Preparation of the Biochip experiment on the EXPOSE-R2 mission outside the International Space Station

F. Vigier [a], A. Le Postollec [b,c,*], G. Coussot [a], D. Chaput [d], H. Cottin [e], T. Berger [f], S. Incerti [g], S. Triqueneaux [h], M. Dobrijevic [b,c], O. Vandenabeele-Trambouze [a]

[a] *Institut des Biomolécules Max Mousseron-IBMM, Centre National de la Recherche Scientifique, Université de Montpellier 1, Université de Montpellier 2, Unité Mixte de Recherche 5247, place E. Bataillon, CC17006, 34095 Montpellier cedex 5, France*
[b] *Univ. Bordeaux, LAB, UMR 5804, F-33270 Floirac, France*
[c] *CNRS, LAB, UMR 5804, F-33270 Floirac, France*
[d] *Centre National d'Etudes Spatiales (CNES), Centre Spatial de Toulouse, 18 Av. Edouard Belin, 31401 Toulouse Cedex 9, France*
[e] *Laboratoire Interuniversitaire des Systèmes Atmosphériques (LISA), UMR CNRS 7583, Université Paris Est Créteil et Université Paris Diderot, Institut Pierre Simon Laplace, France*
[f] *German Aerospace Center, Institute of Aerospace Medicine, D-51147 Cologne, Germany*
[g] *Université Bordeaux 1, CNRS, IN2P3, Centre d'Etudes Nucléaires de Bordeaux Gradignan (CENBG), UMR 5797, Gradignan, France*
[h] *Air Liquide Advanced Technologies, 2 Rue de Clémencière, 38360 Sassenage, France*

Abstract

Biochips might be suited for planetary exploration. Indeed, they present great potential for the search for biomarkers – molecules that are the sign of past or present life in space – thanks to their size (miniaturized devices) and sensitivity. Their detection principle is based on the recognition of a target molecule by affinity receptors fixed on a solid surface. Consequently, one of the main concerns when developing such a system is the behavior of the biological receptors in a space environment. In this paper, we describe the preparation of an experiment planned to be part of the EXPOSE-R2 mission, which will be conducted on the EXPOSE-R facility, outside the International Space Station (ISS), in order to study the resistance of biochip models to space constraints (especially cosmic radiation and thermal cycling). This experiment overcomes the limits of ground tests which do not reproduce exactly the space parameters. Indeed, contrary to ground experiments where constraints are applied individually and in a limited time, the biochip models on the ISS will be exposed to cumulated constraints during several months. Finally, this ISS experiment is a necessary step towards planetary exploration as it will help assessing whether a biochip can be used for future exploration missions.
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Keywords: EXPOSE-R2 mission; ISS; Biochip; Antibodies and aptamers; Space constraints; Cosmic radiation

1. Introduction

Several instruments based on the biochip technology are under development in the framework of planetary exploration, in particular in the context of the search for signs of past life in our Solar System. A biochip is a miniaturized device composed of molecular recognition tools ("affinity receptors") like antibodies or aptamers. It allows the detection of hundreds of different compounds in a single assay. Many antibodies and aptamers have already been produced to detect a wide variety of targets from single molecules (including nucleotides, nucleosides, aminoacids, carbohydrates, etc...) to complex mixtures or whole organisms (Nimjee et al., 2005; Tang, 2007). Two space instruments based on this technology and using antibodies are under development: the Life Marker Chip (LMC) (Sims

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* Corresponding author at: Univ. Bordeaux, LAB, UMR 5804, F-33270 Floirac, France. Tel.: +33 557775682; fax: +33 557776110.
E-mail address: lepostollec@obs.u-bordeaux1.fr (A. Le Postollec).

et al., 2012), and the Signs Of LIfe Detector (SOLID) (Parro et al., 2011).

Biochips are known to be very sensitive tools to detect specific target molecules and biochip sensitivity is related to the presence of functional affinity receptors fixed on a solid substrate. In order to develop a "space biochip", it appears necessary to ensure that these biological receptors will survive a full planetary mission. Interplanetary space is a hazardous environment, which combines wide thermal cycles, extreme temperatures, microgravity, vacuum, severe radiations, etc. Moreover, a space mission implies additional constraints such as contamination risks (need of sterilization procedures), long storage times, vibrations and shocks due to launching, landing and transportation. A biochip dedicated to space should take into account all of those constraints and its design – initially that of a regular biochip as used routinely on Earth – should therefore be adapted. In this context, the BiOMAS (Biochip for Organic Matter Analysis in Space) project proposes to study the feasibility of a space biochip combining antibodies and aptamers (Le Postollec et al., 2007; Baqué et al., 2011a,b).

Some studies have been carried out to test the resistance of a biochip-based instrument to different space hazards like low gravity, energetic particle irradiation, thermal cycling, freeze-drying and long time storage (Maule et al., 2003; Thompson et al., 2006; Le Postollec et al., 2009b; Baqué et al., 2011a,b; de Diego-Castilla et al., 2011; Derveni et al., 2012). The main limitation of these works is that each constraint is generally studied individually and for a limited period of time that is not representative of a real space mission. In particular, the effect of cosmic rays is generally studied at a given energy (or a limited range of energies) and for one type of particle in a single experiment. Only one study has been carried out in conditions closer to space missions ones: some biochip reagents have flown on BIOPAN-6 platform, experiencing LEO (Low Earth Orbit) environment during few days (Derveni et al., 2013). Unfortunately, irradiation conditions encountered during this short time experiment (12 days) were still far from those expected for a mission to Mars. In particular, the ionizing dose received by samples was consequently too low. In order to overcome previous works limitations, we suggest testing the resistance of a biochip model outside the International Space Station (ISS) during a long time period (several months).

