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## DEVELOPMENT OF A BIOCHIP DEDICATED TO PLANETARY EXPLORATION. FIRST STEP: RESISTANCE STUDIES TO SPACE CONDITIONS

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P. Nieminen<sup>8</sup>, L. Dartnell<sup>9</sup>, O. Vandenabeele-Trambouze<sup>10</sup> and G. Coussot<sup>11</sup>

**Abstract.** For upcoming exploration missions, space agencies advocate the development of a new promising technique to search for traces of extant or extinct life: the biochip use. As space is a hazardous environment, a main concern relies on the resistance of this device to a panel of harsh constraints. Within the framework of the BiOMAS (Biochip for Organic Matter Analysis in Space) project, our team is currently developing a biochip especially designed for planetary exploration. We present here the methodology adopted and the beginning experiments to select the best constituents, to determine resistance levels and to define well-adapted protection for the biochip.

### 1 Introduction

Detecting life in the Solar System is one of the great challenges of new upcoming space missions. Several techniques are proposed to detect traces of organic matter on extra-terrestrial objects. A new and promising technique based on biochips, and recommended by space agencies (ESA and NASA), is under study in several countries (Bada et al. 2005; Sims et al. 2005; Steele et al. 2001). A biochip is a miniaturized device composed of biological sensitive systems fixed on a slide. In space conditions, a lot of constraints might alter the efficiency of this analytical tool. Designing a biochip that will be efficient in space conditions requires testing the resistance of all the components of this instrument to space conditions.

With the help of the French space agency (CNES), our team currently develops a biochip especially designed for planetary exploration. The BiOMAS (Biochip for Organic Matter Analysis in Space) project, in progress for two years, has an interdisciplinary dimension bringing together specialists evolving in different area (planetologists, physicists, chemists, biologists and materials specialists) and developing complementary competences. Our aim is to optimize the design of a biochip (including the slide, the ligands and the chemical bonds) that meets the space requirements. As a first step, we have begun several experiments aiming to select the best constituents for our "space biochip", to determine its resistance under several conditions and define, if necessary, some well-adapted protections.

In this paper, we briefly present our current work. Focus is made on the methodology; first results will be presented in forthcoming papers. In section 2, we describe biochip concept and functioning, underlying the

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effort that must be done to adapt it to the harsh space environment. In section 3, we present the methodology used to start studies on the biochip resistance under several space constraints.

## 2 A biochip for space exploration

### 2.1 Description: what is a biochip?

A biochip is a miniaturized device composed of biological sensitive systems fixed on a solid substrate. It allows the quantification of hundreds to thousands of target molecules simultaneously (Maule et al. 2005). The biological systems used are called ligands. Antibodies are the most common on biochips. Each ligand is produced to recognize a specific molecule or family of molecules. The main characteristics of a ligand lie in the high affinity and the high specificity toward its target. In our case, target molecules are organic molecules, which can sign the presence of extant or extinct life: they are commonly called biomarkers (Tang 2007).

Geometrically, a biochip looks like a microscope slide. Ligands are fixed on a substrate, which dimensions are typically:  $75 \times 25 \times 1 \text{ mm}^3$ . Each type of ligand is spotted on the slide and the density of 600 spots per  $\text{cm}^2$  can be reached, proof of the high level of miniaturization. A spot measures approximately 90 to 200  $\mu\text{m}$  in diameter and contains 300 to 400 pL of ligands solution. Ligands are linked to the substrate by chemical reactions, mainly by the creation of a covalent link between the ligand and the slide surface.

### 2.2 Principle: how does it work?

To function, a biochip needs fluids. The first step, previous to the analysis itself, consists in using solvents to extract organic molecules from the solid sample of interest. For the most efficient extraction possible, the sample is first crushed to improve the contact surface with the solvent.

The solution, containing the potential organic molecules extracted, is filtered and placed into contact with the biochip. One important point is to be sure that the solution will pass through all the ligands spots in order to have a chance to detect the molecules of interest (if it contains some). The incubation time is mainly function of the affinity constants of the ligands-biomarker complexes and the experimental conditions (temperature, etc).

The biochip is then washed and analyzed. The detection of the complexes ligand-biomarker can take several forms. One of the most current techniques consists of a fluorescent detection. Each ligand is labeled with a fluorescent dye. The biochip is submitted to a laser excitation at the characteristic wavelength of the dye. If the ligand is linked to its target molecule, a fluorescent signal appears. With this technique, we obtain a visual signal to determine which kinds of molecules are contained in the sample tested. The fluorescence intensity of each spot allows a quantitative analysis.

