

Life Sciences in Space Research

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Hydroponics for plant cultivation in space – a white paper

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Summary

The <u>microgravity conditions</u> experienced in space prevent the proper distribution of water throughout root modules of plant growth hardware, and the lack of convective mixing and buoyancy reduces gas exchange. To overcome this problem, cultivation technologies should be designed that take advantage of the unique traits of the <u>spaceflight</u> environment instead of attempting to recreate Earth-like conditions. Such technologies should be adaptable to both the <u>microgravity</u> of spaceflight and the low gravity environments of the lunar and Martian surface. Current space plant cultivation relies on traditional terrestrial practices and uses <u>porous substrates</u> that are nutrient poor and difficult to regenerate, and does not consider the dominance of surface- or thermal gradient-controlled rather than gravity-controlled water flow in space as a potential beneficial property. We propose systems that control water dispensation and removal by parallel but independent means in a soil-free cultivation system that is adaptable and expandable to crops of varying sizes and shallow or deep rooting plants.

Water dispensation and removal in a substrate-free <u>hydroponic</u> system can be achieved through the misting of nutrient solutions combined with special root module geometry and temperature gradients. The use of hydrogels as substrate, and a means of providing required nutrients and water for plant cultivation in space, can aid in the transition to low-gravity systems by eventual incorporation of on-site <u>regolith</u> to establish Earth-like soil.

Keywords

Hydroponics; Hydrogel; Arcillite; Radish

Introduction

The current status of plant cultivation in space is based upon three systems: experimental studies based on petri dishes, often in Biological Research in Canisters (BRIC) hardware, the Veggie unit, and the Advanced Plant Habitat (APH). Of these, Veggie and APH permit cultivation of plants beyond the seedling stage. Despite their differences (Veggie being an open system, APH a closed chamber with environmental control), both systems depend on solid substrate and a watering mechanism that is based upon traditional plant cultivation methods on Earth. They include a vertical arrangement with lights above rather shallow root zones. The substrate is mineral clay (arcillite) with 1–2 mm grain size to provide adequate porosity for water distribution and to mitigate root hypoxia. However, flight experiments and by inference any planting for future experiments or crop production in Veggie or APH, depend on painstaking preparations (sowing together 'pillows' that contain arcillite and slow release fertilizer for Veggie (Morsi et al., 2022), or compacting fertilizer-infused arcillite into science carriers for APH (John et al., 2021) that introduce complications such as mechanically restrictive root space, chemical interactions (ion exchange) between the arcillite matrix, nutrient solution, and watering systems. Efficient plant cultivation in space needs to avoid the high mass cost of arcillite, which also is not easily reusable, and which restricts growth both spatially and chemically because of the restrictive root space and because the arcillite matrix contains high levels of Al and Mg ions. During the second plant growth experiment in the APH, the arcillite weighed 4200 g and produced 420 g biomass in 28 days. This 10:1 ratio illustrates the waste inherent in arcillite-based cultivation, especially since cleaning or recycling of arcillite requires either the equivalent of autoclaving or a high-temperature

furnace, which will sterilize and combust plant and microbial residues, but also produce problematic fumes in the closed environment of a <u>space station</u> or lunar/Martian habitats. Because of these constraints, and to reduce the single-use mass required to produce an equivalent amount of biomass, a cultivation method akin to <u>hydroponics</u> or <u>aeroponics</u> is of paramount importance.

Equally important is a design that operates just as well under weightlessness conditions as it does in the <u>reduced gravity</u> conditions of lunar or Martian outposts. Ideally, the growth system will enable the transition to plant cultivation using in situ <u>regolith</u>. In addition to <u>water management issues</u>, seeds need to be mounted such that <u>imbibition</u> can be initiated and seedling positioning during germination and plant development secured. Space experiments typically rely on floral foam plugs that in dry form accommodate seeds. However, these foam plugs are naturally hydrophobic and must be treated with detergent-like chemicals to establish the necessary <u>hydrophilicity</u>. Unfortunately, the type and quantity of these chemicals often inhibits germination. Therefore, a generic method is needed to secure seeds by means other than floral foam that also provides anchorage for seedlings and expanding plants. The latter point is especially relevant for crops that develop expanding roots or <u>hypocotyls</u> such as radishes or beets, as these plants expand dramatically at the <u>substrate surface</u> during development.