An experiment outside the ISS is a relevant test to argue for the use of a biochip on new upcoming space missions for several reasons. Irradiation conditions will be closer to those that the biochip will face during a real mission than conditions usually applied on ground tests. The biological components will be submitted to a combination of cosmic and solar particles with a predominance of protons and accumulated ionizing doses will be in the same order of magnitude than those simulated for a biochip aboard a typical mission to Mars (Le Postollec et al., 2009a; McKenna-Lawlor et al., 2012). Exposure duration will be very long (from 12 to 18 months) and therefore dose debits will be slower than on beam facilities, which can influence the

components behavior. Moreover, along with irradiation, samples will face thermal cycles, launch constraints, vibrations, storage delays, etc. This will be very representative of real conditions and it will give crucial data about their resistance against these different factors to develop a future prototype of biochip for space purposes.

In the present paper, we describe the preparation process for the experiment that we will perform outside the ISS to study the resistance of a biochip-based instrument to space constraints. This experiment will use the EXPOSE-R facility, which is presented in Section 2. Section 3 details the composition and the conditioning of our samples. The experimental preparation procedure for the biochip models is described in Section 4. The list of controls designed to evaluate the relative significance/effect of each space mission parameter is given in Section 5. A particular attention will be paid to cosmic rays. For that reason, dosimeters will be attached to the samples in order to evaluate the total dose accumulated during the mission, as presented in Section 6.

2. The EXPOSE-R facility

2.1. Presentation of the EXPOSE-R facility

EXPOSE-R is an ESA (European Space Agency) facility intended to be located on the International Space Station (ISS). The core facility will be placed outside the ISS on the Universal Platform D (URM-D platform) of the Russian module Zvezda (Fig. 1A). This facility is intended for scientists willing to perform long term exposure of a given biological or chemical compound to open space environment (combination of radiation from the Sun, cosmic particle radiation, vacuum, temperature variations, microgravity, etc...). The EXPOSE-R2 mission (2014) uses the core facility of previous missions (EXPOSE, EXPOSE-R) (see for instance Cottin et al., 2008; Rabbow et al., 2009, 2012; Bryson et al., 2011; Cottin et al., 2012) but with modified or added features. It will accommodate a Russian experiment designed by the Institute for Biomedical Problems (IBMP) of Moscow and three European scientific experiments: BIOlogy and Mars Experiment (BIOMEX), Biofilm Organisms Surfing Space (BOSS) and Photochemistry on the Space Station (PSS). These astrobiology experiments all aim at evaluating the evolution of organic molecules or at measuring to what extent some chemical, biological or biochemical samples are resistant to long term exposure to the space environment. Our "*Biochip*" experiment is part of the PSS experiment.

2.2. Description of EXPOSE-R design

In the frame of EXPOSE-R2 mission, the EXPOSE-R facility consists in three removable trays (Fig. 1B), each one of them being composed of 4 stainless steel sample carriers that are similar to those from previous missions (EXPOSE-E and -R) but include new improvement
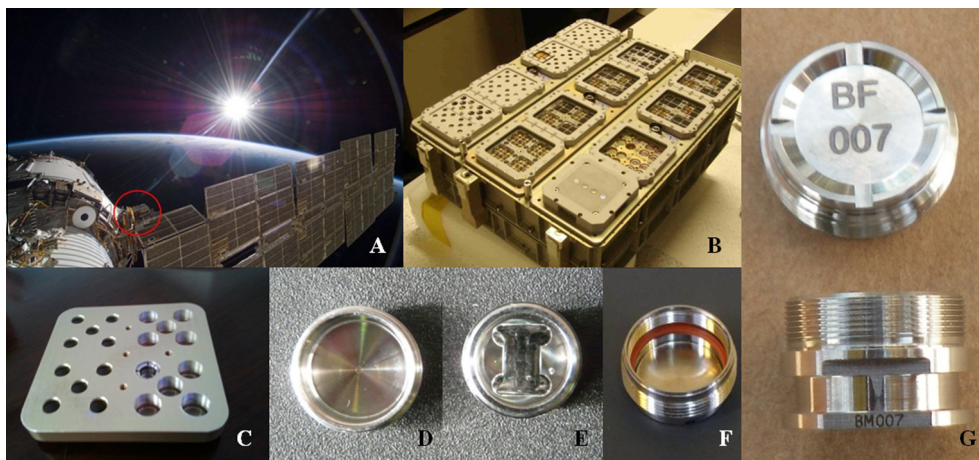
Fig. 1. (A) Location of EXPOSE-R facility outside the ISS (Photo credit: NASA). (B) EXPOSE-R facility with trays integrated. Pictures of (C) a PSS sample carrier and (D), (E), (F), (G) Biochip closed cells. Cell diameter is about 1 cm and, when closed, the cell is about 1 cm height. Both parts of the cell (male and female) are identified (G). An o-ring seal is positioned into the female part to prevent leaks when the cell is closed (F). The bottom of the male part (E) is designed so that two thermoluminescent dosimeters (TLD) can be accommodated under the sample wells.

features. Three sample carriers have been dedicated to the PSS experiment and, on every sample carrier (Fig. 1C), up to 20 samples can be accommodated for the moment. If confirmed by ESA, the final design will allow accommodating 25 samples per sample carrier. The samples are spread into two layers so that they will be irradiated behind two different levels of shielding giving the opportunity to study two different levels of irradiation doses. A cell is a cylindrical stainless steel container of about 1 cm diameter. The closed cells are made of two cylindrical bodies (Fig. 1D, E, G) which can be screwed one into the other with a seal inside the cell (o-ring) to prevent leaks (Fig. 1F). Both parts of the cell have been identified with a laser inscription (Fig. 1G). Sixteen integrally closed cells made of stainless steel will be dedicated to the biochip project (8 cells at each layer). Biochip cells have been designed integrally closed – i.e. without any window – in order to protect samples from UV exposure.

