Fluorescence imaging of microbe-containing particles that had been shot from a two-stage light-gas gun into an aerogel.

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Abstract

We have proposed an experiment (the Tanpopo mission) to capture microbes on the Japan Experimental Module of the International Space Station. An ultra low-density silica aerogel will be exposed to space for more than 1 year. After retrieving the aerogel, particle tracks and particles found in it will be visualized by fluorescence microscopy after staining it with a DNA-specific fluorescence dye. In preparation for this study, we simulated particle trapping in an aerogel so that methods could be developed to visualize the particles and their tracks. During the Tanpopo mission, particles that have an orbital velocity of ~8 km/s are expected to collide with the aerogel. To simulate these collisions, we shot *Deinococcus radiodurans*-containing Lucentite particles into the aerogel from a two-stage light-gas gun (acceleration 4.2 km/s). The shapes of the captured particles, and their tracks and entrance holes were recorded with a microscope/camera system for further analysis. The size distribution of the captured particles was smaller than the original distribution, suggesting that the particles had fragmented. We were able to distinguish between microbial DNA and inorganic compounds after staining the aerogel with the DNA-specific fluorescence dye SYBR green I as the fluorescence of the stained DNA and the autofluorescence of the inorganic particles decay at different rates. The developed methods are suitable to determine if microbes exist at the International Space Station altitude.

Keywords: Aerogel, Space experiment, Hypervelocity impact experiment, DNA-specific fluorescence dye.

Introduction

The existence of terrestrial life under extreme conditions, including high altitudes, has been examined geochemically and geobiologically (reviewed by Rothschild and Mancinelli 2001; Madigan and Orent 1999; Navarro-Gonzáez et al. 2003; Kato et al. 2009; Yang et al. 2009). One notable feature of high altitudes is that solar ultraviolet (UV) radiation is much greater than it is on the ground because of the relative differences in the distances from the sun and the shielding effect of the stratospheric ozone layer, which eliminates UV radiation of <290 nm (Blumthaler et al. 1997; Hallmann and Ley 1998) Consequently, the more intense high-altitude solar radiation probably does substantially more damage to an organism's DNA and its viability (Horneck et al. 2006) than does radiation reaching the earth.

To investigate if microbes exist at high altitudes (up to 77 km above ground level), microbe-capture experiments have been performed in airplanes, balloons, and rockets (reviewed by Yang et al. 2009). These experiments have retrieved radiation-resistant fungal and *Bacillus* spores, and *Deinococci* (Soffen 1965; Fulton 1966; Wainwright et al. 2003; Griffin 2004; Yang et al. 2008a; Yang et al. 2008b; Smith et al. 2009). DeLeon-Rodriguez et al. (2013) suggested that air-borne microbes might be involved in the (bio)chemistry of the atmosphere and hydrological cycles. Microbes may be transferred to high altitude by several mechanisms, e.g., powerful volcanic eruptions (Simkin and Siebert 1994; Robock 2002; Antuña et al. 2003; Oman et al. 2005), meteorite-impact recoil debris (Alvarez et al. 1980; Kring 2000), wind storms (Kellog and Griffin 2006), rocket launches (Bucker and Horneck 1968; Nicholson et al. 2009), and electrostatic forces associated with thunderstorms (Dehel et al. 2008). If microbes exist at altitudes higher than 77 km above the Earth, e.g., in the thermosphere, which corresponds to a low Earth orbit (LEO), it will provide evidence for the expansion of the Earth's biosphere.

