Assessing the Efficacy, Robustness and Safety of Spacecraft Surfaces Incorporating Pylote SAS Mineral Oxide Antimicrobial Technology

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Abstract

Spaceflights induce unique environmental conditions such as cosmic radiation, microgravity, and temperature fluctuations that alter microbial behavior and the human microbiome. These changes include enhanced virulence, increased biofilm formation, and elevated antimicrobial resistance, posing challenges to astronaut health. Microorganisms transferred from Earth to spacecraft can adapt to these conditions, leading to increased pathogenicity. Current antimicrobial measures, including surface disinfectants, can be hazardous and show limited long-term effectiveness against these adapted microorganisms, highlighting the need for novel solutions for microbial control in space environments. This study evaluated the antimicrobial efficiency of Pylote SAS inert mineral oxide microspheres incorporated into varnishes and resins under conditions in line with spaces use to provide a long-term solution for microbial control in spacecraft environments whatever the surfaces materials. The antimicrobial efficacy, robustness, and safety of samples, with and without Pylote technology, were tested using standardized methods. The antimicrobial activity was assessed according to ISO 22196, comparing Pylote-treated samples to controls against *Escherichia coli*, *Staphylococcus epidermidis*, and *Bacillus subtilis*, selected through the MATISS program to represent relevant microorganisms in space environments. The technology's robustness

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test was undertaken under simulated real-use conditions through 1,352 cleaning cycles using chlorhexidine-based wipes. Cytotoxicity was evaluated using ISO 10993-5 standard based on XTT assay regarding leachable substances. Results showed that Pylote-treated samples exhibited superior antimicrobial activity compared to untreated samples, with a log reduction of >2 against the selected strains. After 1,352 cleaning cycles, no bacterial growth was detected after the contact with the Pylote-treated surfaces, confirming the technology's sustainable antimicrobial effect. Cytotoxicity testing revealed no leachable substances at cytotoxic concentrations, ensuring safety for aerospace applications. These findings demonstrate the potential of Pylote' mineral oxide microspheres to provide a innovative, safe, and efficient solution for microbial contamination control on numerous different surfaces, equipment and devices in space environments.

Acronyms and Nomenclature

CFU = Colony forming unit
CNES = Centre National d'Etudes Spatiales
EU = European Union
GC = Gas chromatography
GNB = Gram-negative bacteria
GPB = Gram-positive bacteria
HPLC = High-performance liquid chromatography
ISO = International Organization for Standardization
ISS = International Space Station
JIS = Japanese Industrial Standard
LDPE = Low-density polyethylene
MATISS = Microbial Aerosol Tethering on Innovative Surfaces in the International Space Station
MS = Mass spectrometry
NASA = National Aeronautics and Space Administration
PE = Polyethylene
PEEK = Polyether Ether Ketone
PP = Polypropylene
PVC = Polyvinyl Chloride
ROS = Reactive oxygen species
SAS = Société par Actions Simplifiée
SML = Specific Migration Limits

I. Introduction

The microbial environment in space presents unique challenges for spacecraft integrity and astronaut health[1, 2] . Outer space conditions, characterized by microgravity ranging between 10^{-3} to 10^{-6} g[2-4] , cosmic radiation[3,5] , and altered environmental parameters, significantly impact microbial physiology, behavior, and community dynamics[2-8] . These conditions create selective pressures that drive microbial adaptation, resulting in gene expression modifications[9] and phenotypic changes[10], which can include enhanced virulence and antimicrobial resistance[2, 10-13] . The International Space Station (ISS), orbiting at an average altitude of 400 km in Earth's thermosphere, represents a controlled yet challenging environment where microorganisms experience various stressors[1,2] . Studies have demonstrated that microbial species aboard the ISS undergo significant physiological and genetic adaptations mainly due to the microgravity and cosmic radiation effects[2-5, 8, 10, 14-16].

Biofilm formation in spacecraft environments is of particular concern[2, 9-11]. Multiple Gram-positive and Gram-negative bacterial species demonstrated enhanced aggregation and biofilm formation under low-shear modeled microgravity or simulated microgravity conditions[2, 9, 17-21]. These biofilms can also contribute to material biodegradation, potentially compromising spacecraft structural integrity[22, 23]. The increased resistance of microorganisms in spacecraft environments is a multifaceted issue driven by genetic, physiological, and environmental factors[2]. These drivers include altered gene expression, enhanced biofilm formation, horizontal gene transfer, and unique stress responses, all exacerbated by the microgravity and confined conditions of space habitats[2, 24, 25]. In overextended durations, microorganisms may develop and favor unknown mutations, increased resistance, and enhanced virulence due to repeated disinfectant exposure and adaptation to microgravity conditions[2, 3, 9-11, 26].

Current antimicrobial measures employed on spacecraft, predominantly including surface disinfectants, face limitations in addressing these challenges. Conventional disinfectants require regular reapplication, may be hazardous to handle in the confined spacecraft environment and show diminished effectiveness against microbial communities adapted to space conditions[27-29]. Furthermore, the National Aeronautics and Space Administration (NASA) Science Plan emphasizes understanding the effects of spaceflight on microbial life, processes, and dynamics as critical research priorities[30], highlighting the need for innovative approaches to microbial control in space environments.