3. ISS sample characteristics

3.1. Description of biochip samples

Cells dedicated to the Biochip experiment contain a biomolecule, either an antibody or an aptamer (Baqué et al., 2011a,b). In terms of space biochip development, those affinity receptors were chosen for their capability to recognize biochemical compounds (amino acids, peptides, proteins, carbohydrates, oligonucleotides, etc.) even when only traces are available, thanks to their high level of specificity and affinity (Nimjee et al., 2005; Tang, 2007). Such detection systems were previously challenged concerning their resistance to radiations, storage and freeze-drying (Le Postollec et al., 2007; Le Postollec et al., 2009a,b; Baqué et al., 2011a,b; De Diego-Castilla et al., 2011; Derveni et al., 2012, 2013). In a real mission, affinity

receptors can be used either grafted on a support or free in solution as in the case of LMC and SOLID projects. Therefore, their resistance to space constraints under these two forms will be studied.

One specific antibody and one specific aptamer were chosen as models in the Biochip experiment. The anti-horseradish peroxidase (HRP) antibody was selected for the following reasons: it is a model antibody whose analysis is easily performed (Baqué et al., 2011a) and well known from biochemists, it is cheap and delivery times are short. The aptamer chosen for the ISS mission is an anti-tyrosinamide DNA aptamer labeled with fluorescein that was previously successfully used to evaluate the irradiation effect (Baqué et al., 2011b).

The samples are freeze-dried during their preparation because it is less challenging to send dry samples to space than samples in solution and also to optimize their stability through time (pre-launch storage and long term mission). Indeed, antibodies are known to be quite stable for long time storage when they are freeze-dried (Chang et al., 2005; Wang et al., 2007).

As it was previously mentioned (Section 2.2), 16 cells will be allocated to the Biochip experiment – spread equally on the 2 exposure levels – so that each sample is present in either 2 or 3 replicates on each exposure level (Fig. 2). All 8 biochip model samples will be gathered at one location on the sample carrier because radiation gradients were observed in previous ISS missions (Berger et al., 2012).

3.2. Description of the sample depositing surface

The samples (antibodies or aptamers) are not placed directly into the steel cell but they are deposited in a polystyrene container called a micro-well (Fig. 3A and B). The polymer surface at the bottom of the micro-well is used for fixing the sample. Also, the micro-well is associated with a
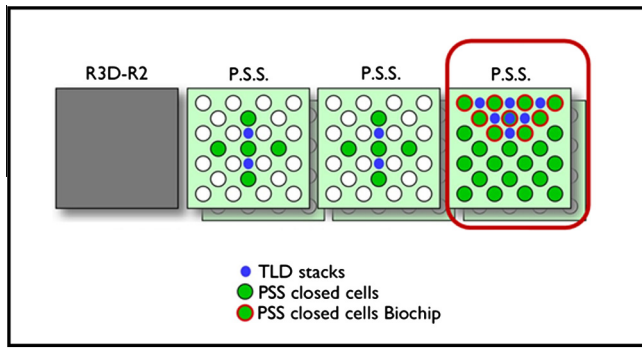
Fig. 2. Configuration of the tray dedicated to PSS experiment. Disposition of biochip cells on the sample carrier (encircled): 8 cells contain samples. The same disposition is applied for the upper and the lower level of the sample carrier. On each level: grafted antibody samples ($n = 3$), free antibody samples ($n = 3$) and free aptamers ($n = 2$). Stacks of thermoluminescent dosimeters (TLD) are located between the biochip cells (see Section 6.3).

Teflon cap and both ensure the sample protection (no contamination) and an optimal recovery (no sample loss).

To fit within the final sample container (the cell), the design of the well has to meet special dimension requirements. When comparing the design of the EXPOSE-R2 cells (Fig. 3C) to that of a regular micro-well on a commercial 96-well plate (Fig. 3A and B), we can see that the well could fit within the closed cell if its height was reduced. We thus use individual micro-wells from commercial strip-well plates (Fig. 3A) to which we perform an additional machining to reduce its height. (Fig. 3B).

3.2.1. Preparation of the sample depositing surface

Many commercial surfaces are available for the grafting of bio-molecules such as antibodies and aptamers, each surface with its own binding properties (covalent bond, adsorption, affinity). The surface is a key parameter in the development of a biochip as it strongly conditions the biochip final properties (sensitivity, stability, etc.) (Moreau et al., 2011, 2013). Several studies demonstrated that the covalent grafting of antibodies using polystyrene microwells covered by N-HydroxySuccinimide ester functions (NHS) is efficient (Baqué et al., 2011a; Moreau et al., 2011, 2013). In addition, Baqué et al. (2011a,b)and Moreau et al. (2013) showed that these surfaces are suitable to

evaluate the biochip performance after exposure to space-related constraints.

Even though we have both samples grafted on a surface (covalent link) or samples free in solution (no link), we chose to use the same well type for all samples for convenient matters. Therefore, NHS functions have to be neutralized for non-grafted samples (free antibodies and free aptamers) to prevent any interaction. We chose to block the NHS functions using BSA before adding the free antibody samples (Fig. 4). For aptamer samples, NHS functions are hydrolyzed rather than covered by BSA in order to avoid any interaction between the positively charged proteins and the negatively charged aptamers.

3.2.2. Control of the machined wells

Using wells with reduced height does not imply a change in common laboratory equipment and procedures. Nevertheless, the machining step that is carried out for well cutting might affect the quality of the commercial surface, whose functions are essential for antibody grafting but strongly sensitive to air exposure (humidity). When NHS-functions hydrolysis occurs, the level of antibody covalent grafting is reduced (Moreau et al., 2011). To assess that machining does not affect surface reactivity, two series of measurements were performed. First, the grafting results of machined surfaces and new surfaces were compared. The amount of grafted antibody is identical indicating that machining doesn't reduce significantly the surface performances (data not shown). Then, intra-batch variability was evaluated to check that surface performances were preserved for all of the batch samples after machining with a number of samples that is consistent with the ISS mission. Two surface features were controlled: the physical properties (for instance, a modification in optical properties could affect blank values) and the biochemical properties (a modification in the surface chemistry could affect the biochip sensitivity). All results (Table 1) show that no general quality loss was observed on any of the functionalized wells after machining so that surface properties are not affected by the machining process (providing that wells do not stay too long in contact with air). Finally we checked that the biochip performances – and especially the percentage of damaged antibody that can be measured – were not
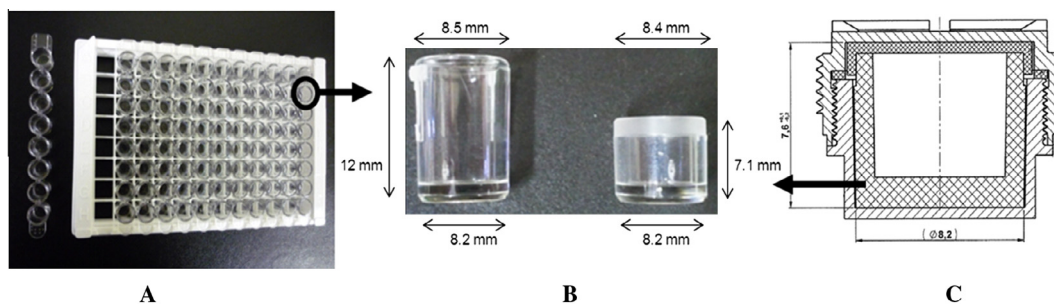