Future plant cultivation on the Moon or Mars will eventually include local <u>regolith</u>. However, the toxic ingredients and apparent inhibitory properties of regolith (Paul et al., 2022) require soil conditioning. This process necessitates the release of nutrients, removal or break-down of toxic compounds such as chlorates and <u>perchlorates</u> (Matsubara et al., 2017; Sutter et al., 2017) and probably includes enriching regolith with microbes and fungi that enhance phosphorus availability (Jansa et al., 2019) and facilitate conditioning of regolith (Crisler et al., 2012; King, 2015). Until sufficient regolith conditioning is achieved, and suitable soil becomes available for crops, plant cultivation likely must rely on hydroponic systems. The transitioning to solid substrate-based plant cultivation will benefit from amendments that comprise low mass and variable water content compatible hydrogels like those currently used in <u>agriculture</u>.

We discuss plant cultivation without solid substrates and the high adaptability for different crops. Hydroponics provides water management under weightlessness as well as low gravity conditions. Substrate-free hydroponic root modules (RM) include features that can be

implemented individually or in concert: the delivery and control of water distribution in a weightless and low gravity (e.g., lunar) environment, the assessment of moisture and water deficit, and the removal of excess water. The application of hydrogels and methods for mounting seeds is included as well.

Current systems

Despite moisture sensors, both Veggie and APH experience difficulties assessing the watering conditions of plants and substrate in microgravity, in part as a result of the position and orientation of moisture sensors. Inconsistencies between substrate (i.e., arcillite) packing, uneven water distribution, and difficulties in assessing evaporation eventually defies automated watering and requires a holistic, observational assessment, of the plant water status and then manual adjustments.

The removal of solid substrate for space cultivation requires reliable, accurate, and reproducible water and nutrient management. In general, water usage primarily consists of dispensation to the seeds during <u>imbibition</u>, followed by nutrient supply to enable growth, and eventually replenishing water that is released by transpiration. These processes vary over time and require adaptable water delivery systems (Fig. 1).



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Fig. 1. Phases of water usage during plant growth. The initial <u>water consumption</u> during germination is relatively small but critical for the development of a healthy seedling.

Subsequent <u>vegetative growth</u> leads to incorporation of water into tissue as well as increasing water transpiration. Senescence is indicated as decreasing water consumption; details, water consumption and time course vary for individual plants and crop species.

Cultivating radishes in the APH showed inconsistent watering control in ground and space studies, suggesting that the system is susceptible to diminished gradients between the sensors and strongly sensitive to variations in local <u>moisture content</u> around the sensor itself. Each APH science carrier unit (quadrant) has two moisture sensors; one is positioned close to the bottom and the second close to the top surface of the container. The natural, gravity-induced <u>moisture gradient</u> (in substrate) would retain higher readings for the bottom sensor over time. However, moisture data showed that the initial condition deteriorated when plants started to grow, and reproducibility was dependent on gravity (Fig. 2). As a result, moisture sensor readings alone are not reliable enough to serve as reference for automated water delivery.





Fig. 2. Recordings of moisture sensors in a science carrier quadrant during the science verification test (SVT) and a space experiment (ISS). The readings show greater synchrony for the space than for the ground experiment. After 10 days the readings (SVT, black) indicate a <u>moisture gradient</u> between the upper and lower sensor but little difference for the space experiment, which unlike the SVT data showed diurnal periodicity with increasing amplitude as the plants and water demand increased.

Therefore, the water and nutrient supply system should be re-evaluated with parameters that focus on space conditions first but allow for adaptation of these components to fractional gravity environments, instead of attempting to recreate Earth-conditions. Similar to well-

established hydroponic or aeroponic systems on Earth that provide plant growth without solid substrate, plant growth in space would benefit from removing substrate to save mass and by delivering water and nutrients directly to the <u>root system</u>. In addition, no recycling or post-processing of arcillite such as root remnant removal and sterilization is necessary, and <u>microbial growth</u> can be better controlled than in a particulate, <u>porous substrate</u>. Difficult to assess <u>microbial loads</u>, combined with the lack of equipment available on the International Space Station to properly sanitize used substrate to a defined initial condition, renders reusing porous substrate impractical.

However, the need to assess water availability does require a moisture sensing system. Capacitive sensors do not have to be exposed to water and therefore can measure moisture in sealed configurations. Capacitance-based sensors rely on the changing capacitance of (high frequency) circuits that are affected by the water content or moisture in the vicinity of a capacitor. The 'capacitive reactance' $Xc = 1/(2\pi CF)$ is measured in Ohms and is a function of frequency F, and capacitance C of the chosen sensor (Rawlins, 2000). These sensors (Fig. 3) are immune to electrode decay and subsequent loss of signal integrity. The current two-blade design mandates horizontal positioning whereas a single blade as in Fig. 3 can be positioned such that the surface is perpendicular to the substrate surface and therefore interferes less with the expanding root system. Regardless of geometry, moisture readings can give misleading results about water availability (Fig.2). Although single blade sensors are less sensitive to small scale fluctuations, they can reliably assess the overall water content of the RM.



