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The SOS-LUX-TOXICITY-Test on the International Space Station

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Abstract

For the safety of astronauts and to ensure the stability and integrity of the genome of microorganisms and plants used in bioregenerative life support systems, it is important to improve our knowledge of the combined action of (space) radiation and microgravity. The SOS-LUX-TOXICITY test, as part of the TRIPLE-LUX project (accepted for flight at Biolab in Columbus on the International Space Station, (ISS)), will provide an estimation of the health risk resulting from exposure of astronauts to the radiation environment of space in microgravity. The project will: (i) increase our knowledge of biological/health threatening action of space radiation and enzymatic DNA repair; (ii) uncover cellular mechanisms of synergistic interaction of microgravity and space radiation; (iii) provide specified biosensors for spacecraft milieu examination; and (iv) provide experimental data on stability and integrity of bacterial DNA in spacecrafts. In the bacterial biosensor "SOS-LUX-Test" developed at DLR (patent), bacteria are transformed with the pBR322-derived plasmid pPLS-1 or the similar, advanced plasmid SWITCH, both carrying the promoterless lux operon of *Photobacterium leiognathi* as the reporter element controlled by a DNA damage-dependent SOS promoter as sensor element. A short description of the space experiment is given, and the current status of adaptation of the SOS-LUX-Test to the ISS, i.e. first results of sterilization, biocompatibility and functional tests performed with the already available hardware and bread board model of the automated space hardware under development, is described here.

Keywords: Space; Bioluminescence; Green fluorescent protein; Radiation; Bioassay; Genotoxicity

1. Introduction

Long-duration space flights expose astronauts to a different regime of radiation, more harmful than on Earth [20,25,27], in addition to microgravity. There is increasing evidence that at least some basic cellular functions are sensitive to microgravity and that microgravity leads to responses at molecular, cellular and systemic levels [7,15], with effects upon cytoskeleton organization of mammalian cells [6,11] and on intracellular signal transduction pathways [24].

For repair of radiation-induced DNA damage under microgravity conditions, only a few and non-uniform data are available. Interactions of radiation and microgravity were investigated in earlier space flight experiments [10,12], and were classified as "additive" (neither sensitization nor protection), "synergistic" (increased radiation effect under microgravity) or "antagonistic" (reduced radiation effect). Exposition to microgravity and cosmic radiation was reported to result in cell inactivation, mutation induction and chromosomal aberrations in a variety of assay systems [8,13]). The mechanisms underlying the observed additive or synergistic phenomena are not yet well understood. Microgravity might interfere with the operation of some cellular repair processes which would result in an augmentation of the radiation response, though a direct effect on enzymatic mechanisms can be excluded on thermodynamic reasons. Cellular signal transduction systems, which control repair processes and induce transcriptional activity, are controlled by environmental parameters, and presumably also by gravity. Modifications of cellular repair can therefore not be excluded per se. In order to test this hypothesis, DNA repair studies in space have been performed on bacteria [9,14], yeast cells [16]

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and human fibroblasts [10], but as in most of the experiments on DNA repair conducted in space, radiation was applied before flight and the samples were kept inactive (e.g. frozen, as spores, or at a repair-prohibiting temperature) until incubated in space. The results are contradictory: from inhibition of repair by microgravity [16], to enhancement [14], whereas Horneck et al. [9,10] did not detect any influence of microgravity on repair. To exclude repair initiation, such as gene activation, in 1 g conditions, radiation of the cells has to be conducted directly in space.

In the space experiment utilizing the SOS-LUX-Test developed by the group of G. Horneck [18] and selected to be performed on the Biolab centrifuges in Columbus on the International Space Station (ISS), the response of cells to irradiation in microgravity will be investigated. In the SOS-LUX-Test on the ISS, artificial UV radiation induces bioluminescence by activation of the SOS response in recombinant bacterial cells. *Salmonella typhimurium* 1535 bacteria are transformed with a plasmid pSWITCH (or pPLS-1) carrying a promoter reporter system with the *luxCDABFE* genes (reporter) from *Photobacterium leiognatii* under the control of the SOS-dependent ColD promoter (receptor). In response to the presence of a DNA-damaging agent like UV radiation, the ColD promoter is induced, leading to the expression of the *lux* genes and resulting in emission of measurable bioluminescence proportional to the applied genotoxin [21,22].