Fig. 3. (A) Picture of a regular micro-plate (Stripwell plate). (B) Well dimensions before and after machining. The whiter part on top of machined well is thinner so that the well can be capped. (C) Cross-sectional drawing of a biochip cell.
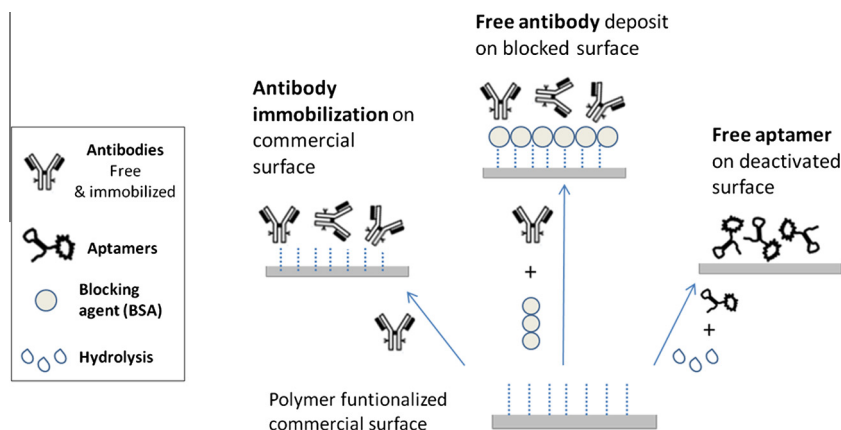
Fig. 4. NHS micro-well surface (bottom) and the 3 uses of its function and reactivity: (1) for antibody covalent grafting (upper left), (2) surface inactivation using a blocking agent (upper middle), (3) surface inactivation using a hydrolysis buffer (upper right). NHS (N-hydroxySuccinimide surface); BSA (bovine serum albumin).

Table 1
Tests on machined wells: physical and biochemical properties. Tests were performed with grafted antibodies for which functionalized surface integrity is essential.

Controlled feature		Description of test	Why test is critical	Result
Physical properties	Visual control	Observe and note any impurities	Process cleanliness conditions both physical and chemical properties	After machining, wells are free of impurities
	Optical properties	Measure absorbance on empty wells	Blank values condition performance test results	All blank values reach normal signals
Biochemical properties: Surface performances	Blocking performance	ADECA[a] (CBB assay)	Blocking agent has a positive effect on antibody samples stability	All wells contain enough blocking agent
	Grafting performance	A2HRP[b] (ELISA assay)	Activity conditions biochip analytical performances	All activity values reach normal signals
Biochemical properties: Analytical performances	Grafting performance	A2HRP[b] (ELISA assay)	Detection levels must not be affected	LOQ level is unchanged after machining

LOQ: limit of quantification, minimum amount of target that can be detected and quantified.
[a] ADECA, Amino Density Estimation by Colorimetric Assay, protocol from Coussot et al., 2011.
[b] A2HRP, Anti HorseRadish Peroxidase Antibody, protocol from Moreau et al., 2011 and Baqué et al., 2011a.

affected by the reduction in sample volume (as implied by height reduction) (data not shown).

For free antibodies and free aptamers, two assays were carried out (data not shown) to control that the use of modified NHS-surfaces gives results identical to those obtained with non activated surfaces as regularly used (Baqué et al., 2011a,b).

NHS surfaces (modified or not) are therefore well adapted for all samples preparation.

4. Experimental procedure for biochip models preparation

Biochip models preparation includes successively well preparation (see Section 4.1), sample preparation (see Section 4.2), freeze-drying step (Section 4.3), and samples conditioning (Section 4.4).

Additional steps that are not performed by our team are briefly presented in Section 4.5.

4.1. Well preparation

This step includes height reduction of the commercial wells, surface preparation (function neutralization) and identification.

4.1.1. Well machining

The functionalized wells used for biochip models preparation are obtained from VWR/Sigma–Aldrich (*DNA Bind Stripwell Plate – N-hydroxysuccinimide modified surface – Costar® Corning*). The machining (height reduction) is performed by Air Liquide (Sassenage, France) as follows: the aluminum pouch containing commercial micro-plates under slight vacuum with neutral gas is opened just before the cutting process. Then, wells are removed from their support, dissociated and cutting process is performed, one well at a time (Fig. 3B). During the whole cutting

process, air humidity content is controlled, and wells are placed on a lint-free paper to prevent any surface degradation (NHS hydrolysis and/or scratching of the well). Once the process is complete, the wells are immediately placed back on their support, then in their pouch which is conditioned with nitrogen gas and heat-sealed. Sixty-six wells have been cut at the same time in order to have the same cutting conditions for wells used for exposed samples, for all controls (see Section 5), for the qualification procedure requested by ESA and for some spares. For the 66 wells needed for this ISS experiment, the whole machining process is performed within two hours.