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Fig. 3. Example of a capacitance-based, single-blade moisture sensor. The picture (https://www.amazon.com/Capacitive-Moisture-Corrosion-Resistant-Detection/dp/B07SYBSHGX/ 7) shows shape and design of these sensors. The required electronics are low power and typically attached directly to the sensor. They are water resistant and function independent of their orientation.

Water delivery:

In the absence of wicking or surface-based water delivery, nutrient solutions must be provided as mist or <u>fine droplets</u>, and the aim to remove solid substrate requires a water delivery system that is effective under low and no gravity conditions. In these variable gravity conditions, mist will 'float' for a longer period of time than it would in Earth-based systems, and therefore would more effectively provide moisture without flooding the roots and causing oxygen deprivation. The nutrient mist can be created by <u>compressed air</u> to disperse fluid (Fig. 4), or by devices that depend on ultrasonic or <u>piezoelectric</u> membranes (Fig. 5).



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Fig. 4. Nebulization by 'flow blurring' depends on gas introduction axially into the solution flow and allows for larger diameter, which prevents clogging (A). Mixing of air (black arrows) and nutrient solution (blue arrows) results in controllable flow and <u>moisture content</u> (B). (www.agilent.com/en/ products/icp-oes/icp-oes-supplies/nebulizers/icp-oes-oneneb-series-2-nebulizer).



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Fig. 5. 20 mm Mist Atomizer DIY <u>Humidifier</u> with <u>PCB</u> 3.7–12 V. These solid-state devices operate at >100 kHz frequencies and are capable of vaporizing more than 300 mL per hour. Available from various retailers.

Water mist can be injected into, or nebulized within, a RM and dispersed by airflow under weightlessness and under partial or standard gravity. Regardless of its generation, the mist will eventually settle or condense on surfaces. Importantly, such designs remove the need for pumps that directly move water or the nutrient solution and are prone to priming issues, especially in microgravity. In addition, the ultrasonic misting process can be made from flexible membranes (Yang et al., 2022) and is useful for antifouling procedures (Kuscer et al., 2017). The saturated air will gradually lead to condensation of water on root surfaces, but this process does not immediately lead to <u>hypoxic conditions</u> and can be controlled via moisture sensors.

Achievable flow and delivery rates vary but are sufficient to provide mist to volumes of several liters per nebulizer. Flow rates for laboratory-sized systems vary from < 1 mL to > 15 mL per minute. However, industrial systems can have manyfold larger pump rates. Venturi based nebulizers are rugged and less complicated than ultrasonic nebulizers and therefore better suited for space or high-volume applications. One disadvantage of conventional nebulizers may be clogging because of their narrow (glass) capillary. However, Teflon-made nebulizers (e.g., OneNeb, Agilent) are more durable and provide fine mist because of a new jet design (Fig. 4). Another advantage of a Teflon-based system is the complete inertness and insensitivity to corrosive fluids (they are routinely used for applications that rely on high concentrations of nitric, hydrochloric, or fluoric acids).

A related design was recently proposed for improved <u>fuel combustion</u> (Akinyemi et al., 2019). Their work illustrates that the principle of nebulization has many applications and that modifications of the <u>gas flow</u> path vary <u>droplet size</u>, distribution, and flow rate (Fig. 6). Therefore, this nebulization design can provide very fine control over nutrient solution and water dispensation in RMs.



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Fig. 6. Top view of a modified flow path of a swirl <u>injector</u> design. The mixing principle is similar to Fig. 4B, but can be modified based on the geometry of <u>nozzle vanes</u> or grooves (from Akinyemi et al., 2019).

Unlike conventional nebulizers, the Venturi-based nebulizers 'pump' liquid based on the pressure differential between a (compressed) nebulizer gas (air) and the <u>ambient pressure</u> of the target chamber, instead of pumping the liquid directly. Thus, the nebulizer not only creates the desired fine mist of nutrient solution, but also supplies air to the root zone and induces mixing, contributing to gas exchange . While conventional nebulizers rely on separate pumps to control fluid flow, the Venturi design changes the flow rate of the nutrient solution by changing the pressure differential between the air supply and root chamber. The required compressor can be utilized for more than one dispensation unit and can be based on diverse designs (scroll, diaphragm, <u>impeller</u>, centrifugation etc.). The many options will enable commercial-off-the-shelf (COTS) units to be used for this purpose.