1.1. Description of the experiment on the ISS as currently planned

The experiment on the ISS will be executed in parallel under microgravity conditions on the non-running centrifuge and on the 1 g reference centrifuge in Biolab. It is started by manual insertion of the frozen stock cultures of *S. typhimurium* TA1535 pSWITCH in stock culture bags (SCBs) into advanced experiment containers (AECs) on the centrifuges. After thawing and incubation, samples of frozen stock solution are transferred by the handling mechanism (HM) to specially designed culture bags (CBs) and mixed with fresh medium to dilute the stock culture and the cryopreservative. Dilution will be repeated twice: the third dilution step transfers the culture samples into a measurement bag (MB) designed to measure absorption and luminescence of the bacterial culture. Stirrers in all sample containers provide homogeneous suspensions, without applying forces higher than 10^{-3} g at a single cell level.

Cell growth of the culture during the incubation period in the MB will be monitored by measuring the optical density with a photometer inside the MB. When mid-log stage of the bacterial culture is reached, the HM transfers three samples from the MB to three additional MBs. Two of them are exposed to two doses of UVC radiation (254 nm); the third MB remains non-irradiated as an in-flight dark control. OD and the luminescence baseline data of the cell culture prior to radiation are recorded. After irradiation, experimental reaction kinetics, i.e. OD of cell growth and luminescence is recorded every 10 min for each test sample over a period of 5 h. Data will be stored on board and downlinked. After 2.5 and 5 h, an aliquot of each sample will be frozen for further investigation on the ground. One SCB will be kept stored at $-20\,^{\circ}$C during the entire mission and returns frozen to the ground.

Special requirements have to be met to perform experiments like these on board the ISS: crew time is limited, size and environment for the experimental set-up are predetermined, as are storage and transportation conditions. Therefore, a test that can be performed fully automatic in miniaturized hardware in a short time, like the SOS-LUX-Test, is preferred. Special hardware has to be developed for the experiment, with a limited choice of space-proof non-hazardous materials due to crew safety considerations. The experimental hardware for the SOS-LUX-Test is specially designed and under development to fit into the Biolab of the Columbus module on the ISS and to perform all necessary requirements for the test itself, including the radiation procedure.

EADS Space Transportation has designed and provided a bread-board (BB) model and sample containers (stock culture bags (SCBs), culture bags (CBs) and measurement bags (MBs), for liquid exchange in microgravity), for tests at DLR Cologne to determine their suitability for the experiment, including resistance to sterilization procedures and biocompatibility of all materials that may come in contact with the bacteria during the experiment.

2. Materials and methods

2.1. Plasmids and bacterial strain

The plasmid pSWITCH is a combined plasmid, consisting of the active regions of the pPLS-1 (lux) and pGFPuv (Clontech Laboratories Inc., CA, USA, 6079-1) plasmids. The construction of the plasmid pPLS-1 (DSM 1033) carrying the *luxCDABFE* genes downstream from a strong SOS-dependent promoter has already been described [17]. This plasmid was combined with the *GFPuv* gene controlled by a lac promoter isolated from the commercially available pGFPuv plasmid. Transformation of the strain *S. typhimurium* TA1535, one of the tester strains in the Ames test [2], was performed with the plasmid as published [18,21]. The strain TA1535 carries an *rfa*-mutation resulting in a deep rough phenotype [23]. Due to its reduced lipopolysaccharide layer and an increased permeability of the cell membrane, it displays a greater sensitivity to mutagens. An additional *uvrB* mutation eliminates the excision DNA damage repair system [1], which additionally triggers a faster SOS response. The transformation of the *S. typhimurium* 1535 strain with this combined pSWITCH plasmid allows the monitoring of the luminescence emission as a signal for DNA damage and therefore for the presence of genotoxic-acting agents in addition to the fluorescence emitted by the constitutively expressed GFP protein as a signal for active intracellular protein synthesis. Fluorescence decrease therefore is an additional reporter for decreasing protein synthesis as result of cytotoxic effects of compounds. In the SOS-LUX-Test of the TRIPLE-LUX space experiment, only the measurement of the luminescence induced by the pSWITCH precursor pPLS-1 was proposed. Therefore in the experiment on the ISS, only the genotoxicity assay of the newly developed pSWITCH will be

used, with absorption measurement of the bacterial culture during the test functioning as a cytotoxicity assay, and to correct the luminescence increase for bacterial growth as already described [21,22].