We have proposed the Tanpopo mission—an experiment to be performed on the Japanese Experiment Module (JEM) of the International Space Station (ISS), which orbits 400 km above the Earth—to investigate possible interplanetary transfer of microbes and organic compounds (Yamagishi et al. 2008). To do so, the experimental protocol is designed to capture micro-particles that might contain microbes. We will expose an ultra low-density silica aerogel, which will serve as the particle trap, to the outside of the ISS for more than **1** year. After retrieving the aerogel, we will determine if particles were trapped in the aerogel. If so, we will first characterize the entrance holes and tracks made by these particles. We will then stain a horizontally cut portion of the interior of the aerogel with a DNA-specific fluorescent dye to detect microbial DNA associated with the particles and their tracks. Any fluorescence arising from the stained DNA will be visualized under a fluorescence microscope. Particles that might potentially contain microbes in the other half of the exposed interior will be subjected to polymerase chain reaction (PCR) amplification of the small subunit ribosomal RNA (rRNA) gene followed by DNA

79 sequencing (Tabata et al. 2011). Comparison between the experimentally determined sequences and
80 known rRNA gene sequences may suggest the origin and properties of the microbial DNA and,
81 consequently, the captured microbes.
82 An ultra low-density silica aerogel is a dried SiO_2 gel with an amorphous structure.
83 Projectiles traveling at hypervelocity (on the order of km/s) are severely damaged when they hit most
84 materials (reviewed in Burchell et al. 2006); however, this type of material offers the advantage that it
85 does much less damage to an impacted projectile and, therefore, can be used to capture particles in a
86 nondestructive manner. It has been used as a cosmic dust collector to capture particles of sub-millimeter
87 size on LEO vehicles, e.g., the European Retrievable Carrier spacecraft of the European Space Agency
88 (Yano and McDonnel 1994), the Micro-Particles Capturer and Space Environment Exposure Device of
89 the National Space Development Agency of Japan (the forerunner of the Japan Aerospace Exploration
90 Agency) at the ISS (Kitazawa et al. 2000; Neish et al. 2005), and the interplanetary dust-capture
91 experiment on the Mir space station (Hörz et al. 2000), and the Stardust mission (Brownlee et al. 2006) of
92 the National Aeronautics and Space Administration. However, a capture experiment of microbes in space
93 has never been attempted. The extremely large kinetic impact energies of projectiles accelerated to
94 hypervelocities causes heat-induced physical transformations including decreases in the volumes and
95 vitrification of the projectiles and target material (Okudaira et al. 2004; Noguchi et al. 2007). Airborne
96 microbes have been transported on dust particles, e.g., clay minerals, through the atmosphere (Kellogg
97 and Griffin 2006; Womack et al. 2010; Smith et al. 2013). If terrestrial microbes at the altitude of the ISS
98 orbit are present, they may be found in dust particle(s) that originated on the Earth. If we assume that
99 particles found at the LEO have an orbital speed of ~8 km/s, the collision speed between an aerogel used
100 to trap a particle and the particle would be at most 16 km/s and dependent on the collision angle.
101 For this report, we assessed if microbes or microbial DNA associated with micro-particles
102 could be captured by an aerogel. Dust particles that are present at LEO altitudes and their sizes have been
103 characterized (McBride et al. 1999; Kitazawa et al. 2000; Hörz et al. 2000), although microbe-containing
104 particles have not been identified to date. LEO dust particle densities are quite small (Hörz et al., 2000).
105 For the Tanpopo mission, we will determine if DNA molecules are present in particles found in the
106 retrieved aerogel as such molecules would indicate the presence of microbes captured by the aerogel. We
107 plan on staining the interior of the aerogel, if it contains particles and their tracks, with the DNA-specific
108 dye SYBR Green I and visualizing by fluorescence microscopy. Similar detection methods have been
109 widely used for environmental samples, e.g., soil, marine sediment, and water. Fluorescence dyes, e.g.,
110 acridine orange (Francisco et al. 1973; Daley and Hobbie 1975), 4',6-diamidino-2-phenylindole (Poter
111 and Feig 1980), SYBR Green I**,** and SYBR Green II (Noble and Fuhrman 1998; Weinbauer et al. 1998;

Patel et al. 2007), have been used to detect environmental microbes. SYBR Green I binds to double-stranded DNA and emits green light when excited by blue light (excitation maximum, 497 nm; emission maximum, 520 nm), and has been used to characterize the sizes, shapes, and number of microbes present in a sample, e.g., marine sediment (Sunamura et al. 2003). However, inorganic, especially vitrified, materials, e.g., soil, minerals, sediment, and rocks, have large autofluorescence signals, which overlap with microbial signals (Morono et al. 2009). The Tanpopo aerogel and captured micro-particles are inorganic materials. Therefore, we needed to develop a method that would distinguish between DNA-associated fluorescence and vitrified inorganic material autofluorescence after retrieval of the aerogel from the ISS.