Water removal:

The efficient control of water and nutrient management requires adding and removing liquid. The sometimes-delicate balance between <u>water consumption</u> and supply necessitates the removal of excess water to avoid waterlogging and root hypoxia of plants, particularly when sensors sensitive to local variation are used to assess moisture content. Even with precise moisture sensors, water dispensation cannot guarantee uniform distribution. Therefore, a water removal mechanism is necessary to drain excess condensation that would otherwise accumulate and overload RMs. Sensors could misrepresent water availability and cause drought or water logging.

While ground applications can simply rely on gravity to collect excess water at the lowest point of a given RM, such convenient collection is not available under weightlessness conditions. There, water flow is independent of gravity but determined by surface interactions and/or <u>temperature gradients</u>. The effect of this condition was demonstrated by the Canadian astronaut Chris Hadfield (https://youtu.be/o8TssbmY-GM). The strict surface interaction can be modified by suitable geometry and hydrophilicity of the surface. The combination of surface tension between acute angled surfaces leads to <u>capillary action</u> that increases as the angle between surfaces decreases, as described in the USPTO Patent # 9962,024 (Weislogel, Pettit, et al.) that states that "such [devices] may be expected to function effectively provided the impacts of surface tension and [cup] geometry are significantly greater than the impact of gravity, allowing for use in standard gravity (e.g. on Earth), sub-standard gravity (e.g. on the Moon, on Mars, on asteroids and/or other fractional bodies), or low to near zero gravity (e.g. free flying in outer space)".

The relationship between angle and fluid cross-section is shown in Fig. 7 and can also be seen in the Hadfield video referenced above. Water is concentrated between acute angles.



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Fig. 7. The interaction of fluid with a (planar) wall that forms an angle. Surface tension, temperature, <u>viscosity</u>, and wall surface properties affect the height h(z,t) of the fluid profile (Concus et al., 2019; Weislogel, 2001).

The fluid behavior illustrated above provides a gravity-independent but geometry-defined water collection system that requires only suitable surfaces and can be further enhanced by the hydrophilicity or <u>hydrophobicity</u> of the surface material. Since the behavior is not restricted to a particular length perpendicular to the wall plane, it can be extended from the interior of the RM to the outside. This external transport will be facilitated using tubing with one or more angular shapes as <u>cross section</u> (Fig. 8). The diameter of the tubing determines this cross section and thus potential flow rate.



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Fig. 8. Example for tubing <u>cross section</u> that guides fluid under space conditions. Based on acute angles water will spread along the length of the tubing. Such tubing can be used to remove excess water from a RM. However, such tubing will not replace the need to pump fluids with greater efficiency. Possible ways of moving liquid depend either on Venturi-based suction (see above, causing movement toward injection point), following a <u>pressure gradient</u> as a result of enhanced airflow (removing liquid from injection point), or by gradients in <u>surface hydrophilicity</u>.

A simple way to maintain control over water distribution in space relies on the principle outlined in Fig. 7. This arrangement does not interfere with gravity-based liquid collection in

partial or standard gravity and works in weightlessness. The concept relies on modified surfaces that will lead to liquid collection at the point of surface intersection with acute angles. An optimization of the concept results in a <u>surface area</u> characterized by pyramid-like extensions that form the desired acute angle between them on all edges (Fig. 9).



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Fig. 9. Surface profile composed of individual tri- or quadrangular pyramids that form acute angles between the lateral surfaces (A). The angle between the surfaces is a function of the height and base of the pyramids. Although the general relationship between base size and height is similar, fewer sides enhance relative groove length and create steeper angles. A good compromise for height profile results from a height to base ratio of about 2 - 2.5 (~ 53 – 43 deg. angle between opposing surfaces, B).

In principle, the profiled surface is not limited to square- or triangular-based structures but can also be achieved with polygons. However, the larger the number of sides, the 'rounder' and therefore less efficient the liquid conduit becomes. Therefore, the most efficient arrangement is a triangular design. However, the square RM design that is widely used now would favor a quadrilateral base-geometry, because of parallel surfaces between the RM walls and pyramidal extensions.