The closed system of the ISS has served as a microbial observatory for the past decade, enabling research on microbial adaptation and survivability in space conditions[2]. Maintaining microbial control is particularly challenging as air and water are constantly recycled, and waste is eliminated when a spacecraft departs for Earth every few months[31, 32]. Cleaning operations take two to four hours of the astronaut's time, with a designated day for cleaning when the crew wipes surfaces, vacuums, and collects waste[31, 32]. In microgravity environments, infectious agents are transported through direct contact, but also aerosols, larger water droplets, and free-floating condensates [26, 33], complicating contamination control efforts. [26,33]

Building on these findings, a sustainable, long-term solution for microbial control on spacecraft surfaces is urgently needed and CNES (Centre National d'Etudes Spatiales) with Spaceship FR Project[34,35] is working to find it. Pylote SAS (France) offers a unique opportunity to address all these challenges through its green, patented mineral technology[36,37]**.** This groundbreaking innovation overcomes many of the limitations associated with traditional antimicrobial surfaces. The technology consists of oxide mineral microspheres, which are non-release, non-ionic, non-metallic, and not nanoparticles-based, making them an environmentally friendly and human-safe solution for prevention innovation.

The PYLOTE SAS technology utilizes a one-step clean manufacturing process called Pyrolyse Pulvérisée™[38]. The microspheres are high-purity ceramic particles with a sphericity coefficient of ≥0.75 and a narrow size distribution centered on few micrometers[38]. The antimicrobial activity of these oxide mineral microspheres operates through a non-release mechanism activated by direct contact with microorganisms. The microspheres possess electron donor properties that, upon contact with H_2O and/or O_2, generate reactive oxygen species (ROS), primarily hydroxyl radicals[39]. This mechanism of action is not photo-activated and relies on surface defects in the oxide minerals known as oxygen vacancies[40]. The highly oxidizing hydroxyl radicals produced on the microsphere surface rapidly destroy a wide range of microorganisms, including Gram-positive bacteria (GPB), Gram-negative bacteria (GNB), viruses, and, to a lesser extent, fungi[38, 41]. This reaction occurs within nanoseconds and is effective within a few dozen nanometers of the material's surface[38] (Figure 1).

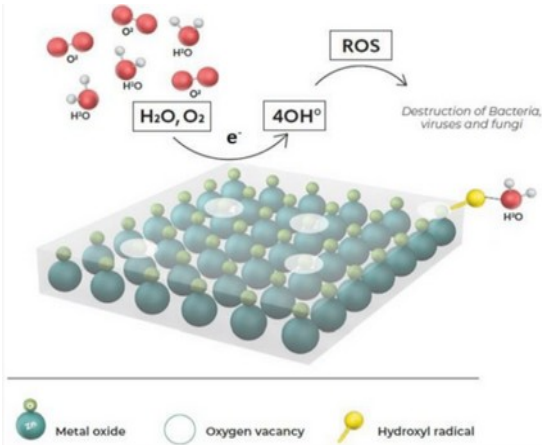

Figure 1. Mechanism of action of oxide mineral microspheres[41]

Microspheres can be integrated into various materials during conventional manufacturing processes without requiring modifications, additional investments, or changes to production lines[41]. Mineral microspheres are authorized as additives for pharmaceutical containers under the European, United States, and Japanese Pharmacopeia standards[38] . This study aims to demonstrate, according to the ISO 22196:2011[42], ISO 10993-05[43], ISO10993-12[44] standards and Regulation EU n°1935/2004 of 27/10/2004[45], the antimicrobial activity efficiency, sustainability, and safety of this breakthrough technology under simulated harsh spacecraft conditions, providing a comprehensive assessment of its potential for long-duration space missions.

II. Material and Methods

The study consisted of a structured seven-phases experimental process to evaluate surfaces with Pylote technology, beginning with matrix selection, technology production, integration, sample production, antimicrobial efficacy testing, sustainability, and safety validation.

A. Step 1: Matrix Selection

Matrix selection for Pylote antimicrobial technology integration was conducted using materials compatible with spacecraft environments. Following industry consultation, matrices were assessed based on space certification requirements, combustibility properties, and functional characteristics. According to the European Space Agency (ESA) standards (ECSS-Q-70), a limited number of polymers are suitable for application in space, including Polyetherimide (PEI), Polypropylene (PP) ,Polyethylene (PE), , and Polyether Ether Ketone (PEEK) were identified as primary polymer candidates due to their prevalent use in space applications, while several materials[46,47], including Polyvinyl Chloride (PVC), were excluded based on space-specific restrictions[48,49]. The paint and varnish matrices testing focused on compatibility with air circulation components and structural elements within the International Space Station (ISS). The final selection criteria prioritized aerospace-grade certification, manufacturing feasibility, and alignment with existing qualified materials.

B. Step 2: Technology Production

The production phase of Pylote technology focused on determining the required quantity of raw materials for both resin and paint-varnish applications. Based on testing requirements, approximately 100 coupons (10cmx10cm) were planned for antimicrobial efficacy testing (Step 5) using the ISO 22196:2011 (JIS Z 2801:2010)[42] protocol, robustness evaluation (Step 6), and safety assessment (Step 7) using ISO 10993-5[43] standard and food contact regulation. The safety testing required approximately 36 cm² of material.

C. Step 3: Integration

The Pylote mineral oxide microspheres were integrated into two distinct matrices. For the resin component, medical-grade LDPE (low-density polyethylene) was chosen as an alternative to aerospace-specific grades due to its comparable rigorous standards for purity, biocompatibility, and performance characteristics. The integration process employed twin-screw extrusion technology, where two parallel counter-rotating screws within a cylindrical chamber facilitated optimal homogenization and uniform distribution of the antimicrobial additive throughout the polymer matrix. Concurrently, for the varnish application, an aviation-compatible acrylic-based varnish formulated for high-contact surfaces was utilized, as aerospace-specific varnish development exceeded project timeline constraints. The integration methodology for the varnish consisted of preliminary sieving of Pylote microspheres followed by incorporation using a mechanical disperser under controlled parameters for mixing speed and duration (Figure 2). Quality assurance protocols included visual inspection of the mixture in the container and at the disperser blade output, with definitive antimicrobial efficacy verification scheduled for subsequent testing in Step 5.