For the study reported herein, we established methods for the characterization of captured particles and their tracks and entrance holes, and for the identification of microbial DNA by fluorescence spectroscopy in an aerogel that had been shot with particles. We first shot *Deinococcus radiodurans* R1-containing Lucentite particles into an aerogel using a two-stage light-gas gun (LGG). The sizes of the captured particles and their exposed tracks were recorded with a microscope/camera and then measured. The interior of the aerogel containing the tracks and particles was then stained with SYBR Green I to develop a method that would allow for the identification of microbial DNA.

Materials and methods

Preparation of projectiles

We prepared micrometer-size particles made of a mixture of the smectite clay Lucentite (Lucentite SWN, Co-op Chemical) and *D. radiodurans* R1 (ATCC 13939). *D. radiodurans* was cultured overnight in 1% (w/v) Bacto Tryptone, 0.6% (w/v) beef extract, and 0.2% (w/v) glucose, pH 7.0 at 30°C with shaking at 150 rpm until the culture reached the stationary phase. *D. radiodurans* cells were collected by centrifugation at 3000 rpm for 10 min at 4°C in a centrifugal concentrator (TOMY, High-Speed Micro-Refrigerated Centrifuge, MR-160). The cells were washed with RT-PCR Grade water (Ambion Co., Ltd., AM9935), and centrifuged as before. The wash process was repeated a total of three times. Lucentite particles, (1 g) were dry-heat sterilized at 180°C for 4 h, suspended in RT-PCR Grade water (1 ml), and mixed with the suspended *D. radiodurans* cells ~10^8 cells/g Lucentite. After the suspension had been centrifuged at room temperature for 10 minutes, the supernatant was removed with a Pasteur pipet, and the precipitate was dried under vacuum in a centrifugal vacuum concentrator (Sakuma Co. Ltd., EC-57CS) for 3 days. The dried precipitate was crushed with a sterile spatula, and particles between 48 and 58 μm in diameter were selected by passing them through mesh (NBC Meshitec). Their dimensions were measured after capturing their images using a microscope

145 equipped with a CCD camera (Olympas Optical Co. Ltd, BX60; Fig. 1a). The average particle weight was
146 estimated by weighing 100 particles on an ultra-microbalance (Sartorius, MC5; n = 3). Lucentite particles
147 that had not been mixed with *D. radiodurans* were prepared in an identical fashion.

148 ***Two-stage LGG experiment***

149 Hypervelocity impact experiments were performed using an LGG (Fig. 2a) at the Institute of
150 Space and Astronautical Science, Japan Aerospace Exploration Agency (ISAS/JAXA). The LGG is a
151 hypervelocity accelerator that uses H_2 (or He) as the accelerating medium. Sample particles were placed
152 into a spherical cavity formed by the inner surfaces of a cylindrical bullet called a "sabot" (Fig. 2b). The
153 bullet was accelerated by the LLG to ~4.2 km/s and stopped by a sabot stopper so that only the particles
154 collided with the targeted aerogel. The aerogel was manufactured as described (Tabata et al. 2010**,** 2012)
155 and consisted of two layers (Tabata et al. 2011) with the upper layer having a density of 0.01 g/cm^3 and
156 the lower layer having a density of 0.03 g/cm^3. When PCR assayed, bacterial contamination was not
157 detected in the aerogel (Tabata et al. 2011). The aerogel (3 cm × 3 cm × 2 cm) was placed into an
158 aluminum holder that had been wiped clean with 70% (v/v) ethanol/water and then the aerogel/holder
159 system was placed in the vacuum chamber (maintained at <10 Pa) of the LLG apparatus. We handled the
160 container and the aerogel with sterile tools.
161
162 ***Fluorescence imaging***

