elements: We previously identified the types of habitats, enclosures and related architectural elements [4] dictated by the Hadley Max scientific objectives [3] as follows: **1. Landing Pads (LP):** For both Human and Robotic missions; like helo pads; flat, devoid of soil backwash contaminant, retroreflector for guidance. **2. Initial Base Structure (IBS):** Living and working habitat; follows the initial stages where there is a landing module (LM). **3. Evolutionary Base Structure (EBS):** Larger scale, separation of work/living activities; increased *in situ* science activities; IBS evolves to dust mitigation structure. **4. Outposts: Remote Science Bases (RSB):** Modeled after IBS, but located >10 km radius from Landing Site. Require up to ~5 RSBs for in depth, *in situ* science activities. Increase number in order of science priority. **5. 'Pony Express' Stations (PEX):** These are the lunar 'pup tents' that will be precursors to the Remote Science Bases (RSB), and then Earth-day sleep-stations on the way to the final Remote Science Bases (RSB). Sample storage stations, geophysical stations; can be resupplied/samples collected by CLPS missions. **6. Robotic Rover Requirements:** a) LRV garage at base for surviving lunar night, re-outfitting; b) Robotic LRV 'pup tents' for surviving lunar night, caching samples. **7. Application to the Artemis Circumpolar Environment (ACE):** How do we optimize these basic requirements and DRM concepts for the harsh conditions of the South Circumpolar Region, and the lunar farside? **8. Assessing Feed-Forward to Mars Exploration:** How does the Mars environment modulate and modify these DRM strategies and architectural elements?

These required structures are the basis for using the Myco-Architecture [6] concept to explore reducing up-mass demands utilizing in situ inflatable structures.

Architectural designs and deployable in situ construction methodologies: In concert with the development of Myco-Architectural biological material, architectural designs and deployable in situ construction methodologies were developed at redhouse studio, including plans, 3d models, section details, and animations of various designs and building processes. After assessing many ways to deploy bio-composites off planet (including lightweight formwork, masonry and additive manufacturing), redhouse arrived at a 'sealed bag' deployment (Fig. 1) as the best option to control the environment for growth, develop the shape of the shelter, and protect the lunar environments from potential contamination. The sealed bag concept will allow the bio-composites to self-assemble in multiple layers of membranes that can provide redundant protection, channel nutrients, and create warm habitable spaces within the framework. This can be achieved at many scales and could be utilized as a platform technology for building any mission structure or object (Fig. 1,2).

<u>Evolution of Architectural Concepts</u>: The design concept started as deployable habitat shell that would grow like a living organism at destination with the aid of in situ resources. This would be less energy intensive and leave a smaller planetary footprint then mining or melting surface material. Intense team study and analysis has enabled the initial concept to grow and evolve new multi-functional facets. We found that the biological functions that enable growth of the materials also provide such benefits as oxygen production and may also be used to generate heat and electricity. Thus , this biomimetic and bio-utilitarian option provides potential options to very high up-mass costs of prefabricated structures that come fully outfitted, and other construction materials. Detailed architectural and design analyses suggested that necessary attributes, such as plumbing lines, stovetops, and floormats, can be folded into the form, plugged-in ready to go, and the floors, walls and windows can be grown in place so that the in situ grown building is comparable to a high-mass Earth-fabricated, and then delivered to the site, structure. In order to accomplish this, however, the challenge is in the packing of the habitat shell into off-planet deliverable cargo geometry constraints. We found that many of the domestic utilities, scientific equipment, furnishings, and fixtures can be built directly into the expandable shell. More detailed assessments showed that such self-contained modules can be wrapped into the larger structure and secondarily de-

ployed once robotic-enabled construction of the shell is finished.

Materialization: The process of making fungal composites includes growing filamentous saprophytic fungi on biomass substrates that can become fused at a cellular level. Our team has demonstrated composites that have structural characteristics superior to wood framing, thermal resistance characteristics superior to fiber-glass batt insulation, and fire resistance equivalent to type-X gypsum board, that is, construction industry standards, all comparable or superior to other ISRU suggested materials.

Construction Methods: In order to save transport mass and still have robustly tested technology and structures the mycotecture team proposes developing an inflatable structure that can fill will living bio-materials (bioterials) that harden in place to form a solid, insulative, radiation-attenuating structure using the inflatable as *permanent formwork* for the grown-in-place building. This enables us to source most (up to 90%) of the mass in situ by way of water, gasses, and trace nutrients that are easily accessible and are planned to be stockpiled by NASA. The design concept of a deployable self-growing structure must take into consideration the viability of growing the constituent organisms at destination. Lunar and Martian environments are very extreme and do not support life. The solution is to use the example of the only living organisms to go to the Moon and return alive as our model. Twelve Americans have walked on the Moon and when they did they were protected from the lunar environment by their A7L spacesuits (designed to provide an astronaut life-sustaining environment during periods of extra vehicular activity and unpressurized spacecraft operation). The suits, made of a white, non-flammable material called beta cloth and Teflon-coated fiberglass, were produced by ILC Dover. These suits and subsequent EVA mobility units have made space conditions bearable by creating an enclosure that mimics Earth environments in atmosphere, temperature, and pressure. The same requirements exist for our microorganisms. The architectural designs produced by this team link these enclosures that are analogous to biological cells with an analog circulatory system. These cells or, lunar optimized bio-reactor enclosures (LOBE), act like space suits for the production of bioterials within the inflated scaffolding. The LOBEs are linked together and are transported in folded inflatable structures to create bio-performative living shells that replace the air and water in the shell cavities as they grow-in-place.

Synthesis: In situ inflatable structures clearly permit significant reductions in upmass penalties, and also offer many other ancillary benefits, such as radiation protection. They also offer significant reduction in environmental impacts of on-site construction from local material. We are continuing to explore innovative ways in which a) in situ Myco-Architecture, combined with b) inflatable structure concepts, can optimize the scientific goals and objectives of long-term missions to the Moon and Mars, as illustrated by the Hadley Max 500-day DRM [1-6].

References: 1. Daniti et al. (2024) LPSC 55. 2. Iqbal et al. (2021) LPSC 52 #1917. 3. Mickey et al. (2024) LPSC 55. 4. Fryd et al. (2024) LPSC 55. 5. Eppler et al. (2024) LPSC 55. 6. Rothschild et al. (2024) LPSC 55. **Acknowledgement:** We gratefully acknowledge support from the NASA NIAC Program and the Brown University UTRA program.

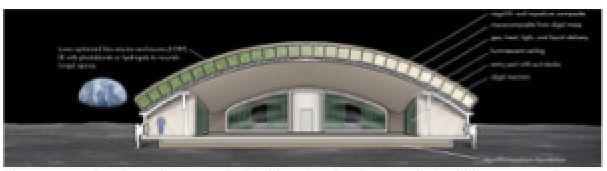

Figure 1: Section through prototypical habitat showing lunar optimized bioreactor enclosures (LOBEs) from left to right first growing photosynthetic organisms that are then converted into a composite bioterial by the growth of fungal mycelium.

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Figure 2: Stills from animation showing the "growth" of a prototypical building.

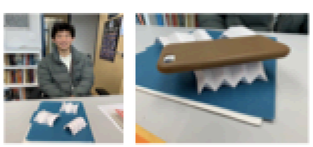


Fig. 3. Left. Brown Undergraduate Christian Wu and his "Flexible Origami" inflatable cargo storage solutions. Right. Strength of "Flexible Origami" (I-Phone atop structure).

4.5.1 Background

HADLEY MAX 500-DAY DESIGN REFERENCE MISSION (DRM) TO THE APOLLO 15 HADLEY-APENNINE REGION: APPLICATION OF SCIENCE GOALS AND OBJECTIVES TO PLANNING LONG DURATION EXPLORATION ARCHITECTURE (1. BACKGROUND). J. Head[1], D. R. Scott[1], B. Boatwright[1], L. Rothschild[2], C. Maurer[3], D. Eppler[4], R. Creel[5], R. Martin[2], W. Mickey[1], D. Fryd[1], M. Daniti[1], C. Wu[1]. [1]Brown University, Providence RI, [2]NASA Ames Research Center, Mountain View CA, [3]redhouse studio, Cleveland OH, [4]San Antonio Mountain Consulting, Houston TX, [5]Huntsville, AL (NASA MSFC Ret.) (james_head@brown.edu).

Introduction: Among the six successful Apollo Lunar Exploration Program landed missions, Apollo 15 was the first Lewis and Clark-like "Scientific Expedition to the Moon" [1]. Experience with Apollo 11, 12 and 14 walking traverses provided fundamental scientific results, demonstrating pinpoint landing techniques, and increasing stay-time, EVA durations, and mobility assistance (such as the Mobile Equipment Transporter, MET, on Apollo 14), but also clearly demonstrated the need for increased numbers of EVAs and mobility in order to reach multiple and more distant scientific objectives [2]. The Apollo 15 mission [1] carried the first Lunar Roving Vehicle (LRV) that enabled Astronauts Dave Scott and

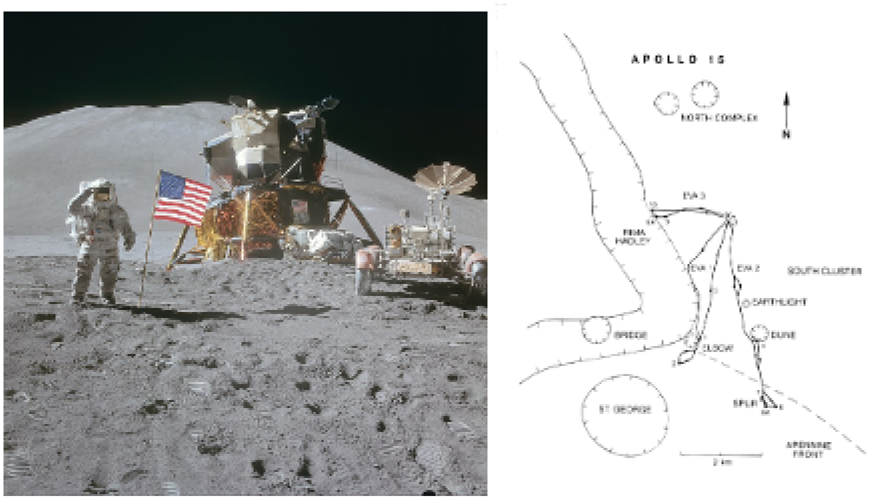


Fig. 1: Left: LMP Jim Irwin, the Lunar Module (LM) and the deployed LRV; Mt Hadley in the background. Right: Completed Apollo 15 Traverses.

