ASTROBIOLOGY SCIENCE COLLABORATION

Interim Report

JPL Task 1041

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A. OBJECTIVES

The objective of this project is to strengthen the scientific research that supports the development of an in situ miniature Focused Ion Beam Secondary Ion Mass Spectrometer (FIB-SIMS), which is supported for the first year of the Astrobiology Science and Technology Instrument Development (ASTID) program. The first phase consists of a feasibility study of the potential of FIB-SIMS analysis of geobiological samples relevant to problems in astrobiology. We are looking for elemental ratios of specific elements (e.g. C, N, P, S and trace metals) in conjunction with high concentrations of Al and Si. Potential biosignatures will be identified by anomalies in the relationships between these elements that are not predicted by chemistry or geology. The second phase (recently funded by the ASTID program) consists of the development of standards for the materials to be analyzed since SIMS is highly dependent on standards due to matrix effects and surface topography. The third phase will consist of the definition of a breadboard in situ miniature FIB-SIMS instrument.

The current work supports the first and second phases by providing analyses of terrestrial samples of interest to astrobiology and standardized elemental analyses of these same samples. We have been investigating samples of rock varnish using the Environmental Scanning Electron Microscope (ESEM) with qualitative Energy Dispersive X-ray Spectroscopy (EDX) at JPL and Electron Probe Microanalysis (EPMA) at JSC. In recent years, the study of rock varnish has become important to astrobiology for two reasons: 1) Rock varnish has been hypothesized to exist on Mars, based on images from the Viking landers and Mars Pathfinder [1, 2]. 2) Rock varnish has been shown to grow in both hot and cold terrestrial deserts and semi-arid regions, and thus provides a potential Martian analog environment for microbial life. Little is known about the mechanisms by which varnish is formed despite almost three decades of research employing both microanalytical and biological techniques [3,4].

B. PROGRESS AND RESULTS

Samples of rock varnish from the Cima volcanic flows in the Mojave Desert and the Whipple Mountains south of Death Valley were collected and cut with a diamond saw to reveal a cross-section of the very thin varnish layer that has grown at the surface of the substrate. Raw samples were used for ESEM analysis with qualitative EDX, and samples were sent to JSC for extensive preparation for EPMA.

1. Environmental Scanning Electron Microscopy

This work is required to pre-identify regions of interest for FIB-SIMS analysis in terms of their composition and morphology at the micron scale. This characterization is required since no analytical FIBs with SIMS capability are known to have the in situ SEM capability needed to identify these regions of interest. Hopefully, this state of affairs will change in the near future through our discussions with the major FIB manufacturers. The lack of this combination requires that the sample be milled while imaging and characterizing the surface, a process that damages the very features we wish to identify. The ESEM is also ideally suited to the analysis of poorly conducting materials, due to the higher operating pressures available. However, EDX is only a qualitative technique, requiring some care in interpretation of results.

Dr. Susanne Douglas has used the ESEM with an energy-dispersive X-ray spectrometer (EDS) to characterize the composition and morphology of the layers in the rough-cut samples of rock varnish. Layering was seen on the scale of several millimeters, including vesicles populated with chasmolithic bacteria (Figure 1). Silica also appears to be deposited on the surfaces of these pores. An EDX spectrum of the cells is compared with an EDX spectrum of the minerals in the sub-varnish region in Figure 2. Some elements, such as C, Na, S, Cl are consistently present in the biological material but not in the host material. In addition, tendril-like formations were observed on the surfaces of the varnish, that appeared to be the remains of upwelling of material from below the surface. Similar features were seen during FIB analyses and were worn away by the ion beam to reveal cracks in the varnish (Figure 3). This is a new observation and may indicate that the varnish is actually formed from processes occurring below the surface of the host rock. Microcolonial fungi in various states of mineralization were also observed (Figure 4).

Figure 1. Backscattered electron image showing details of a typical “vesicle” in the porous region of the rock immediately underneath the varnish coating. Within the vesicle there are chains of chasmolithic bacterial cells (dark material—see especially just above the left side of the scale bar) and diverse crystalline mineral grains. EDS analysis showed these minerals to be calcium phosphates (long needles) and Fe/Zn/Mn oxides (light grains). This chemistry contrasted sharply with that of the basalt substrate, which had inclusions of Fe oxides and Ba/Ce oxides.