163 Lucentite particles with and without *D. radiodurans* were shot at the aerogel at 4.2 km/s.
164 After impact, the regions in the aerogel containing particle tracks were cut out with a sterilized razor
165 blade (Feather Co., Ltd.). The aerogel was cut along the centers of the tracks. The aerogel is a
166 hydrophobic material and is not degraded by water (Tabata et al. 2012). Because a strictly hydrophilic
167 solution could not penetrate the aerogel, we used a SYBR Green I/DMSO/water/acetone solution, which
168 provided fluorescent images with lower backgrounds than did other mixtures (data not shown). The
169 staining solution contained 1 mL of SYBR Green I in DMSO (Invitrogen, Carlsbad, CA), 59 mL of
170 RT-PCR Grade Water (Ambion Co., Ltd., AM9935), and 40 mL of acetone (Wako Pure Chemical
171 Industries, Ltd., Japan). The aerogel, with its tracks exposed, was placed on a glass slide after being
172 stained for 1 h, and then the stained tracks were subjected to fluorescence microscopy (Optical Co. Ltd,
173 BX60). A green (NIBA), red (WIG), or blue (WU) fluorescence filter (Optical Co. Ltd, BX60), or no
174 filter, was used when recording the images**.** Table 1 shows the excitation and fluorescence wavelengths of
175 the filters. Images of 640 × 640 pixels, corresponding to 125.2 × 93.9 μm were recorded. Complete tracks,
176 i.e., from their entry points to their termini, were photographed. The exposure time was 1.0 sec and an
177 image was recorded every 10 or 30 sec for 300 sec. The fluorescence intensities of the pictures were

recorded using ImageJ 1.47n (Abràmoff et al. 2004). The total fluorescence intensity (F_i) of all images taken at a given time after the initial exposure was calculated by integrating the glary value for each pixel. The attenuation rate was defined as the quotient F_i/F_{i0}, where F_{i0} is the total intensity at 0 sec.

Microscopic observation of captured particles and their tracks and entrance holes

Photographs of captured particles, and their tracks and entrance holes were recorded using a microscope equipped with a CCD camera (Optical Co. Ltd, DP72). Their dimensions were measured using ImageJ 1.47n.

Results

Characteristics of the Lucentite particles

Micrographs of the *D. radiodurans*-containing Lucentite particles before impact are shown in Fig. 1a. The particles have irregular shapes but are of similar sizes. Smaller particles adhere to larger particles. The size distributions of the particles before impact are shown in Fig. 1b, with the minimum length and width being 42.0 μm and 28.0 μm, respectively; and the maximum length and width being 80.0 μm and 82.0 μm, respectively. The average mass was 97.1 ± 6.6 ng.

Curation of the captured particles and their tracks

The *D. radiodurans*-containing Lucentite particles were accelerated through the LGG apparatus at 4.2 km/s before colliding with the two-layered aerogel. Examples of the tracks in the aerogel caused by the particles are shown in Fig. 3. The left half of the aerogel had a density of 0.01 g/cm^3 and the right half had a density of 0.03 g/cm^3. Many short tracks overlapped each other (Fig. 3). Because the low-density portion of the aerogel is nearly opaque, not all the tracks could be observed. Entire tracks and the termini are seen in Fig. 4. Each terminus contained a particle, which would probably be a Lucentite particle given its large refraction index. Fig. 5 shows images of the particle entrance holes. The larger holes are associated with longer tracks. Some of the entrance holes are star shaped with fractures at their edges (Figs. 5a and b). Smaller holes and black particles are also observed at the entry surface of the aerogel. These holes were probably made by gun debris; because the debris had a slower speed than did the Lucentite particles, it would not have entered the aerogel. The average dimensions and associated uncertainties of the tracks, entry holes, and the residual particles are shown in Table 2. The average length and width of the residual particles are 48.8 ± 1.2 μm and 48.9 ± 2.4 μm, respectively. The average area of the particles is 1735.8 ± 100.3 μm^2 assuming that they are oval in shape. The average length, width, and area of the entrance holes are 0.58 ± 0.06 mm, 0.62 ± 0.07 mm**,** and 0.20 ± 0.03 mm^2, respectively. The

size distribution of the particles after collision is shown in Fig. 1b. The sizes of the particles are smaller after the collision with the aerogel than before.