D. Step 4: Sample Production

1. *Low-density polyethylene Samples*

Forty plates (10×10×0.2 cm) were manufactured by injection molding using standard LDPE grade (without Pylote technology) and a compound containing Pylote technology. (Figure 2)

Figure 2. LDPE samples: untreated (left) versus treated (Right) coupons

2. *Varnish Samples*

Varnish application was undertaken using screen printing on an adhesive plastic film. The varnish was applied through a screen-printing mesh, enabling precise thickness and coating coverage control. The varnish was subsequently dried to ensure a resistant and durable finish. In this study, the varnish layer thickness was between 10 µm and 15 µm, with a polyethylene liner protecting the adhesive portion to facilitate sample handling. The varnish samples consisted of multiple layers: adhesive base, varnish coating, and protective liner. Twenty A4 sheets of each varnish formulation were produced, including standard and Pylote-treated varnish (Figure 3).

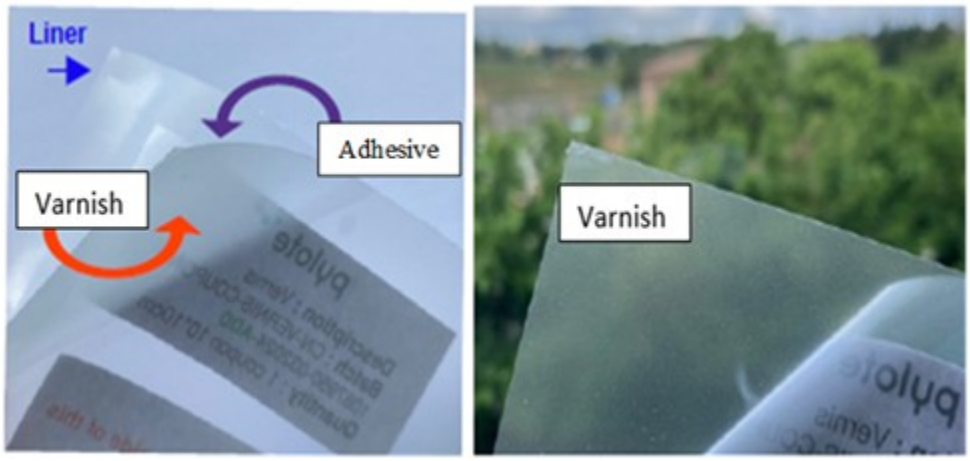

Figure 3.Varnish treated sample

E. Step 5: Antimicrobial Efficiency Testing

The evaluation of the antimicrobial activity followed standardized testing procedures ISO22196:2011 (JIS Z 2801:2010). Microbial inoculum of 10^5 CFU/mL (corresponding to approximately 10^4 CFU/cm²) was applied to test surfaces. A specialized contact recovering film was utilized to ensure proper microbial transfer and recovery for hydrophobic surfaces.

Microbial enumeration was performed in triplicate and reported as log CFU/cm². Two sample groups included control samples without Pylote technology (C) and Pylote-treated assay samples (A). After a 24-hour contact time under standardized conditions, microbial counts were determined for both groups.

The antimicrobial activity was calculated by calculating the logarithmic reduction between the control samples at 24 hours and the Pylote-treated samples at 24 hours (C24h - A24h). Log reduction >2 is considered significant. (Figure 4)

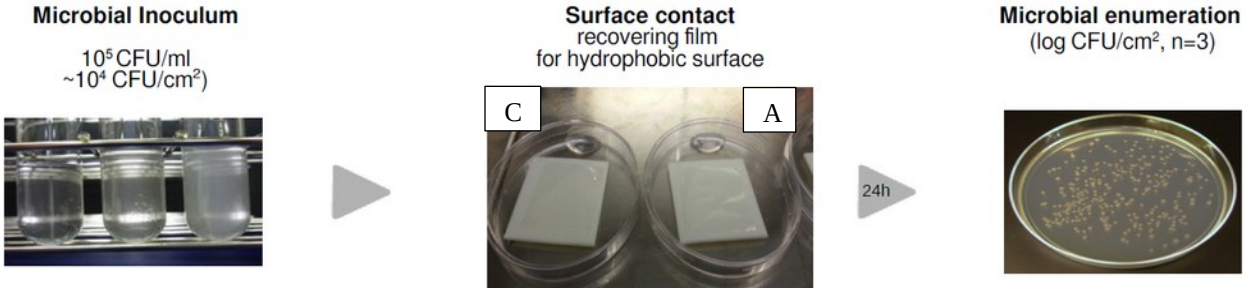

Figure 4. Antimicrobial efficiency testing

F. Step 6: Sustainability Validation

The cleaning procedures routinely performed on ISS surfaces were simulated under the test conditions. The testing protocol encompassed the following steps:

1. *Sample Preparation*

- Two 10cm×10cm LDPE standard plates and two 10cm×10cm LDPE plates with Pylote technology
- Two A4 sheets with standard varnish and two A4 sheets with varnish with Pylote technology
- Identification labels were used on the reverse side of each sample
  Clinell® alcohol-impregnated wipes containing 2% chlorhexidine were utilized for all cleaning procedures

2. *Cleaning Cycle Implementation*

The operator used a chlorhexidine wipe to clean all exposed surfaces of the four LDPE plates and four varnish sheets with a single pass. A drying period of approximately one minute was required between cycles. Subsequent cycles were initiated after complete surface drying. The study was conducted over a two-week period with a maximum of 200 cycles per day.