2.2. Standard laboratory biological set-up for the SOS-LUX-TOXICITY-Test and the SWITCH-Test

2.2.1. Bacterial culture and test measurement conditions

Bacterial culture and measurement conditions have already been described in detail [3,22]. A mid-log phase stage of *S. typhimurium* 1535 transformed with pSWITCH is transferred to a 96-well plate. After induction, luminescence, absorption and fluorescence (not part of the experiment on the ISS), kinetics are measured every 10 min for up to 8 h by the microplate reader "Victor^2" (Multilabel Counter 1420 Victor^2 from EG&G Wallac, Perkin–Elmer, USA). As a genotoxic standard, mitomycin C (MMC, Sigma, Germany) is used. UVC irradiation is performed with a mercury low-pressure lamp (NN8/15, Heraeus) and irradiation is measured with a UVX-radiometer at the sample site before and after each irradiation experiment.

2.3. Sterilization tests of SCBs, CBs and MBs

SCBs are flexible and transparent Latekran bags, whereas CBs and MBs consist of a cuvette part made of polycarbonate for OD and luminescence measurements and an attached bag made of Biofoil 25 (In Vitro Systems & Services). Sterilization ability was tested with the Latekran SCBs and the CBs and MBs by using a certified standard autoclaving procedure (15 min at 121 °C, 1.25 bar, saturated steam atmosphere) in a Technoclav 50 (Technomara). The procedure and results have already been described in detail [26].

2.4. Biocompatibility tests performed with the materials in contact with bacterial cultures

As described in detail by Stojicic et al. [26], the biocompatibility of the bag material (Latekran) and part of the silicon fixative used to connect parts of the hardware components were tested. 210 mg of Latekran granules and 500 mg granules of the silicon sample were incubated together with the bacterial culture. Bacterial cell culture growth was measured as the increase in its absorbance at 600 nm in comparison with a control culture in a Hitachi double ray double monochromator spectrophotometer.

2.5. Biocompatibility test of CBs and MBs using the combined cyto- and genotoxicity test (SWITCH-Test)

Geno- and cytotoxicity of the CB and MB material compared to standard laboratory test tubes were investigated using the combined cyto- and genotoxicity test SWITCH-Test [4,22]. The genotoxicity part of this test is identical to the SOS-LUX-Test performed on the ISS. Aliquots of bacterial cultures incubated inside the CMs, MBs and standard test tubes were transferred into a 96-well plate every 60 min for 5 h. The test itself was performed as described [3,4,22].

2.6. Test of the BB model

A model (BB) using the above investigated CBs and MBs was developed by EADS and tested at DLR. Two photomultipliers (PMTs) are integrated into the BB to detect the UV-induced luminescence and OD, and a LED as light source. The data for luminescence and transmission measurement are measured by PMT 1 with changing of the PMT GAIN from a high level for luminescence measurement (PMT 1 GAIN = 0.8 V for 5 min and 1.0 V for 5 min and LED off) and with a low level (PMT 1 GAIN = 0.5 V and LED = 3.8 V) for the transmission of light measurement. For the BB test, luminescence of bacterial culture was induced by irradiation with 84.4 J m^{-2} of UVC, 254 nm. Luminescence and absorption of the culture was measured for 3 h, and data were monitored every 10 min with the BB and compared to the standard laboratory set-up and measurement in Victor^2 performed in parallel.

3. Results

3.1. Sterilization tests of SCBs, CBs and MBs

The polycarbonate CBs and MBs with their attached Biofoil 25 (In Vitro Systems & Services) bags showed no signs of destruction or corrosion, even after several rounds of autoclaving with the standard procedure, whereas the Latekran material of the SCBs fused. The test is also described in detail by Stojicic et al. [26].

3.2. Biocompatibility tests performed with the materials in contact with bacterial cultures

Bacterial cell culture growth in the presence of 210 mg of Latekran granules and 500 mg granules of the silicon sample, respectively, in comparison with a control was measured as an increase in its absorbance in comparison with a control culture. No growth-inhibiting effect of the Latekran or the silicon was detected [26]. Data are shown in Fig. 1.