Jim Irwin to reach distant objectives and traverse out to a radial distance of 7 km [3] (Fig. 1), the maximum distance permitted by the need for them to walk back if the rover failed (the 'walkback' constraint).

The initial Standup EVA (SEVA), was inserted as a result of Commander Scott's desire to get an initial overview of the terrain (due to the fact that only 20 m resolution images were available for pre-mission planning) [4]. The following three periods of EVA visited four of the five major mission objectives (lunar maria, lunar highlands (Hadley Delta), Hadley Rille, secondary crater cluster, and North Complex). The Apollo 15 mission [1,5,6] completed 19.7 hours of surface exploration, deployed a complex set of scientific instruments, drilled a 2.4 m drill core, traversed 27.9 km of the lunar surface, and returned 77 kg of samples [7], 56% more than the previous Apollo 14 mission. The results of this very successful exploration mission [8] addressed many fundamental scientific questions [1,7], and an entire science conference dedicated to Apollo 15 mission results was held in Houston. As is usually the case in science and exploration, the findings from Apollo 15 [1,7] led to a whole new set of questions, and posed an additional set of exploration destinations in the larger area of the Hadley-Apennine landing site (Fig. 2).

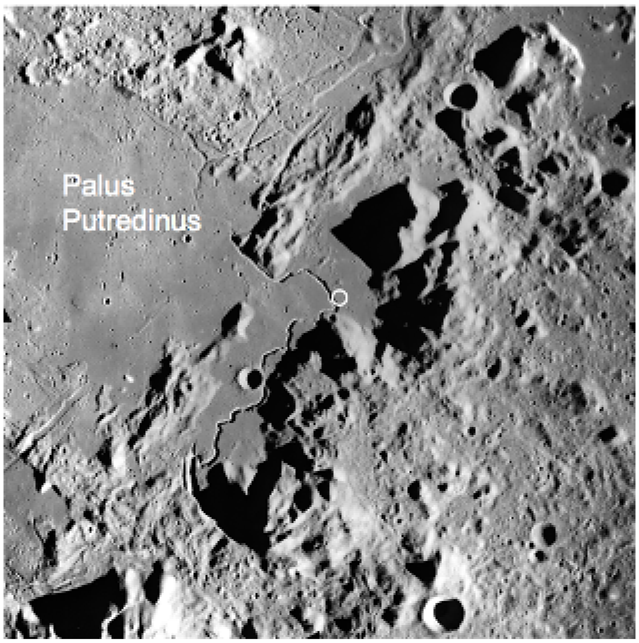


Fig. 2. The broader Hadley-Apennine region showing multiple points of geological interest beyond the 7 km exploration radius around the Apollo 15 landing site (white circle) (Fig. 1, right).

In the years subsequent to the Apollo Lunar Exploration Program, orbital remote sensing missions (such as Lunar Prospector, Clementine, Lunar Reconnaissance Orbiter, Chandrayaan-1) permitted the extension of the Apollo and Luna Mission sample return results to the entire Moon, and a much more refined vision of the Moon as a planetary body emerged [9]. Concurrently, robotic exploration of Mars with landers, orbiters and rovers was providing a view of a planet that appeared to have been Earth-like in its earliest history, but had then evolved to an extremely cold hyperarid polar desert that we see today [10]. Could Mars have harbored life in its earlier "warm and wet" climate history? These emerging results obviously kindled an interest in NASA in the human exploration of Mars. Several committees were formed and engineering studies were undertaken to assess scientific goals and objectives and mission architecture. The following type of questions were posed to these groups: What will be the state of robotic Mars exploration and Mars knowledge in 2030? What are the key science questions that humans can address that will not be addressed by robotic missions by 2030? What is the best way to deploy/utilize humans on Mars? What is the role of IT/robotics alongside humans?

In a 2007 study [11], one of the first issues that arose in reference to the mission architecture was the question of duration of the Mars mission surface stay time for the astronauts. Did scientists want to go and return in one access opportunity, during which the approximate stay time would be ~30 days, or stay on Mars and return at the following access opportunity, during which the approximate stay time would be ~500 days?

The skepticism of the engineers ("What would you be doing on Mars for 500 days???") was met with euphoria from the geologists and other scientists ("Wow! Let us show you what we could do!"). The ensuing reports [11] outlined the results of these studies, identifying 40 candidate Mars human exploration sites Fig. 3, top), designed around MEPAG Goal III: 'Determine the Evolution of the Surface and Interior', and showing Design Reference Missions

(DRMs) for scientific points of interest and traverses that would enable the astronauts to reach them (Fig. 3, bottom).

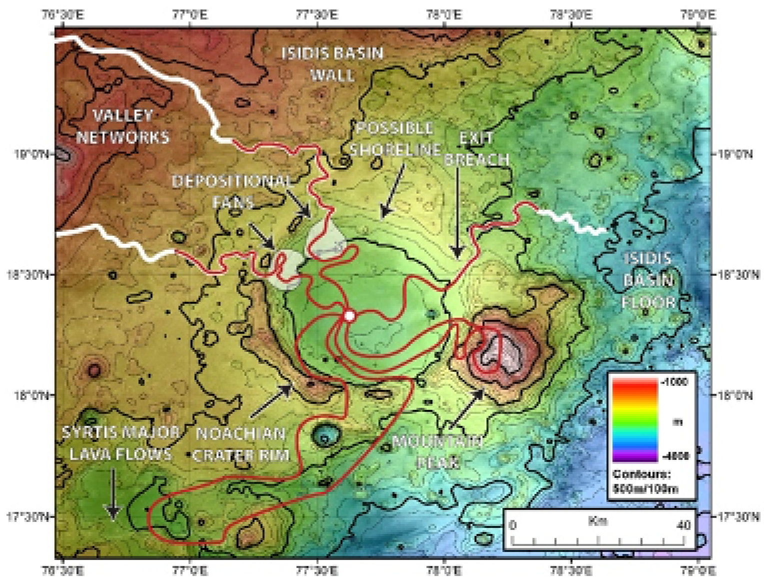


Fig. 3. Top: Forty scientific human exploration landing sites identified on Mars. Bottom: Example DRM and traverses at Jezero Crater (from [12]).

Subsequent studies periodically revisited these issues [12,13], most recently, the MEPAG Human Exploration of Mars Science Objectives (HMSOTT) report [14]. In response to the challenges from the NASA engineers in 2007 ("What would you be doing on Mars for 500 days???"), we reasoned that presenting a more familiar and previously successful mission scenario, but expanded to a 500-day duration, would help both the engineers and scientists develop a design reference mission (DRM) that would mutually educate, as well as identify the key mission parameters and technology requirements for a 500-day mission to Mars. In addition, the several attempts at sustained human lunar exploration (SEI, Constellation) underlined the need for similar thinking approaches for the Moon. And in 2017, the announcement of the NASA Artemis Moon to Mars initiative linked the importance of such an approach.

Thus was born the 500-day Human Exploration Mission back to the Hadley-Apennine region (Fig. 2), the landing site region of the Apollo 15 first scientific expedition to the Moon. Named the "Hadley Max" mission by Apollo 15 Commander Dave Scott, this Design Reference Mission (DRM) concept called on utilizing the Apollo mission planning and execution experience of two of the co-authors (Scott and Head), mission operations LRV design and thermal control planning and execution experience (Creel), crew training and operations experience (Eppler), Myco-Architecture as in situ building materials (Rothschild) [15], and the enthusiasm and creativity of Brown University geology, physics, engineering and computer science students (a team that has grown over the years as the Hadley Max DRM concept has evolved).

The major goals and objectives of the "Hadley Max" 500-Day Hadley-Apennine Design Reference Mission (DRM) project were to identify the key mission elements and requirements in the following areas: 1) Background and motivation (this abstract); 2) Scientific Goals and Objectives; 3) Implications for Mission Design and Architecture; 4) Traverse Design & Implementation, 5) Implications for Habitat Requirements-Role of Myco-Architecture; and 6) Implications for Habitat Design and Construction, each treated in separate LPSC 55 contributions.

References: [1] Apollo 15 Preliminary Science Report (1973) NASA SP-289. [2] J. W. Head (1970) An Analysis of the Scientific Objectives and Proposed Landing Sites in the Hadley-Apennine Region, Bellcomm Memorandum B70 10cJ29. [3] J. W. Head (1971) Status Report on Preliminary Traverse Planning for Apollo 15 Hadley-Apennine, Bellcomm Memorandum B71 02026. [4] J. W. Head (1971) Scientific Benefits of a Pre-Traverse Stand-Up EVA (SEVA) at Hadley-Apennine, Bellcomm Memorandum B71 03031. [5] D. Woods and F. O'Brien (2020) https://history.nasa.gov/afj/ap15fj/index.html, Apollo 15 Flight Journal, [6] E. M. Jones (2020) Apollo 15 Lunar Surface Journal, https://history.nasa. gov/alsj/a15/a15.html. [7] Apollo 15 PET (1972) The Apollo 15 lunar samples: A preliminary description. Science 175, 363. [8] Apollo 15 Mission Report (1971) NASA-TM-I-68394, 325 p. [9] Jolliff, B.L., et al. (2006) New views of the Moon, Reviews in Mineralogy and Geochemistry (2006) 60. [10] Carr, M.H. and Head, J.W. (2010) Geologic history of Mars. EPSL 294, 185. [11] Levine, J., Garvin, J., and Head, J. (2010) Planning for the scientific exploration of Mars by humans, Part 2, Journal of Cosmology 12, 3636. [12] Beaty, D. et al. (2015) MEPAG HSO-SAG. [13] EZ Workshop for Human Missions to Mars (2015) Houston, TX. [14] MEPAG HEMSOTT (2023) https://mepag.jpl.nasa.gov/reports.cfm. [15] Brandić Lipińska M., et al. (2022), Biological growth as an alternative approach to on and off-Earth construction. Front. Built Environ. 8:965145.doi: 10.3389/fbuil.2022.965145. Acknowledgement: We gratefully acknowledge support from the NASA NIAC Program and the Brown University UTRA program.