3. *Antimicrobial Activity Verification*

Prior to laboratory submission for ISO22196:2011 (JIS Z 2801:2010) testing, samples were cut into 5cm×5cm coupons and thoroughly washed with ethanol to remove any chlorhexidine residue. The primary objective of this analysis was to demonstrate retained antimicrobial activity in the samples even after undergoing 1,352 cleaning cycles, simulating the long-term durability expected in space applications.

G. Step 7: Safety validation

1. *Cytotoxicity Evaluation*

The cytotoxicity testing was undertaken according to the ISO 10993-5 standard. The XTT analysis method was applied for this assessment. Sample preparation followed standardized protocols, including appropriate sectioning of non-sterilized samples. Extraction was conducted by incubating the samples in a cell culture medium at 37°C for 24 hours to facilitate the release of potentially toxic substances. Mouse fibroblasts L929 cells (ATCC® CCL-1™, NCTC clone 929 (connective tissue mouse), clone of strain L (DSMZ)) were used due to their high sensitivity to toxins.

L929 cells were incubated with the test extract for 24-26 hours. The resulting concentration corresponded to the ISO 10993-5 and 10993-12 recommendations with surface/volume ratio of 3 cm²/mL (LDPE coupons) and 6 cm²/mL (Varnish coupons). After incubation, XTT reagent was added to the cells. This reagent is reduced by active mitochondrial enzymes in viable cells to form a water-soluble colored product (formazan).

Absorbance measurements were taken using a plate reader at a wavelength of 450-490 nm. The color intensity is directly proportional to the viable cell count. Results were analyzed by comparing the absorbance of test samples with control values (unexposed cells and cells exposed to a positive cytotoxicity control). According to the ISO standard, materials or rather leachable substances from material were classified as cytotoxic if cell viability fell below 70% of the negative control viability.

2. *Food Contact Safety Assessment*

Specific migration testing according to EU Regulation 10/2011 was conducted to evaluate plastic materials intended for food contact applications. These tests ensure that potentially migratory substances from plastic materials do not exceed established safety limits. The testing procedure comprised several key steps:

Substance selection for testing was determined based on specific migration limits (SML) specified in the regulation for substances used in plastic material manufacturing. As Pylote technology is ZnO-based, zinc was selected as the test substance with an established specific migration limit of 5 mg/kg.

Simulated contact conditions were designed to reproduce realistic interactions between plastic materials and foodstuffs, considering temperature, contact duration, and food characteristics (acidic, fatty, aqueous, alcoholic). Standard usage conditions of 2 hours at 20°C were selected for this study.

Rather than using actual foodstuffs, food simulants were employed based on food type and contact conditions. Options include distilled or tap water for aqueous foods, 3% acetic acid for acidic foods, various ethanol

concentrations for alcoholic foods, and vegetable oil or other fatty substances for fatty foods. Acetic acid was selected for our analyses as it represents the most aggressive simulant for metal migration.
The testing procedure involved preparing plastic samples and placing them in contact with food simulants under regulatory-defined conditions (temperature, duration). Samples were incubated in food simulants under specific conditions simulating worst-case usage scenarios. Post-incubation, simulants were analyzed to detect and quantify migratory substances using analytical techniques such as high-performance liquid chromatography (HPLC) and gas chromatography (GC), often coupled with mass spectrometry (MS). Results were evaluated by comparing concentrations of migrated substances in simulants with specific migration limits (SML) established by regulation. Materials with concentrations below SML values were deemed compliant.

III. Results

A. Antimicrobial Efficiency

Table 1 indicated significant antimicrobial efficiency for treated resin and varnish coupons compared with untreated samples, exhibiting substantial reductions of bacterial load across all three tested strains: *Escherichia coli* CIP 53.126, *Staphylococcus epidermidis* CIP 68.21, and *Bacillus subtilis* CIP 52.62 (Institute Pasteur Collection; Paris, France). According to ISO 22196:2011 (JIS Z 2801:2010) standards, a log reduction >2 is considered significant [42].

Table 1. Antimicrobial activity of Pylote technology treated resin and varnish coupons under simulated space conditions

ISO 22196:2011 (JIS Z 2801:2010)				
Bacterial strain	**Inoculum / piece**	**Resin coupons untreated**	**Resin coupons treated**	**Antimicrobial activity (Log CFU/cm²)** (C24h-A24h)
		C24 (Log CFU/cm²)	A24 (Log CFU/cm²)	
E. coli CIP 53.126	$1.02.10^4$	5.98	0.80	**5.18****
S. epidermidis CIP 68.21	$1.20.10^4$	2.86	0	**2.86****
Bacillus subtilis CIP 52.62	$6.1.10^3$	3.39	0.13	**3.26****
Bacterial strain	**Inoculum / piece**	**Varnish coupons untreated**	**Varnish coupons treated**	**Antimicrobial activity (Log CFU/cm²)** (C24-A24)
		C24h (Log CFU/cm²)	A24h (Log CFU/cm²)	
E. coli CIP 53.126	$9.0.10^3$	5.95	0.63	**5.32****
S. epidermidis CIP 68.21	$8.5.10^3$	2.58	0	**2.58****
Bacillus subtilis CIP 52.62	$5.4.10^3$	2.97	0	**2.97****

**Significant reduction with log >2 according to JIS Z 2801 :2010 standard ; C24h : after 24h contact time without Pylote technology ; A24 : after 24h contact with Pylote technology

B. Sustainability Validation

Table 2 showed the sustainable antimicrobial efficiency of resin (LDPE) and varnish samples following 1352 cleaning cycles with Chlorhexidine. Despite thorough ethanol washing of samples post-cleaning regimen, residual Chlorhexidine effects were likely still present on untreated samples. Pylote-treated samples exhibited complete bacterial elimination with no detectable colonies (<1 CFU/cm²). This finding confirms that Pylote antimicrobial technology maintains its efficacy even after extensive cleaning cycles equivalent to 26 years of weekly disinfection protocols on the ISS, demonstrating exceptional durability for long-term applications in challenging environments.