The collection of water or nutrient solutions between the surface structures from the humidified air inside the RM can further be augmented by <u>thermal gradients</u> to force condensation directly onto the pyramidal extensions or RM walls. The most efficient implementation of thermal gradients relies on Peltier-cooled elements that can be attached to the external surface of the RM or the interior of pyramids. Energy-efficient cooling benefits from the <u>higher thermal conductivity</u> of metals. Therefore, the RM should be made from (anodized) <u>aluminum</u>, which is lightweight and non-corrosive if the internal surface is

properly coated. Thermal cooling elements are inexpensive, can be obtained in many different shapes and sizes, and can be low power since they would not have to generate temperature differences between the cooling element and humidified air. Cooling by as little as a fraction of a degree Celsius is sufficient for condensation.

Although the precise calculation of the <u>dew point</u> is challenging, for elevated (>50 % <u>relative</u> <u>humidity</u>, a common approximation is sufficient. The <u>dew point</u> Dc \approx Tc-(100-RH)/5; which indicates that at about 95 % RH and 28 C (common plant growth conditions) the dew point would be 27 C. However, as the expected <u>relative humidity</u> in the RM is close to 100 %, condensation would occur on temperature differentials as close as 0.1 C. Because of such small differences, the forced (i.e., Peltier) cooling may not even be necessary. Rather, simple external air circulation around the RM that provides external cooling may be sufficient to induce condensation on internal surfaces and subsequent collection in channels. This cooling would also benefit from the high <u>thermal conductivity</u> provided by an anodized aluminum container.

Cooling by a Peltier system does not have moving parts, is energy efficient, and will cause condensation of liquid inside the RM. The small size, controllable cooling capacity, and <u>low power consumption</u> are properties that provide flexibility and automated control of condensation and water movement. Although thermo-based water flow control is not designed to move large volumes, it does apply and reach all locations in a closed chamber for which a temperature gradient exists. In this context it is important to point out that illumination of a <u>growth chamber</u> provides a natural thermal gradient between a warmer canopy region and cooler root zone, that will assist with condensation to the desired surfaces. Although condensation will occur directly to roots, water distribution depends on surface interactions. Since the RM initially has a larger surface than developing roots and can have an induced temperature gradient, mist will condense on the wall surfaces necessitating a method for water removal.

For a more comprehensive description of water collection systems, other solutions than the one outlined so far are possible but often mechanically challenging and costly. The most intuitive approach to collect water includes short-term centrifugation. Brief accelerations can concentrate liquid from the root zone volume such that the solution can then be collected, essentially enabling space-based cultivation similar to the ones that are developed for aeroponics and vertical farming on Earth (Hosseinzadeh et al., 2017; Lakhiar et al., 2018). Such

arrangements would likely rely on a circular arrangement of balanced RMs around a central <u>light source</u> (Fig. 10). However, the complex design, limited light intensities (because of centrally located light banks), and rotational support structures are bulky and increase system mass; and is only a meaningful solution during periods of weightlessness, but not on Moon or Mars where partial gravity will enable water collection.



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Fig. 10. Approximate geometry of a centrifugal system that could be rotated to force water in individual growth container (only two shown) to the outer walls. Such a system has the advantage that short (even manual) rotations can collect water that then can be recirculated.

The alternative that is presented here, liquid collection based on surface interactions, also provides water removal and therefore limits root hypoxia. While the acute angle between surfaces facilitates water flow as outlined above, it may require additional manipulation to enhance the efficiency of water collection. Application of thermal gradients to the design displayed in Fig. 11 using a simplified series of pyramidal extensions eliminates some of the complex surfaces. However, tests must be performed which can provide answers as to which approach is most efficient.



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Fig. 11. Concept of a simplified profile of grooves in a RM designed to collect water in the acute-angled grooves (as in Fig. 7 & 9) with a central drain to which angular tubing (as in Fig. 8) can be connected. The RM can be designed in many sizes and accommodates the planting tray (PT, Fig. 15) in unambiguous orientation based on lateral profiles that also serve as guides which allow for changing distances from the base, and thus the RM volume, of the PT. Additionally shown are moisture sensors (blue) and ultrasonic misters (red).

Importantly, hydro- or aeroponic plant cultivation has many unanswered questions (Eldridge et al., 2020). Instead of liquid-based cultures, alternate materials can address problems of water retention, distribution, and management and have lower single-use mass, greater versatility and <u>recyclability</u> than current substrate like arcillite.