### 2.3 Using a biochip for space exploration

Nowadays, biochips are mainly used in the medical field. However, the space community feels more and more interested by this new technology which could be an interesting alternative to traditional techniques usually used to search for organic matter.

#### 2.3.1 Advantages

Obviously, one of its main advantages for space missions lies on its high level of miniaturization. This is a very powerful tool, which weights only 4 g for a volume of less than  $2 \text{ cm}^3$ . Another advantage, compared to actual techniques, is the very high sensitivity of the biochip, which relies on the particularity of ligand/biomarker binding. The detection limit can go down to a very low-level concentration.

#### 2.3.2 Our approach

Instead of developing a biochip by adapting current biochip to space requirements, we have decided to study a new concept of biochip by optimizing all the constituents of this analytical tool in respect to space constraints. This work is in progress and will be presented in depth in forthcoming papers. In particular, analytical developments are not discussed in the present paper, which focus only on space constraints.

### 2.3.3 Space mission constraints and potential damaging effects on a biochip

Interplanetary space is a hazardous environment which combines large thermal cycles, extremes temperatures, microgravity, vacuum, radiations, etc. Moreover, a whole space mission adds new constraints such as contamination avoidance, long storage time, sterilization procedures, vibrations and shocks due to launching, landing and transportation. A biochip dedicated to space should take into account all this panel of constraints and its design, obviously, should be adapted compared to actual biochip used on Earth. All those constraints bring a lot of questions.

Extreme temperatures can destroy ligands, modify substrate geometry (expansion/shrinkage) and create residual stresses in the material. The problem of fluids state is also crucial. Vibration and shocks can produce cracks on the substrate and complicate the detection procedure, which needs a very precise positioning. Vacuum can be damaging from a contamination point of view. It can also weaken the substrate.

Considering all those potential damages, we have engaged works on different aspects to determine and quantify, if possible, the impact of harsh conditions on the biochip components.

## 3 Resistance studies: ongoing work

### 3.1 Substrate specifications

Different types of material can be used as a biochip substrate: glass, thermoplastics, elastomers, etc. Therefore, one of our first works was to define which criteria would be relevant to select the best substrate for a “space biochip”.

A specification folder was established, presenting analytical and space constraints criteria. For each criterion, we tried to give the level to reach and the degree of flexibility that can be acceptable. For instance, we consider that the material should resist a temperature range between  $-70^{\circ}\text{C}$  and  $70^{\circ}\text{C}$ . However, this criterion is relatively flexible as we can imagine an insulating system protecting the biochip and reducing the temperature variability.

Here after, we detail works we have already begun on four of these criteria: outgassing behavior, resistance to solvents, thermo-mechanical behavior and radiation resistance.

### 3.2 Outgassing behavior

A majority of materials outgas when they are placed under low pressure and/or high temperatures. As these two parameters are combined during a space mission, it seems relevant, in the design process of a biochip dedicated to space exploration, to take into account the outgassing properties of the substrate. Outgassing process consists in a loss of matter, which evaporates from the solid: gases encapsulated into the material escape, the most volatile elements desorb, etc. This loss of matter can be critical for the functioning of the biochip from a contamination point of view. Elements outgassed can alter ligands performances. They can link with the ligands and create a false signal, they can modify pH conditions necessary to the survivability of the ligands, they can deform ligands structure, etc.

Therefore, in collaboration with the French space agency, we are performing experiments on different types of potential substrate materials. To better know their outgassing behavior will help us to select the most adapted material for our biochip. Moreover, as outgassing rejects can not be completely avoided, our better understanding of the phenomenon will give us the means to lower contamination risks.

### 3.3 Resistance to solvents

To analyze a sample of soil, we need to extract organic molecules that could be present in this sample. The principle consists in crushing the sample, mixing it with a solvent (organic or not) and getting back the solution to analyze it. Therefore, the substrate has to resist organic solvents that could be necessary to extract molecules of interest. We performed a first battery of experiments in IMM (Institut Max Mousseron, Montpellier) to compare the resistance of different materials to solvents generally considered as relevant for analytical chemistry in the context of exobiological investigations.

Several parameters, such as temperature, solvents concentrations, mixing proportions, contact time, etc, can have an influence on the material resistance. Several damages should be checked after the immersion in the solvent: surface degradation, porosity modification, variation of the autofluorescence level, etc. As everybody

knows, thermoplastics are especially sensitive to organic solvents and their behavior, after an exposure to some of them, must be carefully investigated.