4.5.2 Science Goals and Objectives

HADLEY MAX 500-DAY DESIGN REFERENCE MISSION (DRM) TO THE APOLLO 15 HADLEY-APENNINE REGION: (2. SCIENCE GOALS AND OBJECTIVES).
J. W. Head[1], D. R. Scott[1], B. Boatwright[1], L. Rothschild[2], C. Maurer[3], D. Eppler[4], R. Creel[5], R. Martin[2], W. Mickey[1], D. Fryd[1], M. Daniti[1], C. Wu[1], C. van der Bogert[6], H. Hiesinger[6], W. Iqbal[6]. [1]Brown University, Providence RI, [2]NASA Ames Research Center, Mountain View CA, [3]redhouse studio, Cleveland OH, [4]San Antonio Mountain Consulting, Houston TX, [5]Huntsville, AL (NASA MSFC Ret.) [6]University of Münster, Münster Germany. (james_head@brown.edu).

Science Goals and Objectives: We supplemented the initial five major Apollo 15 scientific goals and objectives (Imbrium impact basin; Sinuous rille origin; Mare history; Ejecta from distant craters; Regolith history), with several additional objectives related to questions raised by Apollo 15 crew exploration/observations, analysis of the Apollo 15 sample suite, and subsequent mapping [1-4].

1) Lunar Maria Lava Flow Emplacement: Prior to Apollo 15, it was thought that Mare Imbrium lavas might be the rapid response of Imbrium basin mantle uplift and resulting massive pressure-release melting, and thus have formed nearly contemporaneously with Imbrium; however Apollo 15 Imbrium ejecta and mare basalt radiometric dates (3.3 Ga) showed that they were ~600 Ma apart, disproving this hypothesis. The mare basalt dates did reveal evidence for two different flow sequences emplaced a few tens of Ma apart, and somewhat different in composition (quartz-normative and olivine-normative). Visual descriptions and high-resolution images by D. Scott revealed distinctive layering in the W rille wall, and portions of these layers were sampled in blocky probable outcrops along the E rille rim. Irwin noted marginal steps along the base of Mount Hadley, suggesting topographic decrease of the mare surface after initial flooding due to lava drainage or and/or solidification. Scott and Irwin discovered clods of green glass beads, an entirely unexpected finding that suggested dense clouds of fine liquid droplets from either pyroclastic or impact crater events, apparently temporally unassociated with the mare basalts, and later shown to be of pyroclastic origin and contain unexpected amounts of H_2O. Intrigued by the question of volatiles in the basalts, D. Scott observed a lone, very vesicular rock (15016) on the maria and made an unscheduled stop to sample it for assessment of volatiles in the lab. Additional crew observations and sampling of highly vesicular mare rocks added new insights about the role of magmatic volatiles in mare basalts and pyroclastic eruptions. But what was the origin of the green glass beads, shown to have originated ~400 km deep in the mantle, their geological context and sequence and relation to the vesicular basalts, and how were they related to the Hadley Rille vent dozens of km to the south, and to the origin of the rille? Apollo 12 had sampled an unusual KREEP-rich breccia (12013) of uncertain provenance and perhaps related to a Procellarum-KREEP Terrane (PKT) crustal province on the NW nearside. Apollo 15 discovered KREEP basalts (15382, 15386) with a crystallization age of 3.9 Ga, indistinguishable for the age of the Imbrium basin, reopening the question of whether the Imbrium impact event induced basaltic volcanism, perhaps contaminated by passing through PKT crust. Outstanding Questions: Does the layering and vertical structure observed in the walls of Hadley Rille correspond to the two sampled lava flows? What is the vertical structure of the two lava flows (vesicle distribution, cooling behavior and history, mineralogical segregation, etc.) and the nature of their interface

(thickness of regolith between them, atop)? Which of these lava flows are associated with the origin of Hadley Rille and in what manner? Do these two flows show evidence of differences in magnetization and field orientation during this critical period? Is there evidence for additional flows (and pre-mare units) deeper in Hadley Rille? What is the regional distribution of the green glass, its associations with the vesicular basalts, and its distribution relative to the Hadley Rille vent? Are either basalt group petrogenetically correlated with the green glass beads? What is the origin and provenance of the KREEP basalts, where do they outcrop, are they related to basalts of different ages outside the Apollo 15 sampling area [4], are they related to the high-albedo smooth plains in the Archimedes area (Apennine Bench Fm.), and are they extrusive basalts or ponded Imbrium basin impact melt? What is full range of basalt compositions and ages in the SE Imbrium basin [4] and where and how does Hadley Rille fit? What is the origin and significance of the marginal terraces observed by Irwin? What is the origin of the several irregularly shaped craters in the Apollo 15 landing region? Do these represent additional vents for lavas seen at Apollo 15?

2) Nature and Origin of Hadley Rille: Early hypotheses ranged from iced-over aqueous fluvial channel erosion to lava-related (open lava flow median channel, highly turbulent thermal erosion, collapsed subsurface lava tubes). Astronaut observations and samples significantly improved our understanding, supporting a lava-related origin, but the actual rille origin remains enigmatic, due primarily to its great length (>135 km) and lack of access to its source vent to the SSW. The broad, low-elevation rim described by Irwin, and the W rille wall stratigraphy documented by Scott, are consistent with an open lava channel and/or thermal erosion, but very narrow portions of the rille elsewhere suggest a collapsed lave tube. Later global documentation of sinuous rilles shows that Hadley rille is anomalously long compared to the global population, and quantitative modeling suggest that the shorter rilles formed by turbulent lava flow and thermal erosion of the substrate, but the formation of the significantly longer, deeper and wider Hadley Rille remains enigmatic. Outstanding Questions: What is the nature of the two Hadley Rille source vents, are they surrounded by pyroclastic deposits (the green glass?), and what is their relationship to the rille? Do they represent separate eruptions or two phases of a single eruption? What are the proximal to distal characteristics of the rille (rim deposits, width, depth, wall-rock stratigraphy, evidence for roof narrowing and collapse)? How does the morphology and structure of the rille differ between straight segments and bends in the rille? What is the thickness of the lava and is pre-lava substrate (Apennine Bench Fm?) exposed? What is the petrogenetic relationship of any circum-vent pyroclastics and adjacent lava flows? Could the marginal terraces observed by Irwin be related to flooding of the valley and drainage of lava into Palus Putredinis? What is the nature and origin of the Schaber Hills (North Complex): volcanic construct or highland lava-covered kipuka?

3) Imbrium Impact Basin: Apollo 14 was targeted to the ejecta deposit of the Imbrium Basin (Fra Mauro Fm.) and Apollo 15 to the rim of the Imbrium Basin (Apennine Mts.) to sample the deepest ejecta (deep, slow-cooling magma ocean anorthosites), date the Imbrium event, understand the highlands diversity in the ejecta, and search for possible mantle material. At Spur Crater on EVA 2, Scott spotted a perched rock brightly glinting in the sunlight, and stated "Houston, I think we found what we came for!", recognizing from a distance that this glint meant large plagioclase feldspar crystals/twinning, a phenomenon he knew meant deep-seated, slowly cooling highland crust. This sample (15415), dubbed the "Genesis Rock" by the media, did indeed reveal much about the nature and cooling history of the lunar

highlands crust, with an age of 4.1 Ga, probably much older but partially reset by the Imbrium event. Other highland norite/spinel troctolite samples (15445) had ages of 4.28-4.46 Ga and are thought to represent the actual solidification age of the deeper magma ocean. Scott and Irwin observed enigmatic 100 m-scale inclined layering in the Apennine Mts. from afar (Silver Spur), well outside the traverse range. Outstanding Questions: What is diversity of highland samples excavated and exposed by the Imbrium impact? What is the full range of rocks in the highland crust and how do they relate to different models of magma ocean formation and cooling? How does the sample petrology change with elevation, possibly related to target stratigraphy? Did the Imbrium impact event sample: mantle material; the KREEP-rich residual layer at the base of the magma ocean; the source regions of pre-Imbrian magmatism (the Mg-suite); ejecta from the adjacent, earlier Serenitatis basin (Silver Spur)? Are there deposits of pure Imbrium impact melt ponded in the Apennine Mts. summit lows? How do such new results compare to highlands sample collected at other sites? A distinctive highland meter-scale wrinkled texture is observed in orbital images (elephant-hide terrain-EHT) and may explain some of the walking and LRV traverse difficulties in the highlands; what is the origin of the EHT and how can this new understanding be used to increase human and robotic mobility in the highlands?

4) Provenance and Age of Secondary Craters and Clusters: Impact craters excavate material from depth and redistribute it laterally in ejecta deposits, the most distant of which are rays and secondary craters and clusters. Thus, samples of such material provide clues as to distant substrate geology and stratigraphy, and can assist in dating the parent impact crater (ages reset by the event). One of the Apollo 15 traverse targets was the crater cluster S of the landing site, attributed to either Aristillus or Autolycus, two large craters, 100-200 km N of the site. Dune, one of the prominent craters in the South Cluster, was sampled at Station 4, EVA 2, including a prominent regolith breccia boulder with multiple basaltic clasts (15498). The 2.1 Ga age of some samples have been attributed to an Autolycus source, but later analyses show some ambiguity due to the overprint of younger Aristillus ejecta [4]; Spur crater KREEP basalts may be delivered from the Th-rich Aristillus crater. Outstanding Questions: What is the variety of ages in foreign materials delivered to the study region from distal crater substrates, and what are their cosmic ray exposure age in relation to their parent crater AMA? How can these data be integrated to provide a stratigraphy for the broader region surrounding the exploration area?