Hydrogel substrates:

While arcillite at the 1–2 mm grain size provides a suitable mix of water- and air-filled pores in the root zone, the solid clay aggregate fills most of the root volume and constitutes significant amounts of wasted mass for plant cultivation in space. Therefore, replacing arcillite with lightweight and/or multifunctional material such as hydrogels is desirable, as such materials are capable of absorbing and retaining many times their dry weight in water. Hydrogels and other functionalized polymers are commonly marketed as soil amendments to improve <u>water holding capacity</u> or modified to function as slow-release fertilizers and applicators of <u>agrochemicals</u>. In these applications they <u>increase crop yield</u> while reducing the frequency of irrigation and total amount of water required (Agaba et al., 2011; Albalasmeh et al., 2022). Additionally, hydrogels have been used as a tool for observing root development and studying morphological responses to <u>environmental gradients</u> (Ma et al., 2019). For solid substrates like arcillite, both water transport and gas exchange in the root zone must occur through the void spaces between grains. In fractional and microgravity, the increased importance of surface forces trap air bubbles and significantly reduce or prevent gas exchange, leading to hypoxic conditions. Conducting water through the interior of the substrate, instead of across the surface, can stabilize the formation of air pockets and prevent hypoxic conditions as drying in one region of the bulk substrate would lead to an uptake of water from elsewhere in the root zone. By relegating water storage to interior gel space, the size and geometry of hydrogel beads can then be tuned to optimize gas exchange without compromising water flow and availability.

However, the amount of water absorbed by the gel matrix that is available for re-utilization by plants depends on the difference between the water potential of the gel and absorbing (root) tissue. As long as the <u>root water potential</u> (typically -0.4 - -0.6 MPa) is lower than the water potential of the gel, water is available to plants. The equilibrium between gel and plant implies that some of the water absorbed by the gel would be plant-available. However, any water captured by the hydrogel that is then unavailable for uptake is likely to improve soil characteristics, since microbial water potentials are lower than that of plants (Brown, 1976). Therefore, microbial metabolism can still utilize water that is not available to plants.

Considering a RM filled with regularly sized spherical beads of hydrogel, the maximal volume that can be occupied by close-packed spheres is about 74 %, although random packing tends to reduce that volume towards approximately 63 % (Li et al., 2008). Furthermore, the gas space in this arrangement forms a single contiguous volume and so would allow unobstructed diffusion of gas throughout the entire root zone. Excess water will fill a portion of the available space, particularly where beads contact each other much like the angular surfaces discussed above. However, increasing the bead size will reduce such blockage and slightly increase the available gas volume and efficiency of gas exchange. Because different plants have divergent oxygen demands and root thicknesses, a combination of spherical beads with two or more distinct diameters may be required to balance the necessary air and hydrogel volumes. The complexity of the water demand, bead size(s), and intrinsic water potentials may require testing of these parameters to optimize plant growth.

As hydrogels are mostly water, for spherical beads the individual density is approximately 1 g/cm³, and the bulk density of a hydrogel substrate would decrease proportional with the air content and therefore fall between 0.63 to 0.74 g/cm³, based on the packing density. In contrast, the bulk density of arcillite (around 0.62 g/cm³) is less variable due to the irregular geometry of crushed rock. Since even moderately absorptive hydrogels can be 98 % water by weight, and some compositions (e.g., N,N-dimethylacrylamide) can reach up to 99.97 % (Cipriano et al., 2014), and because water can be reclaimed by evaporation and condensation in recycling systems, the single-use mass would be significantly less compared with arcillite. The 10:1 ratio of substrate to produced <u>plant biomass</u> would shift to a much more favorable ratio and reduce <u>payload mass</u> and storage, because the dry mass and volume of hydrogels can be 2 % or less of the total substrate.

Commercially available hydrogels are typically polymers of acrylamide or sodium/potassium <u>acrylate</u>. Of these, the <u>polyacrylates</u> tend to have a higher <u>water holding capacity</u> due to the ionic nature of their side chains. Importantly, these gels can be conditioned with a fertilizer solution such that ion exchange with <u>root exudates</u> can replace the cations of the structure, which can then be taken up as nutrients. In contrast, unconditioned <u>sodium</u> acrylate polymers will lead to salt-stress and negatively affect plant development. The ion exchange either of <u>potassium</u> polyacrylate, or conditioned hydrogels that contain nutrients, would function like slow-release fertilizers. But the depletion of ions over time and the effect of replacing cations with protons and root exudates may lead to uncontrolled conditions. Therefore, long-term stability, <u>reusability</u>, and the ease of "resetting" substrate to match experimental initial conditions of conditioned <u>polyacrylates</u> are unknown. In contrast, non-ionic polymers such as <u>polyacrylamide</u> (PAA, Fig. 12) are less active in ion exchange, and can be covalently crosslinked and therefore are more stable for repeated use.