3.3. Biocompatibility test of CBs and MBs using the combined cyto- and genotoxicity test (SWITCH-Test)

Geno- and cytotoxicity of the CB and MB material compared to standard laboratory test tubes were investigated using the combined cyto- and genotoxicity test SWITCH-Test.

The emitted luminescence increase with incubation time was lower for bacteria grown in the MBs and CBs than for bacteria cultured inside the standard test tube (Fig. 2). The measured fluorescence showed no decrease during the incubation time, indicating no protein synthesis inhibition (Fig. 3). Data therefore indicated no genotoxic or cytotoxic effects on bacterial growth and therefore proved the biocompatibility of the CBs and MBs.

3.4. Test of the BB model

For the BB test, luminescence of bacterial culture was induced by irradiation with 84.4 J m^{-2} of UVC radiation at

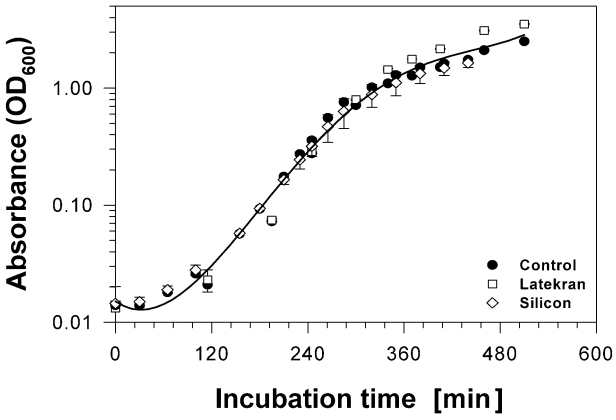


Fig. 1. Bacterial growth curves in the absence and presence of Latekran or silicon fixative.

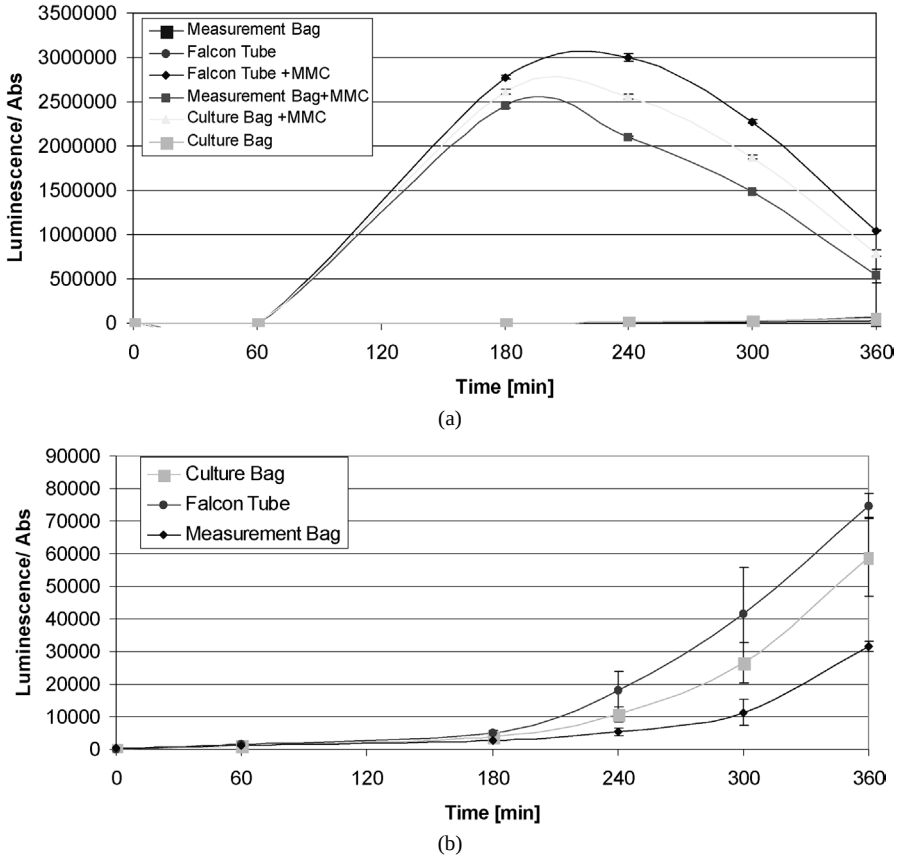


Fig. 2. Luminescence output during incubation of TA1535 pSWITCH in different culture vessels: (b) shows the results of (a) for bacteria incubated without MMC in a higher resolution of the luminescence output.