5) Regolith Stratigraphy: One of the most important questions addressed by Apollo 15 was the vertical stratigraphy of the lunar regolith, its thickness, lateral variability, and implications for processes of mechanical breakdown of the bedrock regolith protolith and chemical alteration induced by impact melting of the transition from bedrock to thick regolith. Scott and Irwin drilled and extracted a 2.4 m core that revealed 42 major textural units and showed conclusively that the regolith was not composed of a homogeneous mixture of impact disrupted materials, but instead was mostly derived from overlapping ejecta layers from nearby craters. Additional drive tube samples were collected along the traverses, and some showed mixing of highland and mare crater ejecta layers. Important informant was gained on the overall changes in regolith development with depth, and the role of lateral mixing near the mare-highland boundary, as well as the role of regolith mass wasting down into the rille. Outstanding Questions: How does regolith stratigraphy vary over a much larger area and are there any regional layers that might act as a chronologic datum? What is the thickness and age of regolith layers on top of buried flows in Hadley rille wall? Can core stratigraphy quantify

lateral mixing degree and processes along the mare-highland boundary? How does regolith stratigraphy differ distal to secondary craters and clusters and what is the proportion of ejecta from the primary and locally excavated material?

Mission Architecture Defined by Science Requirements: In separate contributions, we use these expanded scientific goals and objectives to outline specific mission desired regions of scientific interest (ROSI) and then utilize the ROSI locations to assess implications for the broad architecture of the Hadley Max 500-day Mission.

References: 1) A15 PSR (1973) NASA SP-289. 2) A15 PET (1972) Science 175. 3) Jolliff et al. (2006) RMG 60. 4) Iqbal et al. (2021) LPSC 52 #1917. Acknowledgement: We gratefully acknow-ledge support from the NASA NIAC Program and the Brown University UTRA program.

4.5.3 Mission Architecture Definition

HADLEY MAX 500-DAY DESIGN REFERENCE MISSION (DRM) TO THE APOLLO 15 HADLEY-APENNINE REGION: (3. MISSION ARCHITECTURE DEFINITION).
J. Head[1], D. R. Scott[1], B. Boatwright[1], L. Rothschild[2], C. Maurer[3], D. Eppler[4], R. Creel[5], R. Martin[2], W. Mickey[1], D. Fryd[1], M. Daniti[1], C. Wu[1]. [1]Brown University, Providence RI, [2]NASA Ames Research Center, Mountain View CA, [3]redhouse studio, Cleveland OH, [4]San Antonio Mountain Consulting, Houston TX, [5]Huntsville, AL (NASA MSFC Ret.) (james_head@brown.edu).

Mission Architecture Defined by Science Requirements: We use the expanded broad scientific goals and objectives derived from Apollo 15 mission results [1] and recent regional geologic mapping [2] as a basis to outline specific Hadley Max mission [3] desired regions of scientific interest (ROSI) and more detailed traverse goals and objectives [4]. Here we utilize the distribution of ROSI locations to assess implications for the broad architecture of the Hadley Max 500-day Mission (exploration range, mobility requirements, crew size, number of bases, number of EVAs, upmass and downmass requirements, human-robotic partnership requirements, habitat requirements, etc.).

1. Operational Access Requirements: Landing Sites: On the basis of extending the major goals to a broader exploration and sampling region [2] consistent with current NASA science goals and objectives [5], we locate the primary landing site at the initial Apollo 15 landing site. The 100-200 km radius of operations defined by the distance from the A15 site to the farthest ROSI [4], and uncertainties in the ability to cross Hadley Rille, dictates that two separate landing site/base of operations are required. To optimize the scientific return, we place the second landing site/base of operations to the W of Hadley Rille in mare unit Im3 (Fig. 1).

2. Crew Size, Space Suit and Mobility Requirements: Crew Size: On the basis of the scientific requirement for two landing site bases, and the contingency of crew EVA rescue, we assume 4 crew/base, also an important element of scientific goal division of labor (e.g., one crew rille, one crew highlands) and the possibility of simultaneous EVAs. For the full Hadley Max mission this equals 8 crew on the surface, 4 at each base. Suit Requirements: Minimum is Apollo suit capabilities, assisted by enabling technologies in consumables and mobility, in order to extend traverse time, optimize highland traverses, and expand EVA efficiencies. Mobility Requirements: Minimum is Apollo LRV design and capabilities. Two rovers/landing site (4 total). Enabling technologies include increased efficiency in slope trafficability to ensure exploration of the rille and highlands, ability to carry four astronauts (rescue), ability to survive lunar night ('rover garage' at base), design lifetime >>500 days.

3. Human-Robotic Mission Types and Relationships: Human Mission Types: These include 8-hour EVAs, 10 km radius of operations, with the possible extension to 14 km using the 7 km circumference "outpost" capability (Fig. 1). Robotic Mission Types: It is clear from the Human EVA 10 km radius of operations and the maximum traversable slope constraints (~20° degrees) that a series of parallel robotic missions will be required to meet the scientific objectives, particularly those in the highlands, and at radial distances beyond 10 km from the landing site, and to ensure Human EVA scouting, interpolation and extrapolation [6]. Human-Robotic Mission Relationships: On the basis of Apollo mission planning and astronaut operational

experience, we advise against an Astronaut-tended robotic geologic 'field assistant' as an inefficient use of crew exploration time on the surface. Instead, we strongly urge the development of parallel human-robotic partnerships, generally simultaneous operations to enable precursor, scouting, interpolation, extrapolation, and post mission exploration activities [6].

4. Definition of Required Habitats, Enclosures and Related Architectural Elements: 1. Landing Pads (LP): For both Human and Robotic missions; like helo pads; flat, devoid of soil backwash contaminant, retroreflector for guidance. 2. Initial Base Structure (IBS): Living and working habitat; follows the initial stages where there is a landing module (LM). 3. Evolutionary Base Structure (EBS): Larger scale, separation of work/living activities; increased in situ science activities; IBS evolves to dust mitigation structure. 4. Outposts: Remote Science Bases (RSB): Modeled after IBS, but located >10 km radius from Landing Site. Require up to ~5 RSBs for in depth, in situ science activities. Increase number in order of science priority. 5. 'Pony Express' Stations (PEX): These are the lunar 'pup tents' that will be precursors to the Remote Science Bases (RSB), and then Earth-day sleep-stations on the way to the final Remote Science Bases (RSB). Sample storage stations, geophysical stations; can be resupplied/samples collected by CLPS missions. 6. Robotic Rover Requirements: a) LRV garage at base for surviving lunar night, re-outfitting; b) Robotic LRV 'pup tents' for surviving lunar night, caching samples. 7. Application to the Artemis Circumpolar Environment (ACE): How do we optimize these basic requirements and DRM concepts for the harsh conditions of the South Circumpolar Region, and the lunar farside? 8. Assessing Feed-Forward to Mars Exploration: How does the Mars environment modulate and modify these DRM strategies and architectural elements?

5. Identification of Required Key Enabling Technologies and Operational Concepts: a) Upmass Requirements: The multiple base/outpost (RSB)/pup-tent habitat requirements and their necessary range of complexity and ability to survive lunar day/night cycles, as well as robotic LRV remote servicing stations, places huge mass requirements for delivery of construction materials to the Moon. In order to help alleviate this "upmass roadblock", we pursue two promising technologies: 1) Myco-Architecture [7,8], where building materials can be "grown in situ" in order to significantly minimize upmass penalties, and 2) Inflatable Structural Elements [9], in which low-volume, low-mass inflatables can be combined with Myco-Architecture to produce a wide range of enclosures in situ. b) Human-Robotic Partnerships: The great distances required to reach all ROSI, the increase in area as a function of radius from the base (increasing the need for scouting, interpolation and extrapolation), the steep slopes within the rille and on the highlands, as well as the presence of the Elephant-Hide Terrain (EHT), and the trafficability on these slopes, all dictate a requirement for a robotic LRV (RLRV) operating independently of the human traverses and controlled from the base or the ground. RLRV design and technology challenges include ability to traverse slopes approaching 30°, an advanced suite of remote sensing instruments, constant navigation imaging, near real-time communications with the ground, the ability to collect, document and store individual rock and soil samples, remote operations from base and ground, enclosures (RLRV garages) for lunar night, servicing and sample storage, and a design lifetime >>500 days. c) Supply-Resupply Technology and Infrastructure Requirements: Despite alleviation of upmass construction requirements through Myco-Architecture and Inflatables, significant supply (and resupply) (S/RS) requirements are dictated by the widespread and long-duration exploration strategy. Many dozens of human and robotic S/RS missions to diverse locations, delivering different payloads, and ensuring crew

cycling, are required by the 500-day DRM architecture. Optimal resupply mission require landing, offloading cargo, and onloading crew, rock/soil samples and other materials for return to Earth. d) Mission Operations and Feed-Forward to Mars: Lunar communications latency (~2.5 sec) presented no difficulties during Apollo, but Mars latency (5-20 min) precludes useful direct communications with ground during exploration. In addition, after a few day exploring the lunar surface, astronauts will have by far the best situational awareness and thus be capable of real-time planning and execution of traverses, the goals and objectives of which are planned pre-EVA in consultation with the base/ground. Research into optimal operational frameworks in which Moon-Earth traverse briefings/debrief-ings/planning take place between EVAs, and the highly trained crews are left to execute the pre-planned traverses according to their superior, in situ, situational awareness. Such operational frameworks will be required for Mars exploration. Ground will more likely focus on continuous, parallel operations of the RLRV, and integrating these results into the inter-EVA debriefings and planning sessions.

Synthesis: These Architectural Definition concepts and requirements are now used to explore low-upmass in situ building materials [7,8] and inflatable architectural elements [9] for further conceptualization and design of the Hadley Max 500 day DRM.

References: 1. NASA SP-289 (1973). 2. Iqbal et al. (2021) LPSC 52 #1917. 3. LPSC 55, #****. 4. LPSC 55, #****. 5. NASA M2M Strategy and Objective Development. 6. LPSC 55, #****. 7. Brandić Lipińska et al. (2022), Front. Built Environ. 8. 8. LPSC 55, #****. 9. LPSC 55, #****.
Acknowledgement: We gratefully acknow-ledge support from the NASA NIAC Program and the Brown University UTRA program.