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Fig. 12. Simplified depiction of <u>polyacrylamide</u> gels (without bis-acrylamide bridges) that show the <u>hydrophilic</u> oxygen and amino moieties of the <u>monomer</u> to which water binds.

Because of the direct interaction with water, ion exchange with the polymer reduces the adsorptive binding between water and PAA. This interaction leads to a change in the volume of PAA that is inversely proportional to the ion load. Therefore, the extent of swelling of the gel can directly monitor the ion or fertilizer load of polyacrylamide substrate. As the ionic <u>strength</u> of the imbibed solution increases, the maximum amount of water that can be absorbed decreases. This relationship could be used to provide additional tools for monitoring nutrient availability. As nutrients are depleted, the hydrogel can absorb more water, which leads to increased substrate volume. Depending on the specific chemical composition of the hydrogel, the magnitude of ion load and corresponding volume changes could be monitored visually and provide information about how much fertilizer needs to be replenished. For a closed RM, the change in swelling could be measured by pressure sensors.

Although PAA is chemically stable, root exudates and <u>microbial communities</u> containing extracellular <u>amidases</u> and <u>hydrolases</u> may cause biodegradation over time. Importantly, <u>mechanical degradation</u> of PAA does not result in the accumulation of monomeric acrylamide in crops, and so should not be a human health concern, and the use of non-acrylamide polymers can remove this concern entirely. A suitable substrate based on PAA, acrylamideacrylate <u>copolymers</u>, and other hydrogel polymers can be designed to optimize water holding capacity, release rates of nutrients, hardness, stability, and thus longevity of the substrate. On top of the substantial mass savings compared to single use materials such as arcillite, <u>polymer substrates</u> would aid the transition from plant cultivation in spacecraft to the use of regolith as it exists on the Moon or Mars. They can help with the removal of perchlorates or other toxic compounds as a part of regolith conditioning and facilitate the enrichment of regolith-based soils by the introduction of <u>microbial communities</u>, <u>organic carbon</u> from the <u>polymer backbone</u>, and nutrients.

One of the main obstacles to using in situ regolith is the high concentration of perchlorates (Oze et al., 2021) as they are highly soluble in water. Perchlorate <u>contaminated water</u> is known to inhibit germination and crop growth and is a potential hazard for human health. Remediation and removal of perchlorates from water sources rely on either physical (membrane filtration) or chemical (anion exchange) processes. <u>Sorbents</u> or gels made from chitosan (a <u>polysaccharide</u> composed of β -(1 \rightarrow 4) d-glucosamine and N-acetyl-d-glucosamine) have a strong affinity for perchlorates and can be regenerated for repeated use (Xie et al., 2010). Chitosan also exhibits hydrogel properties due to the presence of amide groups that bind to water as well as perchlorate. Therefore, a copolymer of acrylamide and chitosan could first be used as substrate to grow plants during transit to Mars. The used substrate, which would contain residual nutrients in addition to root fragments and other organic compounds, could then be repurposed to condition regolith for crop production, either by removing perchlorate by treatment with bacteria that express perchlorate <u>reductase</u> (Thrash et al., 2010; Zhang et al., 2002) or iron catalysis (Cao et al., 2005). Microbial communities that reduce perchlorates lead to carbon-enriched substrate and ultimately soil.

Potential benefits to space agriculture of additional modifications to the <u>polymer structure</u> and properties, inspired by plant adaptations, can be investigated as well. The desert shrub <u>Athel tamarisk</u> (*Tamarix aphylla*) is found in hypersaline desert environments and excretes salts and other minerals that remain attached to the plant's surface through <u>hydrogen</u> <u>bonding</u>. During the day, evaporation leaves crystallized hygroscopic deposits at the salt glands. When the relative humidity increases at night, these deposits absorb humidity and rehydrate the plant (Al-Handawi et al., 2023). Although humidity-gathering salts in a hydrogel can leak into the surrounding solution and thus be detrimental to salt-intolerant crops, a copolymer including 2-(N-3-Sulfopropyl-N,N-dimethyl ammonium)ethyl methacrylate (DMAPS) immobilized similar hygroscopic salts, and the resulting gels were capable of absorbing water about 12 times the dry mass, even at relative humidity levels as low as 15 % (Guan et al., 2023). Since plants transpire most of the required water and therefore increase the humidity of their surroundings, humidity levels in the ISS (about 60 %) or in the APH (up to 90 %) is sufficient to rapidly replenish or maintain equilibrium of the water content of such gels. Such gels might be paired with the misting concept of <u>piezoelectric elements</u> and nebulizers that have been mentioned previously. A self-regulating equilibrium between growth chamber humidity, <u>gel water</u> potential, and transpiration rates might be able to provide a substantial proportion of the water requirements for plants, further simplifying monitoring and delivery systems.