Fluorescent staining of microbial DNA

We developed a protocol to detect microbial DNA in the targeted aerogel that uses DNA-bound SYBR Green I fluorescence and that can distinguish microbial DNA from inorganic materials.

Lucentite particles with or without *D. radiodurans* cells were shot at the aerogel. After staining the aerogel with SYBR Green I, we observed the particles in the tracks using a fluorescence microscope equipped with or without a filter. Some small particles that did not contain *D. radiodurans* were observed at the track termini (Fig. 6a). These small particles autofluoresced under each filter (Fig. 6). Fig. 7a shows fluorescence images of aerogel-embedded Lucentite particles at different illumination times. Fig. 7b shows the fluorescence of stained, cultured *D. radiodurans* cells as observed by fluorescent microscopy. Fig. 8a shows the time course of the fluorescent intensity from Lucentite particles and SYBR Green I-DNA complexes. The graphs were plotted using the particle identified by the red arrow in Fig. 7a (open circles) and the average of the data from the three *D. radiodurans* DNA-SYBR Green I complexes identified by blue arrows shown in Fig. 7b (closed squares). In the Fig. 8b, the open squares indicate the average of the fluorescence intensities of the particle shown in Fig. 9a and two similar fast fading particles found in the same track. The closed circles indicate the average of the fluorescence intensities of the particle shown in Fig. 9b and two similar slow fading particles found in the same track. The Lucentite particles autofluoresced longer than 6 min (Fig. 7a, 8a), whereas the fluorescence associated with *D. radiodurans* decreased more rapidly (Fig. 7b, Fig. 8a).

We visualized the tracks made by the *D. radiodurans*-containing Lucentite particles in the presence and absence of a filter (Fig. 10). The shaft of the black arrow is parallel to the direction of the impact in Fig. 10a. Particles (~1 μm in diameter) circled in red were observed only under the green filter (Fig. 10b). Conversely, the particles circled in white were visualized under all filters (Fig. 10).

The fluorescence images of particles in the same track that were observable under the green filter were taken immediately after excitation (Figs. 9a and c). The inserts in Figs. 9a and c are enlargements of the particles shown in the main portions of the respective figures. During the 300 s that the particles were exposed to the excitation light, the fluorescence of the particle circled in red had decayed to a greater extent than had the fluorescence of the particle circled in blue (Figs. 9b and d). The attenuation rate was calculated from a plot of the fluorescence intensity versus the excitation time (Fig. 8b) and was found to be different for the two particles.

We also counted the number of SYBR Green I-stained *D. radiodurans* cells in three un-shot Lucentite particles by fluorescence imaging. The number of cells was 22 ± 3 cells per particle. After the experiment, we found 8, 6, 7, and 8 *D. radiodurans* cells in four different particles (data not shown).

Discussion

Characterization of the captured particles and their tracks in the target aerogel

Lucentite particles of ~60 μm in diameter were used as the model projectile for this hypervelocity experiment. We measured the size of the particles that were found at the ends of the tracks and found that both particle dimensions had decreased after impact (Table 1 and Fig. 1b). Many small particles were observed near the track termini and on the tracks themselves (Fig. 6a), suggesting that the original particles had fragmented. We and others have shown that the interiors of other types of particle projectiles, e.g., serpentine, cronstedtite, and cocoa powder, do not experience temperatures higher than their decomposition temperatures (Okudaira et al. 2004; Noguchi et al. 2007; Spencer et al. 2009), suggesting that any DNA from microbes in the interior of the Tanpopo particles would not be subjected to temperatures that would affect its integrity. Therefore, it should be possible to capture particles of ~60 μm in diameter and to detect microbial DNA in the particles using the aerogel manufactured for the Tanpopo mission.