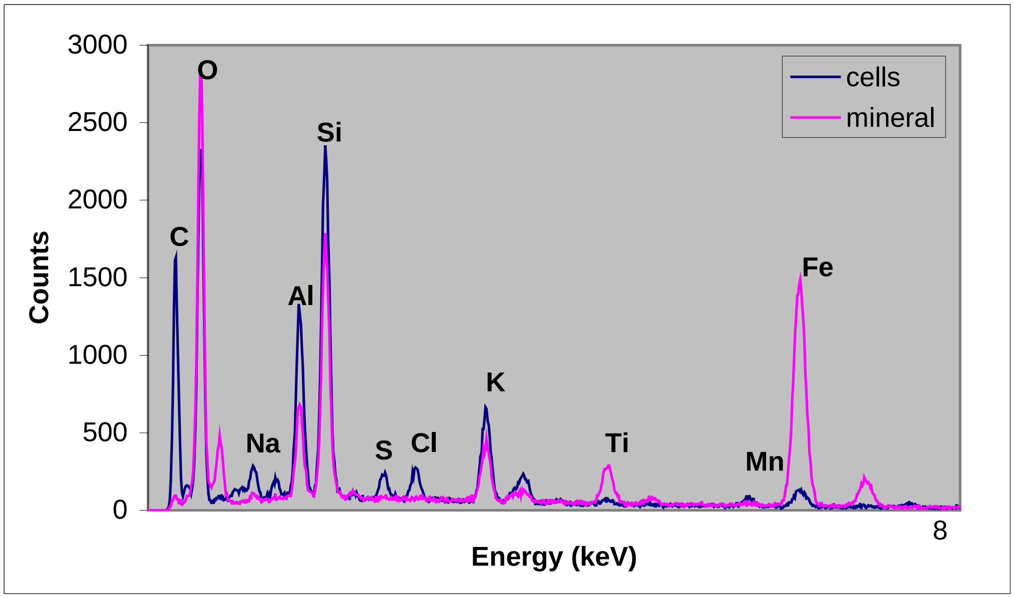


Figure 2. EDS spectrum of cells vs. mineral of sub-varnish region as seen in Figure 2. Some elements, such as C, Na, S, Cl are consistently present in the biological material but not in the mineral.

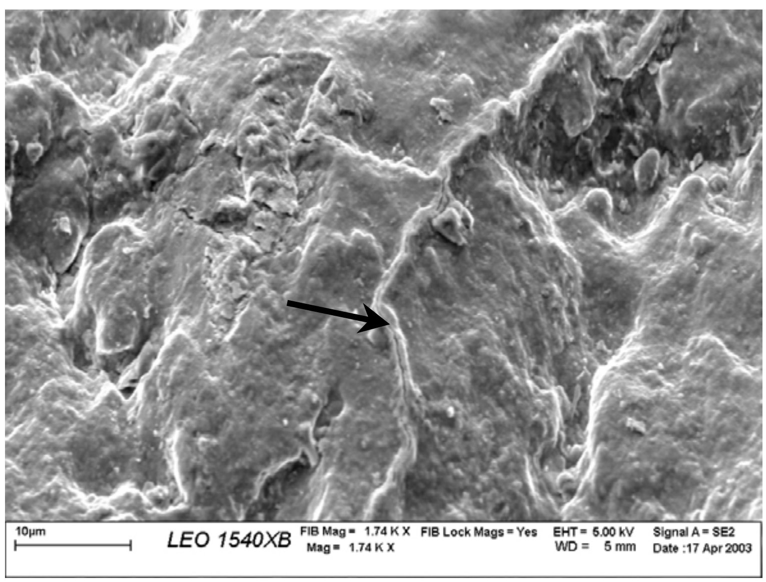


Figure 3. An ion-induced electron image of tendril-like features observed on the surface of rock varnish after damage by ion milling. The arrow indicates an exposed crack under a “tendril”.