Table 2. Antimicrobial efficiency after 1352 cleaning cycles against *Escherichia coli* CIP 53.126

Sample	CFU/mL	CFU	CFU/cm²	log CFU	log CFU/cm²
Resin samples untreated					
Sample 1	4.00×10^{0}	4.00×10^{1}	2.50×10^{0}	1.60	0.40
Sample 2	8.00×10^{0}	8.00×10^{1}	5.00×10^{0}	1.90	0.70
Sample 3	4.40×10^{1}	4.40×10^{2}	2.75×10^{1}	2.64	1.44
Average				2.05	0.85
Resin samples treated					
Sample 1	<1	<10	<1	1.00	0.00
Sample 2	<1	<10	<1	1.00	0.00
Sample 3	<1	<10	<1	1.00	0.00
Average				1.00	0.00
				Red log	**>0.85**
Varnish Samples untreated					
Sample 1	7.001×10^{2}	7.001×10^{2}	4.381×10^{2}	1.85	0.64
Sample 2	2.501×10^{2}	2.501×10^{2}	1.561×10^{2}	2.40	1.19
Sample 3	1.551×10^{2}	1.551×10^{2}	9.691×10^{2}	6.19	4.99
Average				3.48	2.27
Varnish samples treated					
Sample 1	<1	<10	<1	1.00	0.00
Sample 2	<1	<10	<1	1.00	0.00
Sample 3	<1	<10	<1	1.00	0.00
Average				1	0.00
				Red log	**>2.27**

C. Safety Validation

Table 3 revealed that the cytotoxicity evaluation exhibited distinct outcomes for LDPE and varnish coupons. For the LDPE coupons, there was no significant reduction in cell proliferation or viability, with dehydrogenase activity reduced to 89%. Microscopic examination showed no inhibition of cell growth or cell lysis, indicating minimal cytotoxic effects. In contrast, the varnish coupons exhibited a reduction in cell proliferation and viability, with mitochondrial dehydrogenase activity dropping to 44%. Microscopic observations confirmed reduced cell growth and visible cell lysis, suggesting a higher cytotoxic potential. Control experiments validated the study's integrity, with no significant differences between the solvent control (culture medium) and the negative control (extracted polypropylene). The positive control (extract from latex gloves) demonstrated a marked reduction in cell viability and proliferation, with dehydrogenase activity plummeting to 5%.

Given that the tests on the varnish coupons were conducted on a larger surface and on combined varnish and adhesive sample, it is plausible that the adhesive contributed to the release of toxic substances. However, the favorable results from the LDPE tests affirm the safety of the Pylote technology, as no significant cytotoxic effects were observed in these samples.

Table 3. Cytotoxicity testing of LDPE and Varnish coupons with Pylote technology

Replicates		Blank	Solvent control	Positive control †	Negative control ‡	Test extract § 100% V/V
Resin (LDPE) coupons						
Absorption (A450) (raw data)	1	0.170	0.813	0.210	0.902	0.798
	2	0.184	0.856	0.209	0.898	0.770
	3	0.173	0.853	0.208	0.868	0.781
	4	0.164	0.779	0.214	0.864	0.746
	5	0.202	0.897			
	6	0.182	0.893			
Mean (A450)		0.179	0.849	0.210	0.883	0.774
SD		0.012	0.042	0.002	0.017	0.019
Mean (A450)			0.670	0.031	0.704	0.595
Mitochondrial dehydrogenase activity (%)			100	5	105	89
Varnish coupons						
Absorption (A450) (raw data)	1	0.170	0.813	0.210	0.902	0.408
	2	0.184	0.856	0.209	0.898	0.472
	3	0.173	0.853	0.208	0.868	0.471
	4	0.164	0.779	0.214	0.864	0.471
	5	0.202	0.897			
	6	0.182	0.893			
Mean (A450)		0.179	0.849	0.210	0.883	0.473
SD*		0.012	0.042	0.002	0.017	0.004
Mean (A450)			0.670	0.031	0.704	0.294
Mitochondrial dehydrogenase activity (%)			100	5	105	44

Table 4 showed that the LDPE and varnish coupons with Pylote technology comply with the required standards for food contact under the specified test conditions. For the LDPE coupons, the contact result must be ≤ 5 mg/kg to be compliant, and the LDPE support meets this criterion. Similarly, for the varnish coupons, the contact result must also be ≤ 5 mg/kg, and the varnish coupons satisfy this requirement.

Table 4. Food contact safety of LDPE and Varnish coupons with Pylote technology

Testing conditions	**Results (mg/kg)**			
Acetic acid 3% per immersion 2h/20ºC **LDPE coupons**	**Element**	**Migration limit**	**Detection limit**	**Contact**
	Zn	**5**	**0.6**	**1**
Acetic acid 3% per cell 2h/20ºC **Varnish coupons**	**Zn**	**5**	**0.6**	**5**

IV. Discussion

PYLOTE SAS technology represents a significant advancement in this field, offering an innovative and green long-term solution for microbial control and ensuring the safety and integrity of spacecraft and the health of astronauts. The study showed high efficiency against tested bacteria commonly found in spacecraft environments[26, 45]. These results demonstrate that Pylote technology is a critical tool for enhancing crew safety by reducing the risk of infections and maintaining a hygienic environment.