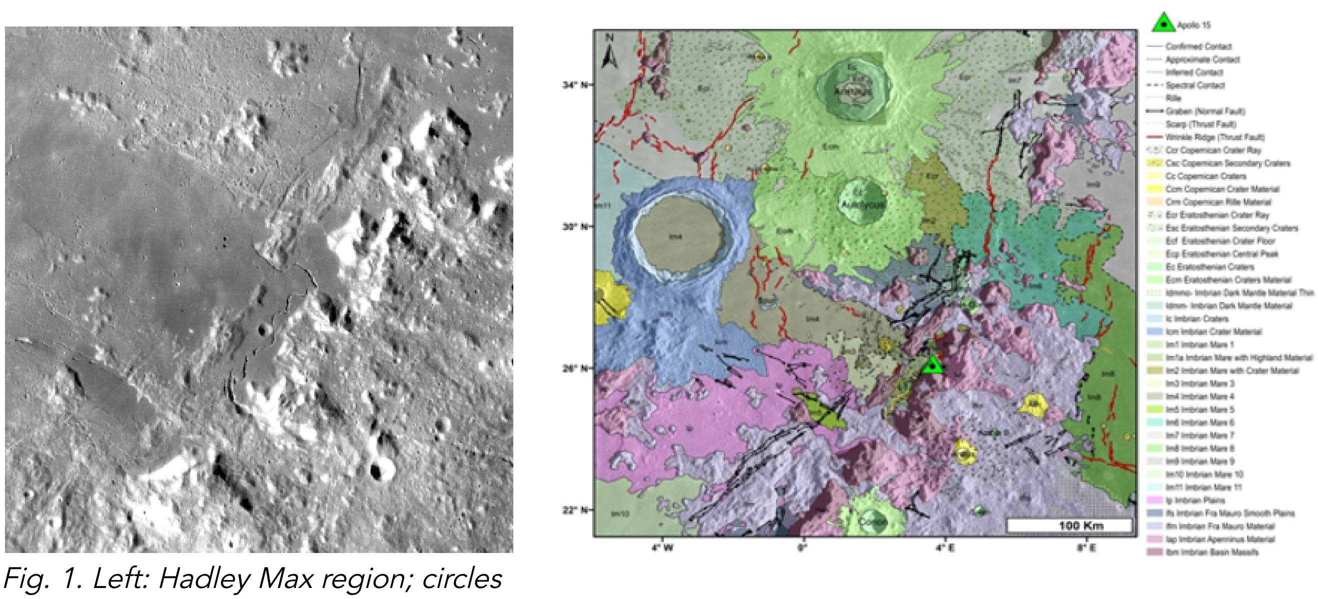


Fig. 1. Left: Hadley Max region; circles show 2 base sites and 10 and 20 km radius around each. Red dots; selected ROSI [4]. Right: Recent geological map [2]; box shows left image (LROC WAC) location.

4.5.4 Traverse Design And Implementation

HADLEY MAX 500-DAY DESIGN REFERENCE MISSION (DRM) TO THE APOLLO 15 HADLEY-APENNINE REGION: (4. TRAVERSE DESIGN AND IMPLEMENTATION). J. Head[1], D. R. Scott[1], B. Boatwright[1], L. Rothschild[2], C. Maurer[3], D. Eppler[4], R. Creel[5], R. Martin[2], W. Mickey[1], D. Fryd[1], M. Daniti[1], C. Wu[1]. [1]Brown University, Providence RI, [2]NASA Ames Research Center, Mountain View CA, [3]redhouse studio, Cleveland OH, [4]San Antonio Mountain Consulting, Houston TX, [5]Huntsville, AL (NASA MSFC Ret.) (james_head@brown.edu).

Traverse Design and Implementation: Beginning with the Hadley Max 500-day Design Reference Mission (DRM) conceptual background [1], we proceeded to utilize Apollo 15 mission scientific goals and objectives, together with expanded broad scientific goals and objectives derived from Apollo 15 mission results and recent regional geologic mapping [2], as a basis to identify the resulting Regions of Scientific Interest (ROSI) for the Hadley Max DRM [3], and used these scientific requirements to define the Mission Architecture (exploration range, mobility requirements, crew size, number of bases, number of EVAs, human-robotic partnership requirements, habitat requirements, etc.) [4], in preparation for more detailed mission design and traverse planning activities, discussed here.
Mission Planning and Operations Guidelines: During a lunar day (~30 Earth days), we assume 15 consecutive Earth days during lunar Night (dedicated base laboratory and traverse debriefing-planning activities), followed by 15 consecutive Earth days in lunar Day (dedicated to EVA operations). In order to optimize human performance, we assume a 6-3-6 Earth day duty cycle, with 3-day 'weekends'. For daily duty cycles, we assume 8-hour sleep periods, and 16 hour work-rest periods. We further assume 8 hour EVAs (further adjusted for changing lighting geometry), and a 10 km radius of operations (walk-back constraint) from the base. Hadley Max Robotic LRV (RLRV) traverses will be designed following the DRM Astronaut traverses with the goal of complementing these with scouting, interpolation, and extrapolation RLRV campaigns. Landing Sites: On the basis of the desire to first build on the Apollo 15 crew observations and analysis of the returned samples, and secondly to extend the major goals to a broader exploration and sampling region [2], we chose the Apollo 15 landing site as the prime landing site (Fig. 1a,c). Using the 100-200 km radius of operations defined by the distance from the A15 site to the farthest ROSI (Fig. 1a), we chose the second landing site/base of operations to the W of Hadley Rille in order to access all ROSI and explore the full range of mission scientific goals and objectives. EVA Radius of Operations: This was defined by the 'walkback distance' (~7 km) during Apollo, the maximum distance that the astronauts could walk back to the LM in the case of LRV mobility failure (in turn constrained by consumable supply and astronaut metabolic rates). For Hadley Max, we assume an improved suit capability to deliver a 10 km radius of operations, but identify this as a key enabling technology requirement. We also introduce and explore the concept of doubling the radial distance of operations by having "Human Outposts" at key locations within the 10-20 km radial circumference from each site, installations that would permit outpost pressurization, human overnight stays and suit consumable recharge, all requiring key technology and design developments. We utilize this, and related concepts (e.g., 'pup' tents, simultaneous and parallel robotic operations, etc.) developed in the Mission Architecture contribution [4]. EVA Station Duration: During Apollo 15-17, the average station duration was 38.7 min, largely defined by the multiple objectives and their intervening separation distances. The longest duration stations were A15-EVA

2-Station 6/6a (highlands boulder, steep slope; 1 hr 20'), A16-EVA 3-Station 13 (Shadow Rock; 1 hr 19') and A17-EVA 3-Station 6 (large boulder; 1 hr 14'). Due to the improved scientific understanding and more focused questions for Hadley Max, we assume a typical station duration of 1 hour. EVA Duration Duty Cycles: Typical A15-17 EVA durations were ~7 hours each, a number constrained by human physiology/diurnal cycles. We assume modest improvements in suit efficiency and mobility speed and adopt an EVA duration of 8 hours for Hadley Max. Station Duration: A15-17 on-station times averaged 38.7' and we adopt 40' for a typical station for Hadley Max. Stations per EVA: Assuming 8 hour EVAs, we adopt a planning number of 5 stations/EVA (the A15-17 average for 3 EVAs was ~12 stations, 4/EVA). Drive Times Between Stations and Average LRV speeds: Average A15-17 drive times between stations were ~17' and average LRV speeds ~7.3 km/hr: given increased efficiency in terrain knowledge, route planning algorithms, and improved LRV design, we adopt 15' and 10 km/hr for Hadley Max. EVA Planning Strategy: On the basis of the above considerations, for Hadley Max average traverse planning guidelines, we assume 5 stations/EVA (5 hours), ~15' travel between stations (1.5 hours) and 1.5 hours for flexibility, for a total of 8 hours. During Apollo, due to the walkback constraint and consumable consumption, EVAs were designed to visit the most distance station first, and then work back toward the LM. We adopt a similar strategy for Hadley Max.

Ability to Traverse Slopes: A major scientific goal is to explore the lunar highlands and the Hadley Rille floor and wall. Experience with Apollo 15 shows that traversing steeper slopes at the base of Hadley Delta (EVA2-S2) (Fig. 1b,c) had a major effect on LRV mobility (wheel slippage, etc.) and Astronaut mobility and metabolic rate. On the basis of data from Apollo and Lunokhod [5] we adopt a maximum traversable slope of 20°, and increase non-mare station times by ~20%. We identify improved human and robotic rover capability on slopes and improved astronaut slope exploration and sampling strategies, as necessary key enabling technologies. One of the major unknowns in traversing and sampling the highlands is the origin of the "elephant-hide terrain", a wrinkled, terrain-parallel morphological texture associated with highland slopes; [6] found that the majority of slopes steeper than 6°–8° in their analysis are covered with EHT. Ridge separation distances are estimated at meters-scale and heights <~ a meter, but the origin of the ridges, their grain-size, and mobility characteristics are unknown. Thus, understanding the EHT is one of the highest operational and scientific priorities in highlands exploration.

Improved Data for Human and Robotic Traverse Planning and Analysis: Apollo 15 site selection and traverse planning were accomplished utilizing 20 m-resolution LO-V images available at the time [1]. The NASA Lunar Reconnaissance Orbiter Mission has operated in lunar orbit for the last 14 years and has provided extremely high resolution images, altimetry, stereo photogrammetry, thermal inertia, water detection and radar data [7]. These fundamental data sets can be readily utilized to produce very high resolution image, topography, roughness, blockiness, slope and evolving lighting conditions maps that are essential ingredients to determining detailed science objectives, station locations, and traverse planning routes for optimal EVA planning. For example, Fig. 2a shows a slope map for one section of Hadley Rille and adjacent highlands, and Fig. 2b shows a slope map for defining human-robotic traverse access from the 20° slope constraint, data essential for human/robotic traverse design.
In addition, the advent of sophisticated mission and traverse planning software that can ingest and maintain cognizance of these multiple spatial data sets in real-time has revolutionized both

pre-mission traverse design and planning, and real-time traverse assessment and contingency planning. For example, recent developments in mathematical frameworks for reasoning under uncertainty (Partially Observable Markov Decision Processes; POMDPs) have been applied to automated decision support frameworks for planetary exploration. Such applications include SHERPA (System Health Enabled Real-time Planning Adviser) [8] that is designed to take different sources of uncertainty into account when generating decision recommendations for traverse planning and real-time operations. We are currently exploring a range of recently described algorithms for optimizing traverse planning. In the next stages of the Hadley Max DRM project, we plan to test and assess some of these for optimizing human-robotic performance and science return.