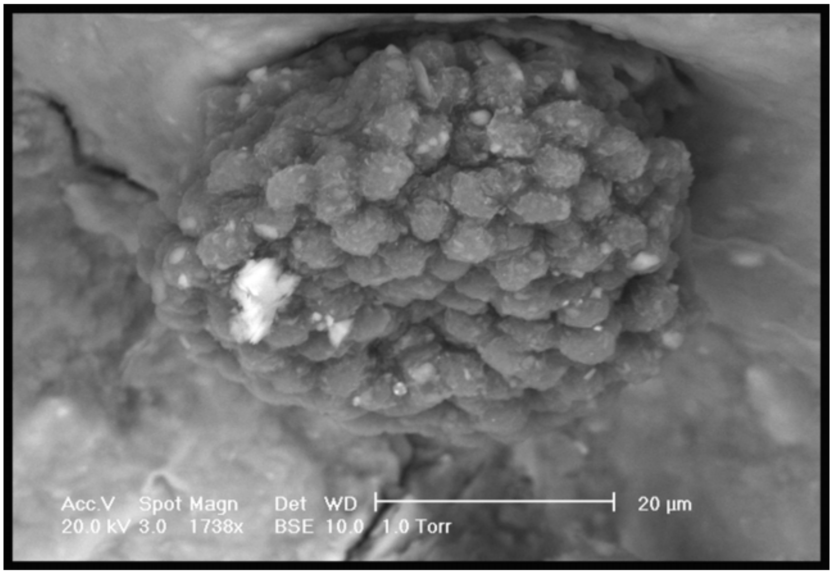


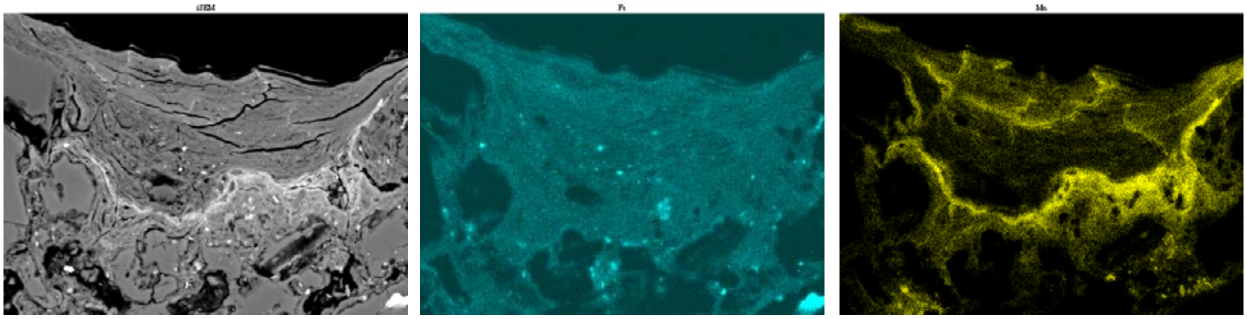
Figure 4. Partially mineralized microcolonial fungi observed on the surface of rock varnish.

2. Electron Probe Microanalysis

Dr. David McKay, NASA Johnson Space Center, has created petrographic thin cross-sections of the varnish samples for analysis using quantitative EPMA. These samples and the data obtained will be used in the currently funded ASTID for optical microscopy and determination of the extent to which the EDX on the ESEM is quantitative. These samples will also form the basis of standardized FIB-SIMS and time-of-flight SIMS (TOF-SIMS) analyses that will be conducted as part of the new ASTID work. Example results of an EPMA analysis of varnish from the Cima volcanic flow are shown in Table 1. An SEM image of this sample with corresponding Fe and Mn images are shown in Figure 5. The spatial resolution of EPMA (like EDX) is limited to approximately 1 micron by the interaction volume of the electrons with the material.

Table 1. An EPMA analysis of a varnish from the Cima volcanic flow.

Component	**Mole Conc.**	**Conc.**	**Units**	
Na_2O	0.765	0.607	wt.%	
MgO	7.099	3.660	wt.%	
Al_2O_3	16.783	21.892	wt.%	
SiO_2	46.524	35.762	wt.%	
P_2O_5	1.827	3.318	wt.%	
Cl	0.106	0.048	wt.%	
K_2O	1.817	2.190	wt.%	
CaO	1.565	1.123	wt.%	
TiO_2	2.640	2.697	wt.%	
MnO_2	14.979	16.659	wt.%	
$Fe2O_3$	5.895	12.043	wt.%	
	100.000	100.000	wt.%	Total



(a) (b) (c)

Figure 5. EPMA analysis of a polished cross-section of rock varnish from the Cima volcanic flow. (a) SEM image, (b) Fe and (c) Mn. A scale bar was not available at the time of this report.