### 3.4 Effects of radiations

One of the most critical parameter during a space mission should be the resistance to cosmic rays irradiation. Even if some shielding could be implemented around the device, it is impossible to completely stop all the charged particles: protection can not be perfect. This is the reason why, studying the resistance of the biochip under different levels of irradiation is essential.

In a first step, we decided to simulate the radiation environment a biochip would face during a typical mission to Mars. The objective is to determine particle types and energy ranges the most relevant for experiments on dedicated irradiation beam facilities. With this simulation work, collaboration is born with the Nuclear Studies Center of Bordeaux-Gradignan (CENBG). We worked on a Monte-Carlo simulation tool which allows the simulation of the particles interaction with matter. We focused on ionizing dose calculations and on determining particles fluences around the biochip.

#### 3.4.1 Simulations strategies

We divided our simulations into two distinct phases.

The first phase was the simulation of the interaction between particle fluxes and a rover traveling from Earth to Mars. During this phase, the primary incident spectra interact directly with the rover.

The second phase consisted in simulating irradiation received on Mars. We considered a rover exploring Mars surface during one month. This second phase was more complicated than the first one because of the interaction of the particles with Mars atmosphere and soil. To simplify, and clarify, we adopted a two-step strategy.

First, we aimed to define the particle environment the rover would encounter on Mars surface. Therefore, we simulated the interaction of primary particle fluxes with Mars environment, defining atmosphere and soil parameters. We generated primary incident particles on top of the atmosphere and analyzed particle fluxes above the surface. Both, downward and upward (backscattered) fluxes were taken into account.

Then, we used those recorded fluxes as primary fluxes into a new simulation to calculate the ionizing dose received by the biochip during a one month stay on Mars surface.

This two-step strategy allows us to free from the scale effect. Simulating interactions with a planet needs kilometers square area whereas our biochip is only few centimeters square large. If the simulation is down at once, you need to generate a lot of primary events to hit the biochip at least once: simulations are really too slow and statistics are really poor. A two-step simulation is a good way to save time and improve clarity.

#### 3.4.2 Incident particles

A special care was taken to choose primary incident particle fluxes to use on our simulations. We considered two different types of primary fluxes: Galactic Cosmic Rays (GCR) and Solar Energetic Particles (SEP). GCR consist of charged particles coming from outside of our solar system. Therefore, in our case, the flux can be considered as isotropic. The radiation energy is proportionally opposite to solar activity: when the Sun activity is minimum, GCR energy is maximum, which represents 4 years of the 11 years solar activity cycle. SEP events consist mainly of protons fluxes due to solar flares or coronal mass ejections. They are punctual in time and can last few hours to few days. They constitute one of the most severe environment that space systems should face (particle fluxes are especially deleterious for electronics). The radiation flux is clearly directional and obviously correlated to the Sun activity. It is also function of the distance between the Sun and the location of interest.

As Monte Carlo simulations can be run with only one type of primary particle, we had to select the most abundant particles in the primary incident spectrum in order to be the most representative with the fewest number of simulations.

Thanks to integration calculations, on energy and on flux, of CREME96 particle fluxes (Tylka et al. 1997), we chose to consider protons, alphas, carbon ions and oxygen ions as a representative sample of the global spectrum of GCR. For SPE, protons and alphas are largely majority.

Details and results of these simulations will be published in a forthcoming paper (Le Postollec et al., in preparation).

### 3.4.3 Irradiation experiments objectives

Simulations give us a precise idea of the radiation environment the biochip will encounter. Therefore it drives our future experiments on dedicated beam facilities and helps us to reproduce in the best way fluxes that can be deleterious to the whole system. Our objectives are to determine how the biochip, and especially the biological systems, will evolve under irradiation. Which constituents are the most sensitive? Which modifications will be induced on ligands or substrate properties? What are the thresholds not to overcome to be still functional? Which shielding could be efficient? A lot of questions have to be investigated and we've already managed to obtain beam time in a French laboratory to test some particular conditions.

### 3.5 Thermo-mechanical studies

A biochip placed on space exploration mission will surely face temperature variations and high vibration and shock levels. Studying the thermo-mechanical characteristics of the substrate appears clearly inescapable. We have started thoughts on this point to determine crucial experiments to perform to compare the different materials in the best way, still with the objective to select the most adapted. Minimize expansion, avoid cracks, keep planarity, for instance, appear as key points.

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