References: 1. LPSC 55, #***(HM-Concept). 2. Iqbal et al. (2021) LPSC 52 #1917. 3. LPSC 55, #*** (HM ROSI). 4. LPSC 55, #**** (HM Architecture). 5.Basilevsky et al. (2019) SSRes 53, 383. 6. Kreslavsky et al. (2021) LPSC 52, #1826. 7. Chin et al. (2007) SSRev 129, 391. 8. Baliban et al. (2018) AIAA SciTech Forum, 10.2514/6.2018-1150. Acknowledgement: We gratefully acknowledge support from the NASA NIAC Program and the Brown University UTRA program.

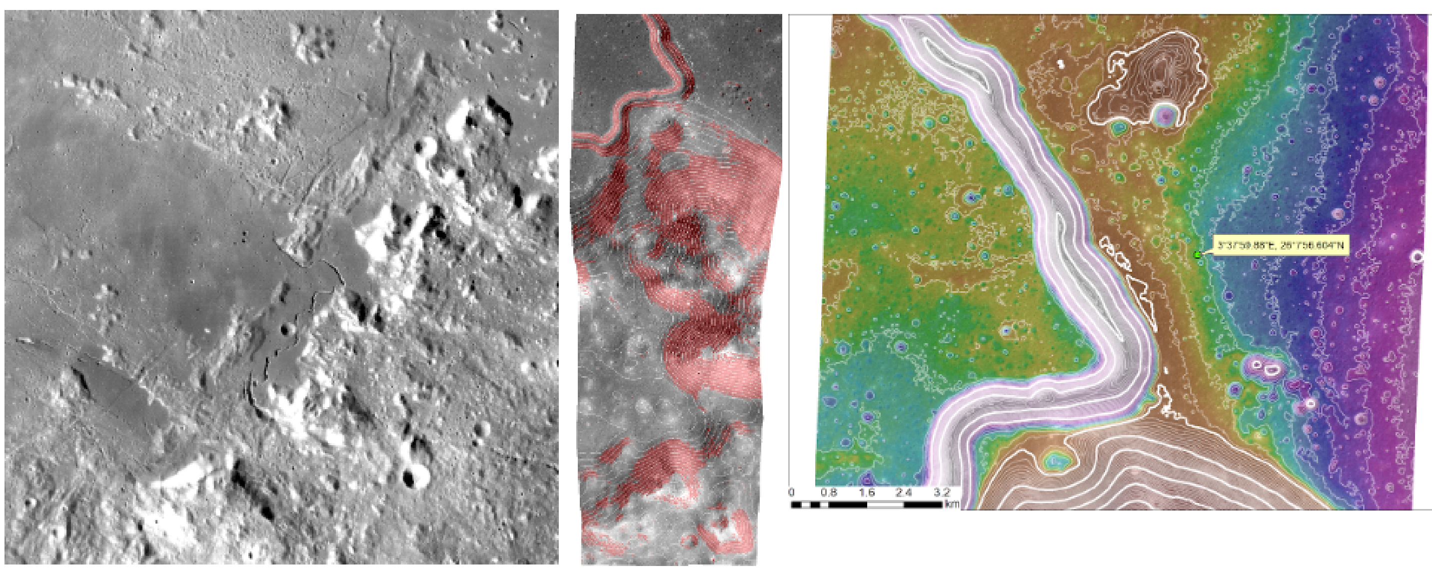


Fig. 1. (a) Left: Hadley Max exploration region showing two base locations surrounded by 5 & 10 km radius circles. Dots represent selected ROSI locations, the scientific input into human/robotic traverse design. (b) Middle: Slope map for a section of the Hadley Max A15 base location, showing the 20° slope constraint (red) on human and robotic rover access. (c) Right: Topographic contour map (thick lines 100 m, thin 10 m) of the rille-mare-highlands area near the A15 site Hadley Max base (green dot).

4.5.5 Reducing Upmass Demands With *In Sit*u Myco-Architecture

HADLEY MAX 500-DAY DESIGN REFERENCE MISSION (DRM) TO THE APOLLO 15 HADLEY-APENNINE REGION: (5. REDUCING UPMASS DEMANDS WITH IN SITU MYCO-ARCHITECTURE). J. Head[1], D. R. Scott[1], B. Boatwright[1], L. Rothschild[2], C. Maurer[3], D. Eppler[4], R. Creel[5], R. Martin[2], W. Mickey[1], D. Fryd[1], M. Daniti[1], C. Wu[1]. [1]Brown University, Providence RI, [2]NASA Ames Research Center, Mountain View CA, [3]redhouse studio, Cleveland OH, [4]San Antonio Mountain Consulting, Houston TX, [5]Huntsville, AL (NASA MSFC Ret.) (james_head@brown.edu).

Working Toward a Solution to the Upmass Problem: We began with the Hadley Max 500-day Design Reference Mission (DRM) conceptual background [1], and proceeded to call on Apollo 15 (A15) mission scientific goals and objectives, combined with expanded scientific goals and objectives derived from A15 mission results and recent regional geologic mapping [2]. We then identified the Regions of Scientific Interest (ROSI) for the Hadley Max DRM [3], and the used these scientific requirements to define the Mission Architecture [4], and more detailed Hadley Max mission design and traverse planning activities [5]. Here we address one of the most significant problems for long-duration and sustained human presence on the Moon and concurrent scientific exploration success: the Key Enabling Technology to alleviate the huge and continuous upmass requirements necessary to support the base and exploration infrastructure [4]. In order to help alleviate this "upmass roadblock", we have pursued two promising technologies: 1) Myco-Architecture [6-9], where building materials can be "grown in situ" in order to significantly minimize upmass penalties, and 2) Inflatable Structural Elements [10], in which low-volume, low-mass inflatables can be combined with Myco-Architecture to produce a wide range of enclosures in situ. Here we outline the evolution of our progress on "Myco-Architecture" and future goals and objectives.

Definition of Required Habitats, Enclosures and Related Architectural Elements: As a baseline for required architectural elements, we called on the Hadley Max DRM Architecture [4] and Traverse Planning [5] studies that produced these baseline elements. 1. Landing Pads (LP): For both Human and Robotic missions; like helo pads, flat, devoid of soil backwash contaminant. 2. Initial Base Structure (IBS): Living and working habitat; follows the initial stages where there is a landing module (LM). 3. Evolutionary Base Structure (EBS): Larger scale, separation of work/living activities; increased in situ science activities; IBS evolves to dust mitigation structure. 4. Outposts: Remote Science Bases (RSB): Modeled after IBS, but located >10 km radius from Landing Site. Require up to ~5 RSBs for in depth, in situ science activities. Increase number in order of science priority. 5. 'Pony Express' Stations (PEX): These are the lunar 'pup tents' that will be precursors to the Remote Science Bases (RSB), and then Earth-day sleep-stations on the way to the final Remote Science Bases (RSB). Sample storage stations, geophysical stations; can be resupplied/samples collected by CLPS missions. 6. Robotic Rover Requirements: a) LRV garage at base for surviving lunar night, re-outfitting; b) Robotic LRV 'pup tents' for surviving lunar night, caching samples. 7. Application to the Artemis Circumpolar Environment (ACE): How do we optimize these basic requirements and DRM concepts for the harsh conditions of the South Circumpolar Region. 8. Assessing Feed-Forward to Mars Exploration: How does the Mars environment modulate and modify these DRM strategies and architectural elements? Here we investigate elements 1-6, and explore how producing construction materials in situ on the Moon can help alleviate the upmass problem. We plan to treat 7 and 8 in future analyses.

Background and Approach: Transporting materials beyond Earth, such as spacecraft, Astronauts, and construction materials, is limited by mass constraints. Yet long-term residence, operation and scientific exploration on the lunar surface requires an extensive infrastructure, and a significant part -nd major large-mass component -of this is in habitats, designed to protect crew and equipment from radiation, extreme temperatures and micrometeorite bombardment. There is a significant mismatch between habitat requirements at destination and what can realistically transported there. Infrastructure for human survival is not automatically "user ready" on the Moon. Habitats could be built with locally sourced regolith or ice materials by In situ Resource Utilization (ISRU), but in the end, even this requires significant upmass. To alleviate this problem, we have been exploring technologies [6-9] that are self-replicating and self-repairing, to assess their utility in circumventing the upmass problem. Lfe meets these technological criteria for space utilization, and in addition can be reprogrammed through synthetic biology. In this quest, we look to exploit the genetic hardware store inherent in our vast biodiversity, moving capabilities from familiar forms such as trees for wood, to a more tractable space-faring chassis such as yeast or bacteria.

Strategy and Concepts: A critical aspect of human space exploration and eventual settlement is the ability to construct habitats while minimizing payload mass launched from Earth. To respond to this challenge, and as a continuation of our research program initiated under the auspices of the "Myco-architecture Off Planet" NASA NIAC Team, we have explored the use of fungal biocomposites, for example Bio-Bricks, (Fig. 1) for growing extra-terrestrial structures and building materials, directly at the destination, significantly lowering the mass of structural materials transported from Earth and minimizing the need for high mass robotic operations and infrastructure preparations. Currently, the idea of working with living biological organisms, and the phenomenon of growth itself, is of increasing interest in architecture and space applications. Here, we describe the use of mycelium-based composites as an alternative, biological approach for constructing regenerative and adaptive buildings for extraterrestrial habitats. These composites, are fire-resistant and insulating, and do not consist of volatile organic compounds from petrochemical products. These can be used independently or in conjunction with regolith, and could employ the living biological growth in a controlled environment for the process of material fabrication, assembly, maintenance, and repair, providing structures resilient to extra-terrestrial hazards. We explored avenues to make this biological approach feasible, providing new, growing materials for designing and building sustainable habitats for long-duration space missions.