C. SIGNIFICANCE OF RESULTS

Rock varnish is a thin coating (< 500 µm) composed of Mn, Fe and clay minerals that are ubiquitous in deserts [5] and believed by some to exist on Mars [1, 2]. Bacteria have been implicated in the formation of rock varnishes due to the dominance of Mn, but the mechanism of varnish growth remains poorly understood and highly controversial [6]. A recent study of varnish using environmental scanning electron microscopy (ESEM) reports rod-shaped objects within desert varnish with various degrees of degradation [7]. However, it is not clear whether the purported bacterium has a role in the formation of the varnish, or is merely encased in the varnish upon death. Preservation of atmospheric signatures in rock varnish has recently been studied by Bao, et al. (2001) [8]. They concluded that rock varnishes or other surface deposits might provide a record of paleoclimatic information and sulfur biogeochemical cycles. This could be useful for those within the scientific community who have an interest in understanding long-term climate variations.

Our results indicate the possibility of a new mechanism for varnish formation. The appearance of silica and microbes at relatively large distances under the varnished surface, along with the observation of "covered cracks" on the surface of the varnish, force us to consider the possibility that varnish is actually formed by the upwelling of dissolved species from below the surface of the substrate – a "bottom-up paradigm" that has not been considered before. We have not solved the very complicated question of whether or not life is involved in the formation of rock varnish, but we have shown that microbes can exist well below the surface in this extreme, Mars-like environment. When coupled with our ongoing work on the identification and characterization of the metagenome found within varnishes, and correlation of the submicron structure and composition of varnish with dust records found in ice cores (proposed NSF project currently in review), these results and samples will be invaluable in directing future work as well as providing standardized samples for analysis in both the FIB-SIMS and TOF-SIMS.

D. FINANCIAL STATUS

The total funding for this task was $50,000, of which $42,000 has been expended.

E. PERSONNEL

Dr. Robert Anderson (JPL 322) also participated in this work.

F. PUBLICATIONS

[1] Kuhlman, K. R., M. T. La Duc, G. M. Kuhlman, R. C. Anderson, D. A. Newcombe, W. Fusco, T. Steucker, L. Allenbach, C. Ball, and R. L. Crawford (2003) “Preliminary Characterization of a Microbial Community of Rock Varnish from Death Valley, California, ” Third International Mars Polar Science and Exploration Conference, Abstract #8057, Poster Presentation.

G. REFERENCES

[1] Guinness, E.A., R.E. Arvidson, I.H.D. Clark, et al. (1997) "Optical scattering properties of terrestrial varnished basalts compared with rocks and soils at the Viking Lander sites," *Journal of Geophysical Research-Planets*, **102**(E12) 28687-28703.

[2] Israel, E.J., R.E. Arvidson, A. Wang, et al. (1997) "Laser Raman spectroscopy of varnished basalt and implications for in situ measurements of Martian rocks," *Journal of Geophysical Research-Planets*, **102**(E12) 28705-28716.

[3] Dorn, R.I. (1998) *Rock Coatings*. *Developments in Earth Surface Processes*. Vol. 6, Amsterdam: Elsevier pp.

[4] Broecker, W.S. and T.Z. Liu (2001) "Rock varnish: Recorder of desert wetness?," *Geology Today*, **11**(8) 4-10.

[5] Potter, R.M. and G.R. Rossman (1979) "The manganese- and iron-oxide mineralogy of desert varnish," *Chemical Geology*, **25** 79-94.

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[7] Probst, L.W., C.C. Allen, K.L. Thomas-Keprta, et al. (2002) "Desert Varnish - Preservation of Biofabrics and Implications for Mars," in *Lunar and Planetary Science Conference*, Houston, TX: Lunar and Planetary Institute.

[8] Bao, H.M., G.M. Michalski and M.H. Thiemens (2001) "Sulfate oxygen-17 anomalies in desert varnishes," *Geochimica et Cosmochimica Acta*, **65**(13) 2029-2036.