a wavelength of 254 nm. Data obtained with the BB were similar to those derived from the standard laboratory set-up and measurement in Victor^2. Both systems detected a luminescence increase after approximately 1 h incubation and a peak luminescence after approximately 2 h and 15 min (Figs. 4a and 4c). A comparable OD increase was also detected both with the Victor^2 standard laboratory set-up and the BB (Figs. 4b and 4d).

OD increased in Victor^2 from 0.04 to approximately 0.08 during the measurement time of 3 h for the sample irradiated with 84.4 J m^{-2}. OD measured with the BB during that time was slightly higher, but light paths and measurement conditions were not optimized in the BB.

4. Discussion

The SWITCH-Test, like its precursors SOS-LUX and LAC-FLUORO-Test, is a short-term bioassay developed for measuring both cyto- and genotoxic effects of agents. The SWITCH-Test indirectly monitors the kinetics of DNA damage-processing in the SOS system by measuring the luminescence emitted by the genetically altered bacteria. This system enables perform-

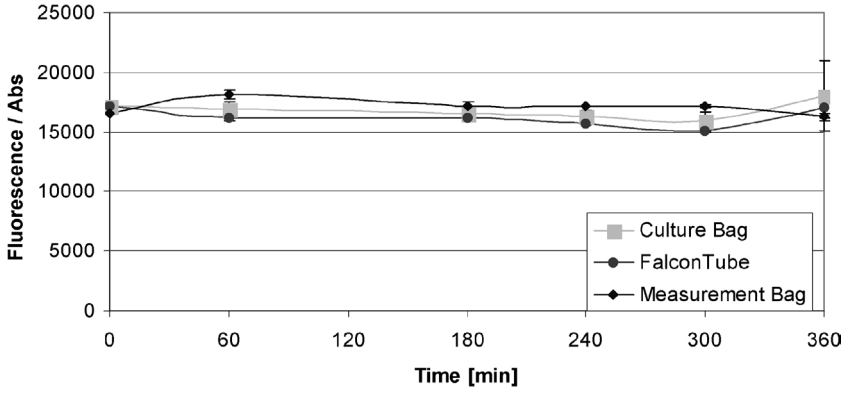
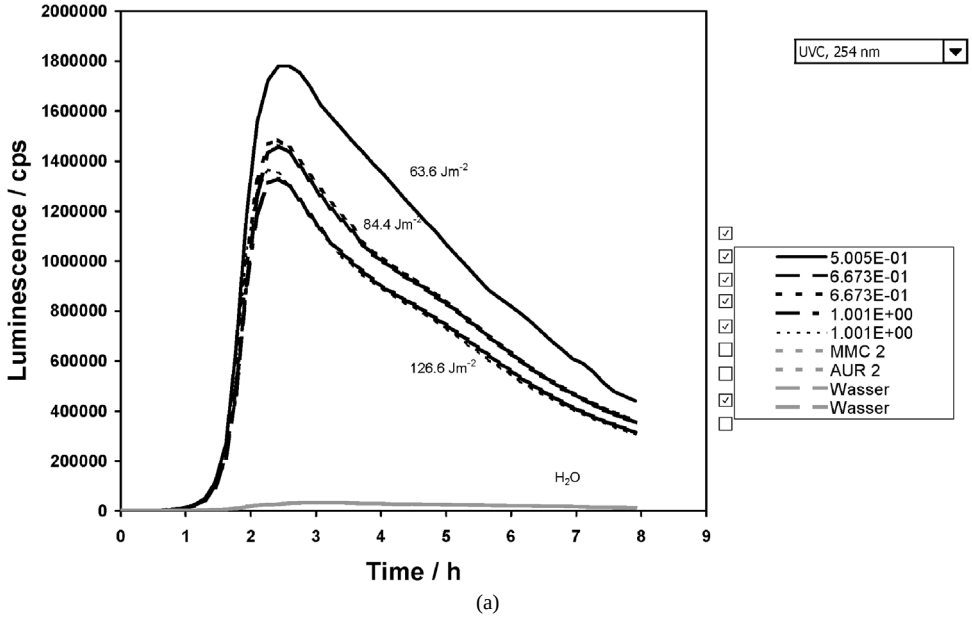
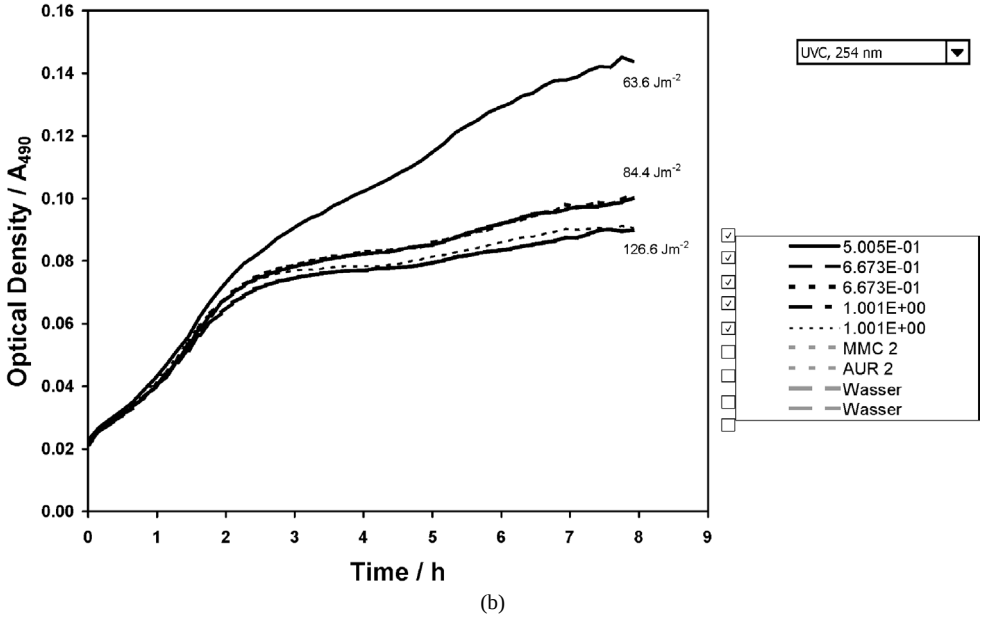