The properties of particles and their tracks and entrance holes in an aerogel provide information regarding the characteristics and impact velocity of the particles (e.g., Burchell et al. 2008). "Hard" projectiles, e.g., soda-lime glass, meteorite samples, and various other minerals, have been used as projectiles (Burchell et al. 1998; Burchell et al. 2001; Burchell et al. 2009; Spencer et al. 2009; Hörz et al. 2006; Kearsley et al. 2012), as have "soft" projectiles, e.g., cocoa powder (an aggregate of organic matter), montmorillonite, which is a clay, glycine crystals, Allende meteorite particles, and soda-lime glass beads (Anderson and Ahrens 1994; Spencer et al. 2009; Tabata et al. 2011; Nixon et al. 2012; Kearsley et al. 2012; Suzuki et al. 2013). Lucentite particles, which had not been used previously as projectiles, created carrot shaped or "gobou" (burdock root)-shaped tracks (Tsuchiyama et al. 2009). The same type of track was found when Allende meteorite particles (Spencer et al. 2009), soda-lime glass beads (Tabata et al. 2011; Kearsley et al. 2012), and Al_2O_3 (Hörz et al. 2009) were used as projectiles. Certain tracks created by comet dust from the Stardust mission had a similar shape (Brownlee et al. 2006; Tsuchiyama et al. 2009; Iida et al. 2010). Therefore, Lucentite particles act as "soft" projectiles.

The Lucentite particles created jagged entrance holes (Fig. 5), which was expected because the cross-sections of the particles were not circular in shape (Fig. 1a). The entry holes had deeply indented margins (Figs. 5a and d) such as were seen for aerogels impacted by glycine (e.g., Nixon et al.

277 2012). The features of these holes might be ascribed to the low density of the aerogel. To identify the type
278 of particles that might collide with the aerogel used for the Tanpopo mission, we need to survey other
279 types of particles, with different diameters and different accelerations, to characterize the types of tracks
280 and entrance holes that they would produce.
281
282 ***Detection of microbial DNA in the aerogel by fluorescence staining***
283 The ability to detect microbes in a complex environment by fluorescent imaging has
284 improved with the development of new stains and extraction methods (Noble and Fuhrman 1998; Shopov
285 et al. 2000; Boenigk 2004; Zhou et al. 2007; Kallmeyer et al. 2008). In the aforementioned reports,
286 fluorescence spectroscopy in conjunction with SYBR Green I staining was used to distinguish microbial
287 DNA from inorganic particles. Sunamura et al. (2003) reported that the fluorescence of SYBR II-stained
288 microbial DNA could be differentiated from autofluorescing substances. Fluorescent signals from SYBR
289 Green I-stained microbes and non-specific background signals from inorganic particles have different
290 colors under a long-pass filter, which is transparent over a wide wavelength range (Morono et al. 2009).
291 Pure Lucentite particles intensely autofluoresce under the red, blue, and green filters (Fig. 6), whereas
292 SYBR Green I-stained DNA fluorescence is observed through only the green filter. The track circled in
293 white autofluoresced and could be visualized through all filters (Fig. 10). This fluorescence was, therefore,
294 probably emitted by vitrified Lucentite fragments and/or the aerogel. Spectral discrimination was used to
295 detect microbes present in low abundance in marine sediment (Sunamura et al. 2003). Because the
296 number of particles captured in space is expected to be very limited, fluorescent imaging should provide
297 sufficient sensitivity.
298 When SYBR Green I binds to double-stranded DNA, it undergoes a conformational change
299 that increases its fluorescent intensity (Zipper et al. 2004). Attenuation of SYBR Green I fluorescence
300 intensity occurs when the molecule is damaged by oxygen radicals, increased temperature, and extremes
301 in pH. Generally, detection of microbes by fluorescence microscopy is hampered by quenching of the
302 DNA-bound fluorophore. However, the quenching rate can be used to distinguish SYBR Green I
303 fluorescence from fluorescence by vitrified material. The fluorescence intensity of the vitrified particles
304 in the aerogel decayed much more slowly than did that of the SYBR Green I-DNA complex (Fig. 8a),
305 indicating that the difference in the decay kinetics can be used to distinguish the two types of
306 fluorescence. We also measured the fluorescence decay (Fig. 8b) of the two particles shown in Fig. 10.
307 The fluorescence of the particle that decayed more rapidly is most likely associated with SYBR Green
308 I-DNA complexes (Fig. 9). The fluorescence of the particle that decayed more slowly is most likely
309 associated with a vitrified particle and/or aerogel material.