4.1.2. Well identification

The identification of samples and wells is a critical point that is checked throughout the whole sample preparation and conditioning process in order to prevent results misinterpretation after ISS samples return. Several quality controls are performed in order to prevent well confusion. First, prior to the use of the machined wells, each of them is identified on its circumference using an extra thin permanent marker. Indeed, mechanic printing would be too long for individual identification with regard to chemistry moisture sensitivity. The correspondence between the well, its content and the cell are reported on several procedures that are kept by all partners at different locations, both on printed and electronic documents.

4.2. Sample preparation

Immediately after opening and identifying, wells are submitted to sample preparation (grafted antibodies, free antibodies or aptamers) following their respective procedures.

4.2.1. Grafted antibodies preparation

The following grafting protocol was adapted from Baqué et al. (2011a). Monoclonal anti-peroxidase antibodies are obtained from MyBioSource, USA. PBS (Phosphate Buffer Saline) is obtained from Euromedex, France (10X solution, pH 7.4, 10^{-2}M, diluted in pure water to obtain 1X final solution), BSA (Bovin Serum Albumine, fraction V, 96–100% protein), Tween® 20 and sodium azide are obtained from Sigma–Aldrich, France. The antibody solution at 200 µg/mL is prepared by dilution of the commercial solution (5.6 mg/mL) in a PBS1X buffer containing 0.05% sodium azide. Antibody grafting is performed in one-step (Fig. 4) by adding 100 µL of this solution per well and leaving overnight at room temperature under smooth agitation using a micro-plate agitator (Titramax, VWR, France), and followed with a washing step where wells are washed five times with PBS buffer containing 0.05% Tween®20 (PBST) and twice with PBS. Then, saturation is performed using 150 µL of a 3% BSA solution in PBS with 0.09% sodium azide during 2 h at room temperature and followed by the same washing sequence as for antibodies.

4.2.2. Free antibodies preparation

Free antibody samples are prepared in two steps (Fig. 4). First the surface is blocked and then antibodies are deposited into the wells. BSA is used as the blocking agent in the same way saturation is performed for grafted antibodies. The antibody solution at 200 µg/mL is prepared by dilution of the commercial solution (5.6 mg/mL) directly in the freeze-drying buffer which composition is detailed in Baqué et al. (2011a), and 100 µL are added to each well.

4.2.3. Aptamers preparation

Aptamer samples are prepared in two steps (Fig. 4). First the surface is hydrolyzed and then the aptamers are deposited into the wells. Carbonate buffer (0.1 M, pH 9.4) is used for hydrolysis: 150 µL are added per well and left overnight at room temperature under smooth agitation using a micro-plate agitato. Then wells are washed five times with ultra pure water. Sodium carbonate and sodium bicarbonate are obtained from Acros, France. The aptamer solution at 500 nM is prepared by dilution of the commercial solution in water as described in Baqué et al. (2011b) and 100 µL are added to each well.

4.3. Freeze-drying

Liquid volumes spotted on commercial biochips are so small that water evaporates instantaneously and freeze-drying step is not necessary (like for the majority of micro-arrays studied by de Diego-Castilla et al.). However, as this experiment is performed with wells, the liquid volumes considered are much bigger and freeze drying step appears unavoidable.

4.3.1. Sample preparation for freeze-drying

Antibodies in solution in water suffer during freeze-drying (exposure to very low temperatures and desiccation) (Wang et al., 2007). A freeze-drying buffer has been developed to limit antibodies physical or chemical degradation during the freeze-drying step. Its composition (including components, pH and additives) is described in Baqué et al. (2011a). Aptamers, however, are less sensitive to freeze-drying and they are simply diluted and freeze-dried in ultra-pure water.

Free antibodies and aptamers are diluted directly in their freeze-drying buffer (respectively freeze-drying buffer and ultra-pure water) but for grafted antibodies it is necessary to add the freeze-drying buffer after the saturation step (120 µL per well).

4.3.2. Freeze-drying procedure

This step is performed using a freeze-dryer (Christ Alpha 2–4), a sealing device and an aluminum case that was especially designed for the experiment to guarantee optimal stability of freeze-dried samples without affecting the freeze-drying process (Fig. 5). Indeed, we have observed that a loss of activity occurs after the
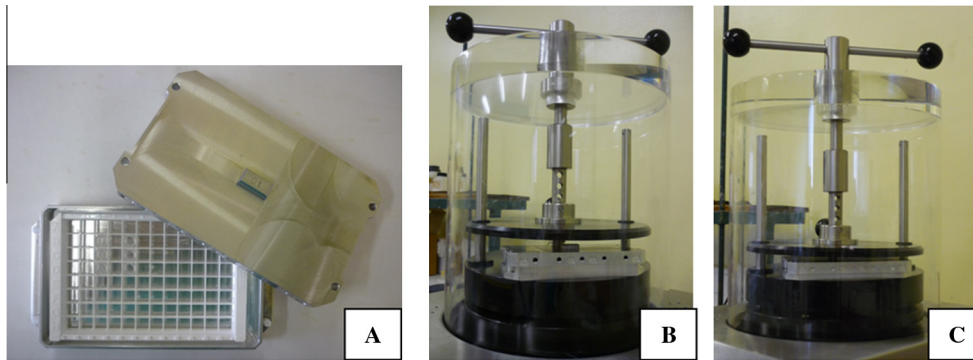
Fig. 5. Pictures of (A) the aluminum case with the sample preparation support; pictures of the sample-containing case within the freeze-dryer, in (B) open or (C) closed configuration; the case is closed by applying a vertical pressure using the sealing device. The whole system ensures both optimal samples freeze-drying in the open configuration and good samples protection with sufficient air-tightness in the close configuration (applied at the end of the cycle).

freeze-drying step which is due to rapid rehydration of the dry samples with air humidity. We thus limit samples damages after freeze-drying by isolating the freeze-dried samples within the closed case before removing them from the interior of the freeze-dryer (up to 15% less degradation).