Previous experiments, MATISS (Microbial Aerosol Tethering on Innovative Surfaces in the International Space Station) 1, 2, and 2.5 provided valuable insights into the behavior of microbial contamination in microgravity through the use of hydrophobic coatings like perfluoro-decyl trichlorosilane (FDTS) [26, 45, 50, 51]. While MATISS experiments have demonstrated the effectiveness of hydrophobic surfaces in repelling water droplets and fine particles, Pylote technology offers a complementary yet distinct approach by actively targeting microorganisms through a non-release, sustainable embedded contact-based mechanism, with high efficiency demonstrated on high-touch surfaces[41].

The technology's innovative design ensures that it can be uniquely integrated into any kind of surfaces, from high-touch areas like handles and equipment to air circulation ducts and storage containers, without requiring significant modifications to existing manufacturing processes[38].

The durability and robustness of the antimicrobial activity were demonstrated under simulated harsh conditions of spacecraft disinfection protocols. These findings ensure that Pylote can provide long-term protection against microbial contamination and transmission, reducing the need for frequent reapplication of disinfectants minimizing the burden on astronauts.

The cytotoxicity testing according to ISO 10993-5 and food contact compliance assessments under EU Regulations No. 1935 and No. 10/2011 confirm that PYLOTE-treated materials are non-toxic and safe for use in environments where they may come into contact with food or drinking water. The findings showed that the technology aligns with the stringent safety standards for space habitats.

Future research should focus on long-term spaceflight testing to validate PYLOTE's performance *in-situ* a full range of environmental conditions encountered during long-duration spaceflight. Expanding microbial testing and conducting longitudinal studies on crew health impacts during extended missions will further position PYLOTE SAS as a leading technology in microbial control, providing safe and hygienic space habitats.

V. Conclusion

Pylote technology showed promising results for microbial control applications in spacecraft environments. Its efficacy, durability, safety, and versatility meet numerous challenges faced in controlled spacecraft conditions, offering potential solutions to pave the way toward a sustainable, green solutions for spaceflight microbial control and astronauts' safety.

References

[1]Castro, S. L., Smith, D. J., & Ott, C. M. (2014). Researcher's Guide to: International Space tation Microbial Research. *National Aeronautics and Space Administration, Johnson Space Center, ISS Program Science Office*, 1-44.

[2]Bijlani, S., Stephens, E., Singh, N. K., Venkateswaran, K., & Wang, C. C. (2021). Advances in space microbiology. *Iscience*, *24*(5). https://doi.org/10.1016/j.isci.2021.102395

[3]Huang, B., Li, D. G., Huang, Y., & Liu, C. T. (2018). Effects of spaceflight and simulated microgravity on microbial growth and secondary metabolism. *Military Medical Research*, *5*, 1-14. https://doi.org/10.1186/s40779-018-0162-9

[4]Herranz, R., Anken, R., Boonstra, J., Braun, M., Christianen, P. C., de Geest, M., ... & Hemmersbach, R. (2013). Ground-based facilities for simulation of microgravity: organism-specific recommendations for their use, and recommended terminology. *Astrobiology*, *13*(1), 1-17. https://doi.org/10.1089/ast.2012.0876

[5]Horneck, G., Klaus, D. M., & Mancinelli, R. L. (2010). Space microbiology. *Microbiology and molecular biology reviews*, *74*(1), 121-156. https://doi.org/10.1128/mmbr.00016-09

[6]Checinska, A., Probst, A. J., Vaishampayan, P., White, J. R., Kumar, D., Stepanov, V. G., ... & Venkateswaran, K. (2015). Microbiomes of the dust particles collected from the International Space Station and Spacecraft Assembly Facilities. *Microbiome*, *3*, 1-18. https://doi.org/10.1186/s40168-015-0116-3

[7]Checinska Sielaff, A., Urbaniak, C., Mohan, G. B. M., Stepanov, V. G., Tran, Q., Wood, J. M., ... & Venkateswaran, K. (2019). Characterization of the total and viable bacterial and fungal communities associated with the International Space Station surfaces. *Microbiome*, *7*, 1-21. https://doi.org/10.1186/s40168-019-0666-x

[8]Senatore, G., Mastroleo, F., Leys, N., & Mauriello, G. (2018). Effect of microgravity & space radiation on microbes. *Future microbiology*, *13*(7), 831-847. https://doi.org/10.2217/fmb-2017-0251

[9] Wilson, J. W., Ott, C. M., Zu Bentrup, K. H., Ramamurthy, R., Quick, L., Porwollik, S., ... & Nickerson, C. A. (2007). Space flight alters bacterial gene expression and virulence and reveals a role for global regulator Hfq. *Proceedings of the National Academy of Sciences*, *104*(41), 16299-16304. https://doi.org/10.1073/pnas.0707155104

[10] Zea, L., Larsen, M., Estante, F., Qvortrup, K., Moeller, R., Dias de Oliveira, S., ... & Klaus, D. (2017). Phenotypic changes exhibited by E. coli cultured in space. *Frontiers in Microbiology*, *8*, 1598. https://doi.org/10.3389/fmicb.2017.01598

[11]Vaishampayan, A., & Grohmann, E. (2019). Multi-resistant biofilm-forming pathogens on the International Space Station. *Journal of biosciences*, *44*, 1-5. https://doi.org/10.1007/s12038-019-9929-8