Fig. 3. Fluorescence output during the incubation of TA1535 pSWITCH in the tested culture containers.

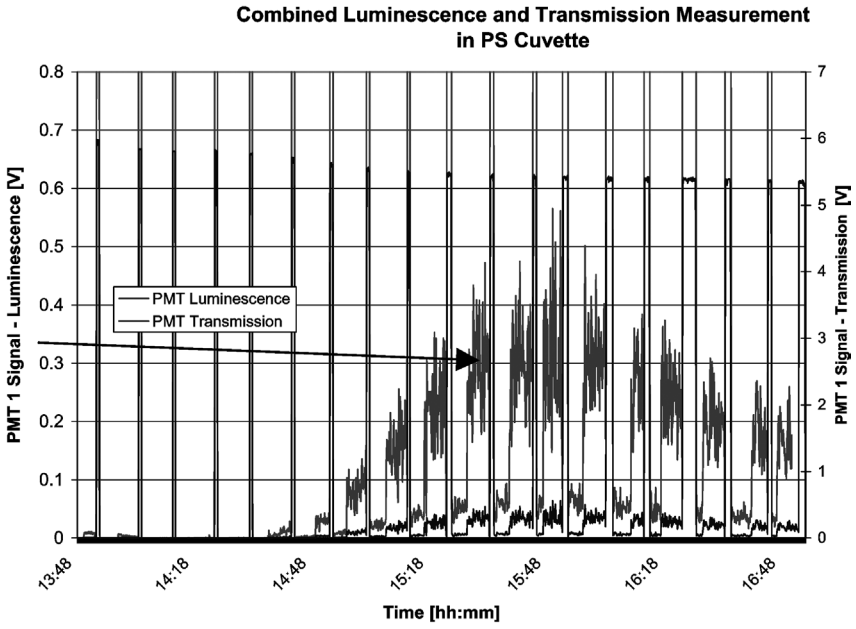


(a)