After being cooled down with the liquid nitrogen, the wells on their support are placed in the case and then on the sealing device shelf in the open configuration (Fig. 5). It is left overnight at −80 °C and 0.05 mbar and then removed from the freeze-dryer as follows: when the freeze-drying process is complete, the chamber of the freeze-dryer is filled with nitrogen gas to limit the contact with humidity-charged air, and using the sealing device, the sample-containing case is shut before removing from the chamber.

4.4. Conditioning

As described hereafter, the samples are conditioned in hermetically closed cells filled with a mixture of helium and argon and fixed on a supporting device that is placed in a tube to secure transportation.

All conditioning tools (well caps, cells, cell screwing socket, cell supporting devices and transport tubes) are manufactured and provided by Air Liquide (Sassenage, France). Cells (both half parts) and transport tubes are engraved with laser for identification. Following delivery in lab, all tools are cleaned and stored in proper conditions that prevent any contamination.

4.4.1. Integration of samples within cells
When removed from the freeze-dryer, the sample-containing case is opened in a glove box pre-equilibrated with a mixture of inert gas made of 10% helium in argon (Air Liquide, France). Such gas mixture was chosen because following its conditioning the cell integrity will be controlled with helium leak tests.

Once inside the box, the freeze-dried samples can equilibrate to room temperature in a dry atmosphere (gas mixture

contains $H_2O < 3$ ppm and, after box equilibration, relative humidity < 15%) so that no condensation occurs.

After opening the case, the sample-containing wells are covered one by one with a Teflon cap using a vacuum suction pen (Fig. 6A). Then the capped wells are picked one at a time and placed in the corresponding cell taking good care to match perfectly the right sample to the right cell according to well and cell respective identification. Note that the cells are opened within the glove box prior to equilibration with gas to ensure that they contain no trace of humidity detrimental for the dry samples. For a more convenient manipulation inside the glove box, fine-tip tweezers are used (Fig. 6B). Following well addition, the cells are closed partly manually (Fig. 6C) and partly using a screwing socket combined with a torque wrench (Fig. 6D). To ensure that they are all screwed properly and identically, the final tightening is performed at a given torque of 0.7 Nm. At that step, the cells are on a supporting device to make their manipulation and shipment easier.

Once all cells are closed, each supporting device is taken out of the glove box and placed within a transport tube which is filled with a mixture of argon and helium and hermetically closed.

4.4.2. Final sample conditioning and storage
Once conditioning is complete, the transport tubes are stored in a fridge at +4 °C waiting for final conditioning.

The final conditioning is performed by Air Liquide by taking out the cells from their tube and welding with laser the male and female parts together in order to make the cell completely tight. Before and after welding, helium leak tests are performed to check for cell integrity. Finally, cell supporting devices are placed back in transport tubes; the tubes are flushed with gas and stored in the lab (Montpellier, France) in a dedicated fridge at +4 °C.

4.5. Data on samples preservation

Several experiments have been performed on ground to ensure that the preparation process preserves grafted
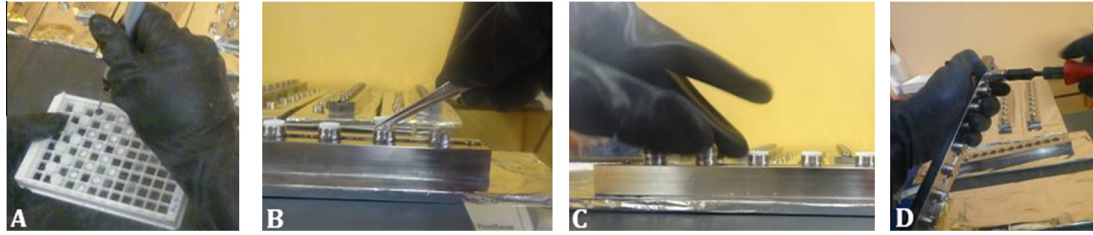
Fig. 6. Pictures of the different steps of cell integration for freeze-dried samples. (A) Covering of every sample-containing well with a cap, (B) integration of capped well to corresponding cell, cells are placed on a supporting tool, (C) manual closing of cells, (D) final closing of cells with proper torque using a dynamometric wrench.

antibodies performances. The behavior of samples has been also studied through long time storage and varying thermal environment.

Grafted antibodies activity loss after the freeze-drying step (using the specific aluminum case) was determined around 20% (5 different tests with 5 repetitions each time). Preservation of samples during long time storage was studied with several samples stored in a fridge at 4 °C. Six tests were performed along one year and they demonstrate that the performance loss remains under 15% after 12 months storage (Moreau et al., 2013). We can notice that the flight samples are stored under inert gas whereas samples tested were just sealed in a plastic bag, therefore the preservation of functionality will certainly be better for flight samples. Thermal shifts from −10 °C to +45 °C were applied to antibodies samples in order to assess their behavior under the different thermal shifts that they could face during transport and manipulation on Earth and during the flight. We measured an activity loss of 16% (Moreau et al., 2013). Thanks to our several studies, we know that we can precisely measure samples activity until 91% of reduction. Therefore, samples are still perfectly acceptable to be exposed to harsh space conditions after the unavoidable activity reduction due to preparation steps, storage and transport.

4.6. Additional steps before loading on the ISS

Before sample delivery to ESA, cells will be transferred from their initial supporting device to the corresponding sample carriers, either ground sample carriers or flight sample carriers. That step is performed at LISA (Laboratoire Interuniversitaire des Systèmes Atmosphériques, Paris, France) under the responsibility of the PSS experiments leader. Sample carriers will be loaded on trays at MUSC – DLR (Microgravity User Support Center, Cologne, Germany) and trays will be closed with the appropriate gas conditions and transported to launch site (Baikonour, Kazakhstan). Finally, samples will be shipped to the ISS using Progress spacecraft. Once there, the trays will be installed into EXPOSE-R core facility and stored within the ISS for a few weeks at maximum. Then, EXPOSE-R will be placed at outer surface of the ISS on the Universal platform D.