Our research has explored the potential and challenges of using mycelium-based biocomposites for space applications. The approach of using biological growth for the off-Earth construction, similarly to other researched ISRU-based approaches, is designed to lower the mass of materials needed to be transported from Earth. In addition, it focuses on lowering the energetic costs of the construction of in situ habitats, such as the work required to assemble the habitat. In the long-term, using biological materials and growth as a construction method, opens up the potential of ELMs (Engineered materials composed of Living cells that form or assemble the Material itself or modulate the functional performance of the material in some manner) [11].) to potentially provide supplementary capabilities, such as sensing and responding to environmental stimuli, self-healing, etc. Such developments could make the habitats even more flexible and reliable. The further development of research on ELMs and mycelium-biocomposites will allow for advancements in the field of biotechnology and habitat construction. These concepts employ living biological growth in a controlled environment for the process of material fabrication, assembly, and maintenance. Positive attributes of these approaches and techniques include the modest upmass requirements of a few spores, nutrition for mycelial growth, and a growth framework, along with the potential to reproduce using in situ resources, the ability to grow to accommodate on-site terrain, and the potential additional control provided by the tunability of the materials. We see myriad possibilities for mycotecture utilization off planet. Because the research is still in an early stage, one of our major goals once the enabling technologies are identified, is to use the Hadley Max 500-day DRM Architecture to develop a technology roadmap and recommendations for further development.

References: 1. LPSC 55, #***(HM-Concept). 2. Iqbal et al. (2021) LPSC 52 #1917. 3. LPSC 55, #*** (HM ROSI). 4. LPSC 55, #**** (HM Architecture). 5. LPSC 55, #**** (HM Traverse Planning). 6. Rothschild et al. (2017) Astrobio. Sci. Conf. #3720. 7. Rothschild et al. (2022) LPSC 53 #2983. 8. Rothschild et al. (2023) LPSC 54 #2544. 9. Brandić Lipińska et al. (2022), Front. Built Environ. 8. 10. LPSC 55, #***HM In situ construction). 11. Nguyen et al. (2018) Adv. Mat. 30, 1704847.
Acknowledgement: We gratefully acknowledge support from the NASA NIAC Program and the Brown University UTRA program.

Fig. 1. Left: Bio-Brick made of Myco-Architecture Materials (Courtesy Chris Maurer): This brick is a composite of wood and fungal mycelium. Fungi break down biochemicals like cellulose converting them into their own chitin-rich biomass. By growing mycelium on plant fodder they fuse at a cellular level allowing us to utilize the best characteristics of their respective Kingdoms - Plantae and Fungi. We are currently developing methods to grow these multi-kingdom composites off-planet to save transport cost, reduce energy demands, and utilize bio-performative aspects such as radiosynthesis, that may one day convert space travel's biggest liability, ionizing radiation, into a resource for material production. Bio-Brick dimension is 17 x 12.5 x 5.5 cm. Right: J. Head and NASA Administrator Bill Nelson examine one of our Bio-Bricks at Brown University. Photo by RI Senator Jack Reed.

4.5.6 Reducing Upmass Demands Utilizing In Situ Inflatable Structures

HADLEY MAX 500-DAY DESIGN REFERENCE MISSION (DRM) TO THE APOLLO 15 HADLEY-APENNINE REGION: (6. REDUCING UPMASS DEMANDS UTILIZING IN SITU INFLATABLE STRUCTURES). J. Head[1], D. R. Scott[1], B. Boatwright[1], L. Rothschild[2], C. Maurer[3], D. Eppler[4], R. Creel[5], R. Martin[2], W. Mickey[1], D. Fryd[1], M. Daniti[1], C. Wu[1]. [1]Brown University, Providence RI, [2]NASA Ames Research Center, Mountain View CA, [3]redhouse studio, Cleveland OH, [4]San Antonio Mountain Consulting, Houston TX, [5]Huntsville, AL (NASA MSFC Ret.) (james_head@brown.edu).

Introduction: Beginning with the Hadley Max 500-day Design Reference Mission (DRM) conceptual background [1], we proceeded to utilize Apollo 15 mission scientific goals and objectives, together with expanded broad scientific goals and objectives derived from Apollo 15 mission results and recent regional geologic mapping [2], as a basis to identify the resulting Regions of Scientific Interest (ROSI) for the Hadley Max DRM [3], and used these scientific requirements to define the Mission Architecture [4], in preparation for more detailed mission design and traverse planning activities [5]. We then turned to addressing the major upmass challenges revealed by any long-term residence and sustained presence on the Moon, first assessing reduction in upmass demands by employing in situ Myco-Architecture [6], and here exploring reducing upmass demands utilizing in situ inflatable structures.

Definition of Required Habitats, Enclosures and Related Architectural Elements: We previously identified the types of habitats, enclosures and related architectural elements [4] dictated by the Hadley Max scientific objectives [3] as follows: 1. Landing Pads (LP): For both Human and Robotic missions; like helo pads; flat, devoid of soil backwash contaminant, retroreflector for guidance. 2. Initial Base Structure (IBS): Living and working habitat; follows the initial stages where there is a landing module (LM). 3. Evolutionary Base Structure (EBS): Larger scale, separation of work/living activities; increased in situ science activities; IBS evolves to dust mitigation structure. 4. Outposts: Remote Science Bases (RSB): Modeled after IBS, but located >10 km radius from Landing Site. Require up to ~5 RSBs for in depth, in situ science activities. Increase number in order of science priority. 5. 'Pony Express' Stations (PEX): These are the lunar 'pup tents' that will be precursors to the Remote Science Bases (RSB), and then Earth-day sleep-stations on the way to the final Remote Science Bases (RSB). Sample storage stations, geophysical stations; can be resupplied/samples collected by CLPS missions. 6. Robotic Rover Requirements: a) LRV garage at base for surviving lunar night, re-outfitting; b) Robotic LRV 'pup tents' for surviving lunar night, caching samples. 7. Application to the Artemis Circumpolar Environment (ACE): How do we optimize these basic requirements and DRM concepts for the harsh conditions of the South Circumpolar Region, and the lunar farside? 8. Assessing Feed-Forward to Mars Exploration: How does the Mars environment modulate and modify these DRM strategies and architectural elements?

These required structures are the basis for using the Myco-Architecture [6] concept to explore reducing upmass demands utilizing in situ inflatable structures.

Architectural designs and deployable in situ construction methodologies: In concert with the development of Myco-Architectural biological material, architectural designs and deployable in situ construction methodologies were developed at redhouse studio, including plans, 3d models, section details, and animations of various designs and building processes. After assessing many ways to deploy bio-composites off planet (including lightweight formwork, masonry and additive manufacturing), redhouse arrived at a 'sealed bag' deployment (Fig. 1) as the best option to control the environment for growth, develop the shape of the shelter, and protect the lunar environments from potential contamination. The sealed bag concept will allow the bio-composites to self-assemble in multiple layers of membranes that can provide redundant protection, channel nutrients, and create warm habitable spaces within the framework. This can be achieved at many scales and could be utilized as a platform technology for building any mission structure or object (Fig. 1,2).

Evolution of Architectural Concepts: The design concept started as deployable habitat shell that would grow like a living organism at destination with the aid of in situ resources. This would be less energy intensive and leave a smaller planetary footprint then mining or melting surface material. Intense team study and analysis has enabled the initial concept to grow and evolve new multi-functional facets. We found that the biological functions that enable growth of the materials also provide such benefits as oxygen production and may also be used to generate heat and electricity. Thus , this biomimetic and bio-utilitarian option provides potential options to very high up-mass costs of prefabricated structures that come fully outfitted, and other construction materials. Detailed architectural and design analyses suggested that necessary attributes, such as plumbing lines, stovetops, and floormats, can be folded into the form, plugged-in ready to go, and the floors, walls and windows can be grown in place so that the in situ grown building is comparable to a high-mass Earth-fabricated, and then delivered to the site, structure. In order to accomplish this, however, the challenge is in the packing of the habitat shell into off-planet deliverable cargo geometry constraints. We found that many of the domestic utilities, scientific equipment, furnishings, and fixtures can be built directly into the expandable shell. More detailed assessments showed that such self-contained modules can be wrapped into the larger structure and secondarily deployed once robotic-enabled construction of the shell is finished.

Materialization: The process of making fungal composites includes growing filamentous saprophytic fungi on biomass substrates that can become fused at a cellular level. Our team has demonstrated composites that have structural characteristics superior to wood framing, thermal resistance characteristics superior to fiber-glass batt insulation, and fire resistance equivalent to type-X gypsum board, that is, construction industry standards, all comparable or superior to other ISRU suggested materials.

Construction Methods: Building Envelope: The building envelope grows as three pneumatic rings provide initial structure and the circulatory system then delivers nutrients to the building membrane cells. An intersection of three rings inflates to set the structural form. This can be delivered by compressed gasses and/or water, or from compressed canisters embedded within the rings. Rovers can be used to deliver the in situ resources, or the mechanisms could be embedded within the folded skin. The rings will later be filled bio-composites, but as air filled tubes they initially and immediately as serve as scaffolding to let the micro-organism begin permanent construction. The building's circulatory system feeds the membrane cells to grow the bio-composite structure. Cyanobacteria embedded within the cells are fed water, nitrogen, carbon dioxide, and other nutrients sourced in situ. Heat is supplied to the cells creating the right conditions for growth. The organisms grow, releasing oxygen that is stored within a special bladder. Once the cyanobacteria have reached a critical biomass, the nutrient rich substrate is dehydrated to a level that would support myceliation by saprophytic fungi. Oxygen is then released back into the cells and the fungi can feed on the oxygenated algal biomass. The fungi branch between the algal cells and begin to devour them by secreting enzymes and converting the external dissolved cellulosic material into chitin within the fungal cell walls. The algal biomass becomes fused with the mycelium at a cellular level and is heated and compressed by the heat-traced pneumatic membranes. The multilayer system allows for redundant protection and separation of materials in various states of matter. The hydrogen produced allows fenestration while providing radiation protection.