Seedling support:

The water management system would be incomplete without giving consideration to methods for securing seeds, seedlings, and eventually mature plants. Tests in preparation for an APH experiment showed clearly that the need to contain arcillite, provide water, and positioning of seeds are difficult to reconcile. The design of the planting tray (PT) and RM includes a science carrier top with either long slits, or a grid of holes to provide proper plant spacing. Experiments showed that the expansion of the tissue that eventually forms the bulbous radish is sensitive to mechanical restriction by the support material (Capmat) of the science carrier (Fig. 13). To overcome problems with water delivery, seed attachment, and adequate bulb expansion, a 'crossed duckbill' design is proposed (Fig. 14) that should be made of soft and highly elastic <u>silicone</u>.



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Fig. 13. Radish plants grown in the science carrier in preparation for the APH2 space experiment. The constriction resulted from wicking material that surrounded the seed and provided water for <u>imbibition</u>. However, the inelastic behavior of the felt-like material (Capmat) and the science carrier top constricted radish expansion. Subsequent use of 'Oasis' (= floral) foam eliminated these issues but the particulate nature of the readily disintegrating, non-elastic foam required covering of the seeds with gauze.



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Fig. 14. Conceptual sketch of an <u>elastomer</u> plug that can be inserted in RM covers. Key features include a round compartment (1.5–2 mm diameter, center top) that accommodates the seed and a cross design with slits that initially provide water for imbibition by <u>capillary action</u> (as in Fig. 7) before roots extend into the substrate below. The vertical part provides mechanical support but allows expansion of tissue and bulb formation. The base of the plug matches mounting holes in the science carrier top (Fig. 15). The <u>silicone</u> plugs can be easily sanitized for reuse.

Such devices can be molded, and the thickness as well as softness of the <u>silicone material</u> can be optimized. Added benefits of silicone materials include a <u>chemical inertness</u> to water, <u>organic solvents</u>, acids, and minerals. The consistent softness (with low to no hardening over time) is also important for repeated and long-term use. The proposed design includes an indentation such that seeds of different sizes can easily be inserted. The cross-shaped slits expand toward the base and therefore provide capillary interaction similar to Fig. 7, Fig. 8, Fig. 9. Therefore, seeds will be imbibed without the need to provide additional wicking material. The softness of the silicone allows for expansion as the seeds swell in the slits and eventually, at least in the case of radishes, hypocotyls expand. The <u>elastic properties</u> of the silicone can secure seeds in place and remove the need for glue, as is routinely done in current plant experiments on the ISS.

The PT (Fig. 15) forms the top part of the science carrier and contains mounting holes for suitably sized plugs (Fig. 14) and a provision to slide smoothly up and down the base module (Fig. 11) but does not contain any instrumentation, sensors, or other attachments. The mobility of the cover minimizes the RM volume for initial water dispensation but allows for gradual expansion of the root space. Water or nutrient solution will be administered through openings in the base of the unit. This approach simplifies water management, and facilitates plant accessibility, system maintenance, and harvest.



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Fig. 15. PT (science carrier covers) with five and nine growth positions for plants with expanding <u>hypocotyls</u>. Numerous perforations and cut-outs allow for water and air <u>equilibration</u>. The circular cut-outs accommodate <u>elastomer</u> plugs (Fig. 14) and seeds. The PT matches the RM and contains openings for access to the base unit (Fig. 11).

These design suggestions simplify the complexity and maintenance, and facilitate overall construction, of a hydroponic RM. Most importantly, they remove solid substrate such as arcillite, reduce waste and mass, are adaptable to the <u>microgravity conditions</u> of <u>spaceflight</u> and partial gravity of the lunar and Martian surface, and can aid in the transition to in-situ

<u>resource utilization</u> for future space agriculture. We hope that these considerations will contribute to a novel design of plant cultivation systems.

CRediT authorship contribution statement

Karl H. Hasenstein: Conceptualization, Funding acquisition, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Nicholas M. Miklave:** Conceptualization, Funding acquisition, Methodology, Writing – original draft, Writing – review & editing.

Conflict of interest

The authors declare no conflict of interest.

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