[12]Wilson, J. W., Ott, C. M., Quick, L., Davis, R., zu Bentrup, K. H., Crabbé, A., ... & Nickerson, C. A. (2008). Media ion composition controls regulatory and virulence response of Salmonella in spaceflight. *PloS one*, *3*(12), e3923. https://doi.org/10.1371/journal.pone.0003923

[13]Urbaniak, C., van Dam, P., Zaborin, A., Zaborina, O., Gilbert, J. A., Torok, T., ... & Venkateswaran, K. (2019). Genomic characterization and virulence potential of two Fusarium oxysporum isolates cultured from the International Space Station. *Msystems*, *4*(2), 10-1128. https://doi.org/10.1128/msystems.00345-18 https://doi.org/10.1128/msystems.00345-18

[14]Knox, B. P., Blachowicz, A., Palmer, J. M., Romsdahl, J., Huttenlocher, A., Wang, C. C., ... & Venkateswaran, K. (2016). Characterization of Aspergillus fumigatus isolates from air and surfaces of the international space station. *Msphere*, *1*(5), 10-1128. https://doi.org/10.1128/msphere.00227-16

[15]Gilbert, R., Torres, M., Clemens, R., Hateley, S., Hosamani, R., Wade, W., & Bhattacharya, S. (2020). Spaceflight and simulated microgravity conditions increase virulence of Serratia marcescens in the Drosophila melanogaster infection model. *npj Microgravity*, *6*(1), 4. https://doi.org/10.1038/s41526-019-0091-2

[16]Nickerson, C. A., Ott, C. M., Mister, S. J., Morrow, B. J., Burns-Keliher, L., & Pierson, D. L. (2000). Microgravity as a novel environmental signal affecting Salmonella enterica serovar Typhimurium virulence. *Infection and immunity*, *68*(6), 3147-3152. https://doi.org/10.1128/iai.68.6.3147-3152.2000

[17]Kim, W., Tengra, F. K., Young, Z., Shong, J., Marchand, N., Chan, H. K., ... & Collins, C. H. (2013). Spaceflight promotes biofilm formation by Pseudomonas aeruginosa. *PloS one*, *8*(4), e62437. https://doi.org/10.1371/journal.pone.0062437

[18]Wang, H., Yan, Y., Rong, D., Wang, J., Wang, H., Liu, Z., ... & Han, Y. (2016). Increased biofilm formation ability in Klebsiella pneumoniae after short-term exposure to a simulated microgravity environment. *Microbiologyopen*, *5*(5), 793-801. https://doi.org/10.1002/mbo3.370

[19]Lynch, S. V., Mukundakrishnan, K., Benoit, M. R., Ayyaswamy, P. S., & Matin, A. (2006). Escherichia coli biofilms formed under low-shear modeled microgravity in a ground-based system. *Applied and environmental microbiology*, *72*(12), 7701-7710. https://doi.org/10.1128/AEM.01294-06

[20]Orsini, S. S., Lewis, A. M., & Rice, K. C. (2017). Investigation of simulated microgravity effects on Streptococcus mutans physiology and global gene expression. *npj Microgravity*, *3*(1), 4. https://doi.org/10.1038/s41526-016-0006-4

[21]Castro, S. L., Nelman-Gonzalez, M., Nickerson, C. A., & Ott, C. M. (2011). Induction of attachment-independent biofilm formation and repression of Hfq expression by low-fluid-shear culture of Staphylococcus aureus. *Applied and Environmental Microbiology*, *77*(18), 6368-6378. https://doi.org/10.1128/AEM.00175-11

[22]Novikova, N. D. (2004). Review of the knowledge of microbial contamination of the Russian manned spacecraft. *Microbial ecology*, *47*, 127-132. https://doi.org/10.1007/s00248-003-1055-2