310 The inserted image in Fig. 9a shows two particles, ~1 μm in size attached to one another. *D.*
311 *radiodurans* cells tend to form diplococcal structures (Fig. 7b), which supports the idea that the detected
312 particles in Fig. 9a are *D. radiodurans* cells.
313
314 ***Assessment of microbial contamination during the space experiment***
315 We will attempt to minimize microbial contamination of the aerogel prior to its placement on
316 the ISS by appropriately designing the apparatus used to contain it and taking precautions during its
317 handling. However, the potential for microbial contamination remains.
318 To minimize possible contamination, the manufacture of the aerogel and the following
319 procedures will be performed in a clean room. Notably, we have already shown using PCR that an
320 aerogel manufactured in the clean room is sterile within the detection limits of the experiment (Tabata et
321 al 2011). We will place the aerogel in aluminum Capturer Panels (described in Tabata et al. 20014), and
322 store the system in the sterile Ziploc bags (AsahiKASEI, Japan). After transporting the Capture
323 Panels-aerogel system in the Ziploc bags to the ISS, the system will be removed from the bags and
324 attached to the Exposed Experiment Handrail Attachment Mechanism. During this procedure, the aerogel
325 surface may be contaminated by airborne microbes in the ISS pressurized area. The Capture
326 Panels-aerogel system will then be transferred to the outside of the ISS thorough the ISS airlock where it
327 will be attached to a handhold on the Exposure Facility of the JEM-ISS. After more than 1 year, Capture
328 Panels will be transferred to the pressurized area through air lock. Upon pressurization of the air lock,
329 air-bone microbial cells may contaminate any tracks present and the surface of aerogel. We have tested
330 the ability of an aerogel to withstand re-pressurization as the hypervelocity experiment described herein,
331 which was performed under vacuum, whereas the subsequent examination of the aerogel was performed
332 at atmospheric pressure. In addition, we found that microbial contamination on the tracks to be less than
333 the detection limit (Fig. 6 and 7a). An aluminum cover will be placed on the Capture Panel system to
334 protect the aerogel, placed in the sterile Ziploc bags and returned to Earth. We will open the bags in a
335 clean room. Next, we will recover the portion of the aerogel surrounding tracks. Tracks and entrance
336 holes will be visualized because aerogel is transparency. Then we cut the gel so that the tracks were cut
337 lengthwise along their interior and used one half tract for fluorescent imaging. The other half of the
338 aerogel that contains the other half-track interiors is reserved for DNA analysis. Finally, we will stain the
339 tracks with SYBR Green I and perform fluorescence microscopy to assess if any microbial cells are
340 associated with particles that caused the tracks.
341
342 ***Conclusion***

We prepared particles of *D. radiodurans*-containing Lucentite that were shot at an aerogel in an LGG apparatus at 4.2 km/s. The fluorescence of the SYBR Green I-stained microbial DNA could be distinguished from non-biological fluorescence by fluorescence imaging. These results indicate that we can use the developed protocol to identify any microbes found in the aerogel situated at the LEO altitude. We are now establishing methods to isolate microbial DNA from an aerogel and identify the species from which it was obtained by PCR assessment of small-subunit rRNA.

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