<u>Synthesis:</u> In situ inflatable structures clearly permit significant reductions in upmass penalties, and also offer many other ancillary benefits, such as radiation protection. They also offed significant reduction in environmental impacts of on-site construction from local material. We are continuing to explore innovative ways in which a) in situ Myco-Architecture, combined with b) inflatable structure concepts, can optimize the scientific goals and objectives of long-term missions to the Moon and Mars, as illustrated by the Hadley Max 500-day DRM [1-6].
References: 1. LPSC 55, #***(HM-Concept). 2. Iqbal et al. (2021) LPSC 52 #1917. 3. LPSC 55, #*** (HM ROSI). 4. LPSC 55, #**** (HM Architecture). 5. LPSC 55, #*** (HM TRAV). 6. LPSC 55, #*** (HM MYCO). Acknowledgement: We gratefully acknowledge support from the NASA NIAC Program and the Brown University UTRA program.

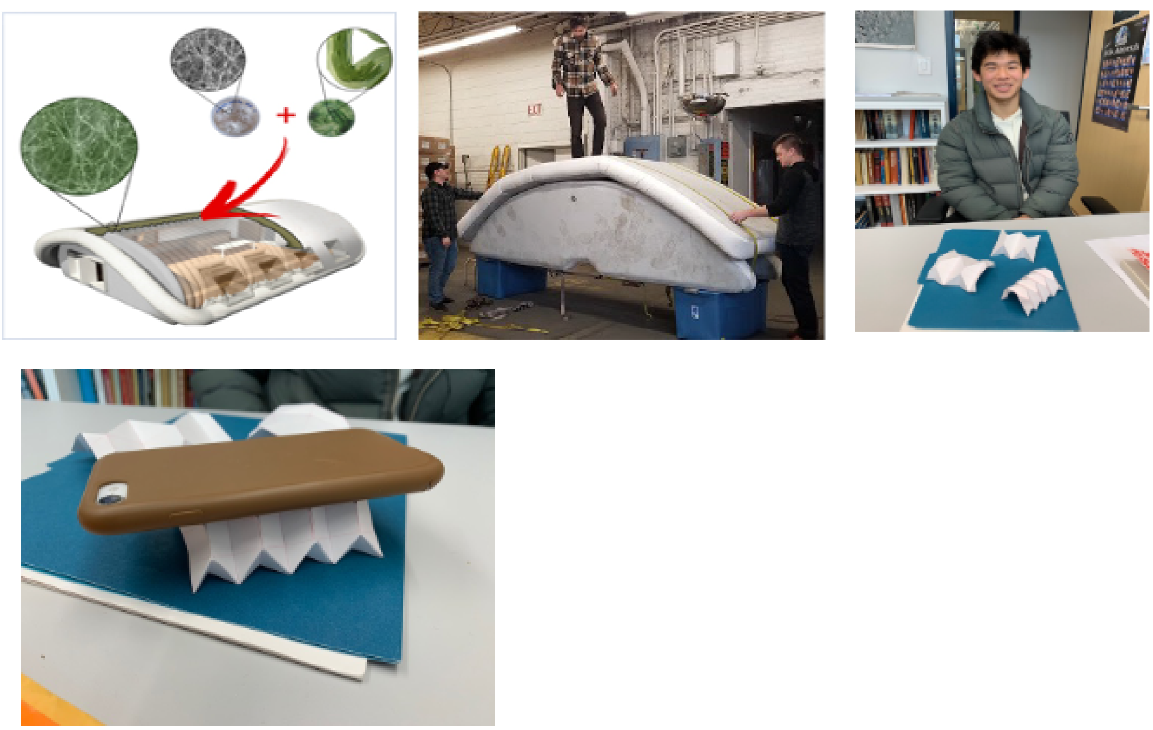
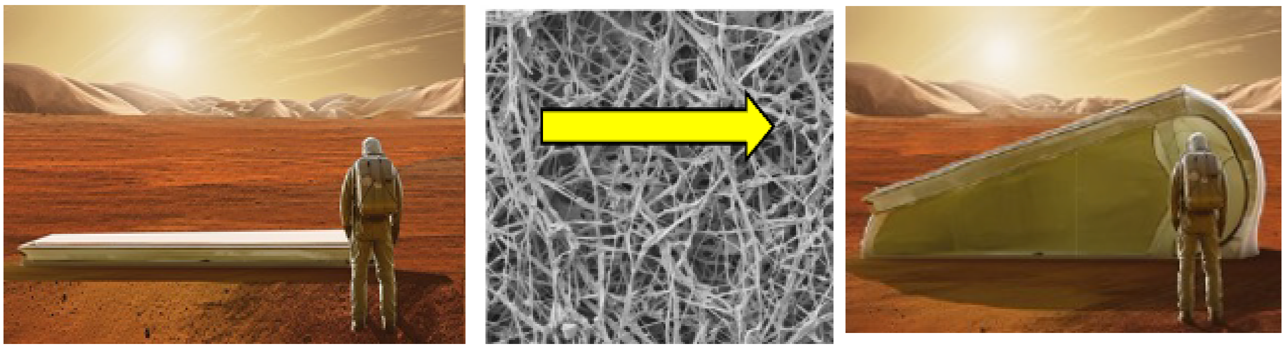


Fig. 1. Left. Initial concept for self-assembled bio-composite in unfolding bag. Left middle: Testing the strength of an inflatable structure at redhouse studio. Right Middle: Brown Undergraduate Christian Wu and his "Flexible Origami" inflatable bag cargo storage solutions. Right. Strength of "Flexible Origami" (I-Phone atop structure).

Fig. 2. Below. Fungal mycelia will produce habitat at destination by deployment in a membrane-bound bag filled with a compressible, lightweight scaffold coated with nutrient-rich hydrogel. Options to include functionized mycelia or B. subtilis*, and use as basis for biocomposite with bioplastic or regolith. Potentially self-repairing. Many other applications.*



("Hadley Apennine 500 Day Design Reference Mission" and "Lunar Orientale Basin Design Reference Mission")

11.5 Posters from programmatic reviews.

2021 (citural)

2022 (Tucson)


Life as Technology
MYCOTECTURE OFF PLANET
NIAC
NASA Innovative Advanced Concepts
mycelia
THE TEAM
Lynn J. Rothschild, NASA Ames Research Center
Chris Mauer, redhouse studios (architect)
Jame W. Head III, Brown University (planetary science)
Ivan Paulino-Lima, Blue Marble Space Institute of Science (microbiology/synbio)
Debbie Senesky, Stanford University (aerospace engineer)
Katheryn Kornegay, Stanford University (materials science grad student)
Martyn Dade-Robertson, Monika Lipińska, University of Newcastle (architects)
Maikel Christian Rheinstädter, McMaster University (planetary simulator)
Christopher Workman, Nicolas Musitu, Technical University of Denmark (fungi)
Eneko Axpe, Stanford Oydrogel, Earth based applications)
David Cadogan, President Moonprint Solutions (aerospace engineer)
Why
★ Humans need habitats for protection, especially off planet
★ Traditional approaches like a turtle: Bring them (e.g., spacecraft or massive 29k kg habitat for nominal Mars
★ New approaches use ISRU: robotic construction of regolith or ice houses
★ Rely on intense construction operation; pros and cons of materials (Tables 1 &2, right).
TABLES 1
Lipińska et al., 2022, Frontiers of the Built Environment
TABLES 2
Lipińska et al., 2021, IAC
What
• We propose using mycotecture: fabrication based on fungal mycelia to grow structures (table, chair, rover shell, garage) AND habitats.
• Currently being explored in "green architecture" because low mass, inexpensive, great material (and psychological), properties, adaptable to site at destination, potentially self-healing, multiple uses, sustainable, compostable.
• Many of same benefits off planet.
stool made <2 weeks fungal growth by the Stanford-Brown-RISD 2018 iGEM
How
• Plastic shell is loaded with fungal spores. Key features: drop stitch construction, embedded fungal mycelia, potentially algal spores, bacillus spores, nutrient solution.
• Deployed at destination. Inflatable with activation by the addition of water.
• Algae grow first, then mycelia and bacillus.
• In situ post-processing possible, for example heat processing taking advantage of lunar day, or drying.
Phase II
• Architectural progress: Grew 4x4 m prototype, designed, built and tested inflatable with drop stitching (Chris, Dave), explored the creation of "sand bricks" and "sand towers" (Monika).
Inflatable (Moonprint)
• Mission context: Integration with bi-weekly architect meetings. Planning exploration scenarios for extended sorties, bases, and "campsites" on the Moon and Mars (Jim).
Sand tower (Monika)
Interlocking bricks ~7x5x3 cm (Monika)
• Fill key technical knowledge gaps such as growth of fungi on cyanobacteria, (Chris, Nicolas), mechanical testing of mycomaterials at Stanford (Debbie, Kat)
Algal bio-composites (redhouse). d) compressed composites that are structural and insulative.
• Expose materials to off planet environments, including planetary simulator (McMaster, Maikel) and ISS (MISSE experiments 12&13, Kim de Grohe, PI).
Kat (Stanford)
Planetary simulator
MISSE
Earth-based applications include creating "NASA corner" for award-winning restaurant, Azurmendi.
Chris founded two companies: one in Africa that turns waste biomass into edible mushrooms and sustainable buildings for refugees, and one biocycler with Black Environmental Leaders in Cleveland to use fungi to recycle and remediate condemned homes
4 interviews, 1 TV filming (NHK Japan), 5 invited talks including Fungal Genetics Society and MIT.
Publications to date: LEAG, LPSC, IAC, plus
Lipińska, M., Maurer, C., Morrow, R., Dade-Robertson, M., Senesky, Magdalini Theodoridou, D., Zhang, M. and Rothschild, L. Biological Growth as an Alternative Approach to On and Off-Earth Construction. Frontiers in Built Environment (in press 8/2022)
image courtesy of Co-I Chris Maurer