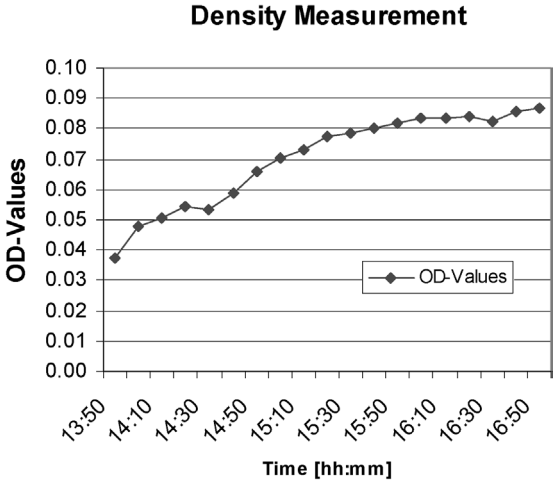


(b)

Fig. 4. (a) Luminescence in cps with time in h for 3 samples each of 63.6, 84.4 and 126.6 J m^{-2}. (b) Absorption with time in h for 3 samples each of 63.6, 84.4 and 126.6 J m^{-2}. (c) Combined luminescence and transmission diagram measured with the BB. (d) OD values measured with the BB.



(c)



(d)

Fig. 4. (*continued*)

ing the test from radiation to data acquisition in microgravity, and therefore the estimation of the effect of the combination of space radiation and microgravity on the living cell, and particularly on the DNA damage repair system. Absorption is measured to provide data for the correction of a growth-related luminescence increase and for the detection of cytotoxicity. Automation and on-line data registration as early as 2 h after the test start satisfy minimal crew time requirements and make this test applicable for use on the ISS [5]. During genotoxicity measurement campaigns, in comparison with other genotoxicity tests (Ames test, Vitotox, etc.) in Mol, Belgium [19], and Koblenz, Germany [4], the test proved its reliability and low genotoxicity detection limit, as well as its short response time, in double-blind tests.

Although, in the meantime, a more sophisticated test system was developed with the SWITCH-Test, leading to the determination of protein synthesis inhibition as a measure of cytotoxicity in addition to luminescence (genotoxicity) and absorption (cytotoxic) measurements, fluorescence measurements will not be applied on the ISS. Because of the numerous safety considerations applicable to a manned space flight experiment, the request for such a major change resulting in complete redesigning of special hardware and repetition of the flight acceptance procedure would increase costs and endanger the flight schedule and the performance of the entire experiment on the ISS. For this experiment, absorption measurement is sufficient to detect cytotoxic reactions.

Due to safety considerations and practicability of the hardware design, UV radiation was chosen as a substitute for cosmic radiation. Though DNA damage induced by the two types of radiation differs, they nevertheless both trigger the SOS repair system of the bacterial test strain, thus inducing the reporter system used here for investigations on DNA damage repair in microgravity.

Sterilization and biocompatibility tests of the already available parts of the hardware showed satisfying results with meas-

urement and culture bags. Sterilization experiments with the Latekran bag suggest the use of γ-sterilized Latekran bags. Hardware components caused no geno- or cytotoxic effects on *S. typhimurium* TA1535 pSWITCH.

Performance tests with the BB proved that the chosen measurement conditions and devices are able to monitor bacterial growth measured as an OD increase in the desired amount and volume of bacteria for the desired period of time. Luminescence measurements are relative and depend on the type and specification of the photo detector used. Therefore, detection of the signal and a relative but comparable kinetics of luminescence measurement data as delivered by the BB compared to the standard laboratory set-up using Victor^2 are sufficient for measurement of the UV-induced luminescence of the SOS-LUX-Test. Deviations between Victor^2 and the BB are explained by differences in the whole set-up, including the amount of bacterial culture, stirring mechanisms and sample geometry, and the not-yet-optimized light pathway in the BB.

All tests performed and described here confirmed the suitability of the hardware components chosen and already developed for the specially designed space experiment hardware still under development.

To define the final set-up of the entire experiment, the final hardware, including the irradiation device, has to be tested under conditions as close to those on the ISS as possible. Delivery of the prototype of the hardware is scheduled for September 2005. Further experiments using both parts, the SOS-LUX-Test and the LAC-FLUORO-Test, of the SWITCH-Test on the ISS will be proposed.

Acknowledgements

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