5. Controls

5.1. Ground controls

Ground controls correspond to the samples that stay on Earth during the whole ISS mission. They follow the same preparing process than ISS samples in the lab and almost the same transporting process except that they don't go all the way to the launching site but stay in the German Aerospace Center (DLR) after sample uploading on trays. They will thus be used as references when analyzing the ISS samples returning from space. Two types of ground controls are prepared (Table 2). One will be kept at +4 °C, as a reference for the combination of all space constraints. Another control called the mission ground reference (MGR) will be exposed to thermal cycling as close as possible to that of the flight samples. The temperature cycles will be defined based on data collected on the ISS during EXPOSE-R2 mission so that MGR samples undergo the same thermal history as ISS samples, almost instantaneously (data processing time).

5.2. Presentation of lab controls

We chose to have additional controls apart from the 2 ground controls already mentioned. Indeed, due to a limited amount of samples that can be exposed on the ISS, only 16 samples are allocated to the Biochip experiment, equally spread on both exposure levels. Each sample type is thus available in only 2 or 3 replicates (Table 2). It is very important that we are able to interpret the final analysis results despite the low number of samples, hence we need to have a way to control every critical step of the mission.

Two types of lab controls are prepared corresponding to 2 critical steps of the mission: preparation, and transport (Table 2). They are all prepared, stored and transported in parallel to the flight samples so that their analyses give precious information on the flight samples condition at a given moment.

Preparation controls are "t_0" controls which must ensure that samples preparation went well, and that ISS samples quality was not affected by preparation. The results of preparation controls analysis will condition the

Table 2
Sample types and numbers for every mission phase.

		Number of samples		
		Grafted antibodies	Free antibodies	Aptamers
ISS samples	Upper level	3	3	2
	Lower level	3	3	2
Ground controls	MGR (+4 °C)	3	3	2
	MGR (thermal cycling)	3	3	2
Lab controls	Preparation controls	3	3	/
	Transport controls	3	3	/

MGR: mission ground controls.

acceptance or rejection of the prepared set. In case of lot rejection, a new lot will have to be prepared.

Transport controls tell us in what condition the samples are before launching and in what extent they have been affected by transportation throughout all transportation steps from the laboratory to the launching site. The results of transport controls analysis do not condition the following of the mission but have an informative nature.

The controls are only prepared for antibodies samples. Indeed, aptamers are known to be extremely stable to temperature variations (which will be the main transport constraint) and their preparation only includes two steps, thus limiting the risks of degradation (Baqué et al., 2011b). Controls go through the whole preparation procedure together with ISS samples. Preparation controls are analyzed right away after sample preparation. Transport controls are integrated within cells and transports tubes. They are transported to launching site in the same conditions as ISS flight samples and then shipped back to the laboratory for storage. The analysis of transport controls will be performed in parallel to the ISS flight samples, at the end of the mission.

The time course of our experiment, from samples and controls preparation to final analysis is illustrated in Fig. 7.

5.3. Analysis of lab controls

Sample rehydration must be done instantaneously after cell opening. For preparation controls, a biochemical analysis is performed following the protocol published by Baqué et al. (2011a) (antibody direct ELISA or antibody competitive analyses) and by Baqué et al. (2011b) and Ruta et al. (2009) (aptamer analysis). Results obtained with lab control samples are compared to their respective lab reference value: the activity of grafted antibodies must be above 30 ng of HRP and the IC50 (half maximal inhibitory concentration) of free antibodies must be less than 5 μg/ml. Those criteria are based on the following requirement: any result that we wish to analyze must be greater than the limit of quantification (minimum level of interaction that can be detected and quantified). The criteria also include a margin that was determined based on our knowledge of the level of degradation for each critical step. Conclusions drawn from preparation control analyses are generalized to all samples and they condition whether the ISS samples (flight and ground samples) can follow on the mission.
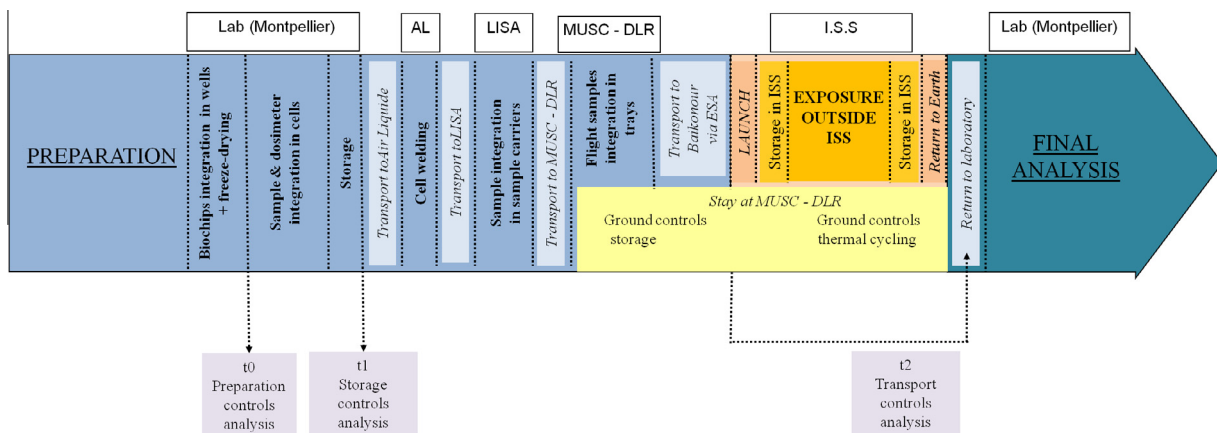

Fig. 7. Time course of the Biochip experiment, from preparation to final analysis (AL = Air Liquide, LISA = Laboratoire Interuniversitaire des Systèmes Atmosphériques, MUSC – DLR = USOC (User Support and Operation Center) of German Aerospace Center).