[23]Klintworth, R., Reher, H. J., Viktorov, A. N., & Bohle, D. (1999). Biological induced corrosion of materials II: new test methods and experiences from MIR station. *Acta astronautica*, *44*(7-12), 569-578. https://doi.org/10.1016/S0094-5765(99)00069-7
[24]Aunins, T. R., Erickson, K. E., Prasad, N., Levy, S. E., Jones, A., Shrestha, S., ... & Chatterjee, A. (2018). Spaceflight modifies Escherichia coli gene expression in response to antibiotic exposure and reveals role of oxidative stress response. *Frontiers in microbiology*, *9*, 310. https://doi.org/10.3389/fmicb.2018.00310
[25]Lynch, S. V., Mukundakrishnan, K., Benoit, M. R., Ayyaswamy, P. S., & Matin, A. (2006). Escherichia coli biofilms formed under low-shear modeled microgravity in a ground-based system. *Applied and environmental microbiology*, *72*(12), 7701-7710. https://doi.org/10.1128/AEM.01294-06
[26]Lemelle, L., Rouquette, S., Mottin, E., Le Tourneau, D., Marcoux, P. R., Thévenot, C., ... & Place, C. (2022). Passive limitation of surface contamination by perFluoroDecylTrichloroSilane coatings in the ISS during the MATISS experiments. *npj Microgravity*, *8*(1), 31. https://doi.org/10.1038/s41526-022-00218-3
[27]Marra, D., Ferraro, R., & Caserta, S. (2024). Biofilm contamination in confined space stations: reduction, coexistence or an opportunity?. *Frontiers in Materials*, *11*, 1374666. https://doi.org/10.3389/fmats.2024.1374666
[28]Li, L., Fu, Y., & Liu, H. (2019). Development of effective and safe compound disinfectant for space cabins. *Acta Astronautica*, *159*, 480-485. https://doi.org/10.1016/j.actaastro.2019.01.037
[29]Mora, M., Mahnert, A., Koskinen, K., Pausan, M. R., Oberauner-Wappis, L., Krause, R., ... & Moissl-Eichinger, C. (2016). Microorganisms in confined habitats: microbial monitoring and control of intensive care units, operating rooms, cleanrooms and the International Space Station. *Frontiers in microbiology*, *7*, 1573. https://doi.org/10.3389/fmicb.2016.01573
[30]Tomko, D., Souza, K., Smith, J., Mains, R., Sato, K., Levine, H., ... & Zeituni, A. (2016). Space Biology Science Plan 2016–2025. *NASA*.
[31]Deep cleaning the International Space Station. Avilable at : https://cmmonline.com/news/deep-cleaning-the-international-space-station#:~:text=Astronauts%20on%20the%20International%20Space,surfaces%2C%20Space.com%20reports
[32]Recycling in Space: Waste Handling in a Microgravity Environment Challenge. Available at: https://www.nasa.gov/missions/station/recycling-in-space-waste-handling-in-a-microgravity-environment-challenge/#:~:text=Astronauts%20can%20process%20small%20pieces,use%20or%20vent%20as%20needed.
[33]Ott, C. M., Bruce, R. J., & Pierson, D. L. (2004). Microbial characterization of free floating condensate aboard the Mir space station. *Microbial ecology*, *47*, 133-136.
[34]CNES, URL.: https://cnes.fr/en/projects/spaceship [accessed March 01, 2025]
[35]Navarro, G., "Spaceship FR Project: How to interface with the different actors to contribute to Space Exploration and Human Spaceflight?", ICES-2024-332.
[36]Marchin, L. Individualised Inorganic Particles. Patent WO2015170060(A1), 2 November 2015.
[37]Marchin, L. Use of Materials Incorporating Microparticles for Avoiding the Proliferation of Contaminants. Patent WO2015197992, 30 December 2015.
[38]Feuillolay, C., Haddioui, L., Verelst, M., Furiga, A., Marchin, L., & Roques, C. (2018). Antimicrobial activity of metal oxide microspheres: an innovative process for homogeneous incorporation into materials. *Journal of Applied Microbiology*, *125*(1), 45-55. https://doi.org/10.1111/jam.13752
[39]Brown Jr, G. E., Henrich, V., Casey, W., Clark, D., Eggleston, C., Andrew Felmy, A. F., ... & Zachara, J. M. (1999). Metal oxide surfaces and their interactions with aqueous solutions and microbial organisms. Doi: 10.1021/cr980011z
[40]Applerot, G., Lipovsky, A., Dror, R., Perkas, N., Nitzan, Y., Lubart, R., & Gedanken, A. (2009). Enhanced antibacterial activity of nanocrystalline ZnO due to increased ROS-mediated cell injury. *Advanced Functional Materials*, *19*(6), 842-852.
[41]Iskandar, K., Pecastaings, S., LeGac, C., Salvatico, S., Feuillolay, C., Guittard, M., ... & Roques, C. (2023). Demonstrating the In Vitro and In Situ Antimicrobial Activity of Oxide Mineral Microspheres: An Innovative Technology to Be Incorporated into Porous and Nonporous Materials. *Pharmaceutics*, *15*(4), 1261. https://doi.org/10.3390/pharmaceutics15041261
[42]*ISO 22196*; 2011—Measurement of Antibacterial Activity on Plastics and Other Non-Porous Surfaces. ISO—International Organization for Standardization: Geneva, Switzerland, 2011.
[43]10993-5, I. S. O. I. (2009). Biological evaluation of medical devices—part 5: tests for in vitro cytotoxicity. https://www.iso.org/standard/36406.html
[44]ISO10993-12. (2021). Biological evaluation of medical devices—part 12: Sample preparation and reference materials. https://www.iso.org/standard/75769.html
[45]Karamfilova, E. (2016). Food contact materials-regulation (EC) 1935/2004. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:32004R1935
[46] Rinaldi, M., Cecchini, F., Pigliaru, L., Ghidini, T., Lumaca, F., & Nanni, F. (2020). Additive manufacturing of polyether ether ketone (PEEK) for space applications: A nanosat polymeric structure. *Polymers*, *13*(1), 11. https://doi.org/10.3390/polym13010011
[47]Kiefer, R. (2011). *Polymeric materials with additives for durability and radiation shielding in space* (No. NF1676L-14040).

[48]Stepnov, A. A., Lopez-Tavera, E., Klauer, R., Lincoln, C. L., Chowreddy, R. R., Beckham, G. T., ... & Vaaje-Kolstad, G. (2024). Revisiting the activity of two poly (vinyl chloride)-and polyethylene-degrading enzymes. *Nature Communications*, *15*(1), 8501. https://doi.org/10.1038/s41467-024-52665-z
[49]Pedley, M. D. (2014). *Flammability Configuration Analysis for Spacecraft Applications* (No. JSC-CN-34581).
[50]Lemelle, L., Campagnolo, L., Mottin, E., Le Tourneau, D., Garre, E., Marcoux, P., ... & Place, C. (2020). Towards a passive limitation of particle surface contamination in the Columbus module (ISS) during the MATISS experiment of the Proxima Mission. *npj Microgravity*, *6*(1), 29. https://doi.org/10.1038/s41526-020-00120-w
[51]Sethi, S. K., & Manik, G. (2018). Recent progress in super hydrophobic/hydrophilic self-cleaning surfaces for various industrial applications: a review. *Polymer-Plastics Technology and Engineering*, *57*(18), 1932-1952. https://doi.org/10.1080/03602559.2018.1447128