6. Dosimetry

6.1. Interest of dosimetry measurement for the experiment

One of the main objectives of this experiment is to study the ionizing radiation impact on our samples. Indeed, ionizing radiation can induce local damages to bio-molecular targets (such as DNA) through local energy deposition (see for e.g. Cucinotta et al., 2003). At the International Space Station Low-Earth orbit, the main contribution arises from primary trapped protons and GCRs, as well as from secondary protons and neutrons. X-rays are usually considered as negligible in view of other incident particles fluences (Ersmark, 2006). Fluences in Low-Earth Orbit have been recently simulated by Matthiä et al. (2013b) using a realistic galactic cosmic ray model (Matthiä et al. 2013a) for the dominant species (H, He, C and Fe); the largest fluences are obtained for H and He, while fluences for C and Fe are smaller by several orders of magnitude. A more specific simulation is under development to assess the ionizing dose that the EXPOSE-R facility and the biochip cells will receive during the exposition phase of the present mission. These simulations use the GEANT4 tool (Agostinelli et al., 2003; Allison et al., 2006).

However, simulations are interesting but they need to be confronted with real measurements. Therefore, to assess the impact of ionizing radiation on our samples, it is necessary to characterize as accurately as possible the radiation environment that they will encounter during the mission.

Two types of radiation dosimetry measurements will be performed on the EXPOSE-R platform.

An active sensor package called R3D, for Radiation Risks Radiometer-Dosimeter, will provide some active dosimetry data for the whole platform. R3D instrument is adapted from similar sensor packages used during previous EXPOSE missions (Dachev et al., 2012). It will provide the history of the accumulated radiation dose (μGy/h), the total dose (Gy), the particle flux (particles/cm^2/s) and the particle fluences with different deposition energies.

Thanks to the German Aerospace Center (DLR) expertise in space dosimetry, the Biochip experiment will benefit from passive dosimetry measurements performed as close as possible to our samples. These different dosimetry measurements will be a precious help to interpret results when samples will be back on Earth as they will give data on the

homogeneity of radiations among all the cells (and all the samples). We will have a good assessment of the difference of radiation level between the upper and the lower tray. We will also be able to determine possible gradients in radiation exposures among the cells of a same tray.

6.2. Thermoluminescent dosimeters (TLD)

Thermoluminescent dosimeters will be used to estimate the dose received by samples. These dosimeters are made of ^{7}LiF with Mg and Ti dopants and are provided by Thermo Fisher Scientific, Inc. (Ohio, USA) under the trade name TLD-700. One of the main advantages of those detectors is their small size as they only measure $3.2 \times 3.2 \times 0.9$ mm (Fig. 8).

They will provide the global dose absorbed during the whole mission. Indeed, the dosimeter stores the energy captured from ionizing radiations in the crystal lattice defects, and releases it as light when heated during laboratory readout. The amount of light is correlated to the radiation exposure. Such dosimeters have already been used on EXPOSE-E facility (Berger et al., 2012). All the TLDs used for our experiment are calibrated and provided by the Institute of Aerospace Medicine of the German Aerospace Center (DLR) which will also perform the post-flight analysis.

6.3. Thermoluminescent dosimeter locations on the sample carrier

TLDs will be integrated in 3 different locations: within the cells, directly in the sample carrier core and beneath the sample carriers.

Two TLDs will be placed in each cell, under the well containing samples. Fig. 8 shows the design of the cell bottom which has been machined to offer two specific adapted locations for the two dosimeters. As they are very close to the sample, they will give a very good estimate of the ionizing dose received.

Also, special holes will be drilled into the sample carrier core to host TLD stacks. Indeed, some columns of eight stacked TLDs will be integrated between cells (Fig. 2) in order to assess radiation distribution in depth.

Finally, some dosimetry modules composed of TLDs and CR39 films will be integrated beneath the sample carriers to determine the dose levels for maximum shielding.

6.4. Thermoluminescent dosimeters integration

TLDs are provided in plastic holders and each TLD is identified by its position in the holder map. The dosimeters are integrated in the cells during sample conditioning within a glove box. All of the cells, including the ISS samples and controls, will contain a pair of dosimeters. To proceed with their integration, they are grabbed with fine tip tweezers and placed in the respective cell following an integration map. At the bottom of the cell, a punch is made at
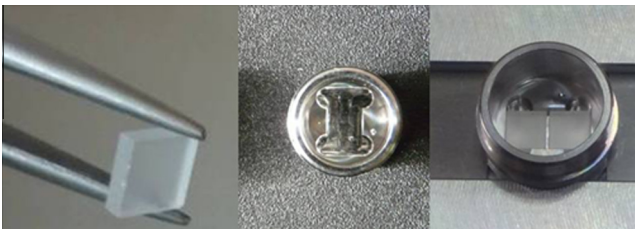

Fig. 8. Pictures of a TLD (left), the specific design of the biochip cell bottom (middle) and two TLDs integrated into a cell (right).

one of the 2 TLD locations during cell manufacturing to differentiate them (Fig. 8). This allows placing every dosimeter back in its original position in the plastic holder at the end of the mission. Indeed, each dosimeter has its own specific calibration factor and should be well identified all along the mission. Once dosimeters are positioned at the bottom of the cell, the sample integration can be carried out.

7. Conclusion

In the present paper, we present the preparation of the Biochip experiment which will be part of the PSS experiment aboard the ISS using the EXPOSE-R facility. For this experiment, two types of biological samples were selected: a model antibody and a model aptamer. The optimized conditioning consists in adding them to a small container under the freeze-dried form. After validation (acceptance) of the prepared batch, the samples will be stored in a controlled atmosphere, waiting launching. Once on the ISS, the samples will undergo different levels of exposure to space constraints (in particular different levels of cosmic radiations). Both the preparation and analysis procedure optimizations are now complete and the samples are ready to fly. EXPOSE-R2 mission should be launched in April 2014 and it is intended to last for around 2 years including 12–18 months of exposure outside the ISS. The scientific objective is to test the resistance of the biochip models to cumulated space constraints. Indeed, even though ground experiments are useful for evaluating the impact of a given space constraint on a biochip-based instrument, it does not take into account the cumulative effect of all constraints.

Nevertheless, both types of experiments (carried on Earth and on ISS) are very useful as they can help the scientific community to optimize the use of a biochip-based instrument for space exploration by developing adapted protections.

Acknowledgments

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