TEST FLIGHT OF A BIOREACTOR MODULE FOR CARTILAGE TISSUE ON MASER 9

Nadine Conza(1), Augusto Cogoli(2), Rita Dreier(3), Peter Bruckner(4), Simona Berardi-Vilei(5),
Jutta Kraemer(6), Ron Huijser(7)

(1) Technical University Delft, N.E.Conza@wbmt.tudelft.nl
(2) ETH Zurich, cogoli@spacebiol.ethz.ch
(3) University of Münster, dreier@uni-muenster.de
(4) University of Münster, pibi@uni-muenster.de
(5) Centerpulse, Winterthur, simona.berardi@centerpulse.com
(6) ESA-ESTEC, Noordwijk, Jutta.Kraemer@esa.int
(7) Dutch Space, Leiden, r.huijser@dutchspace.nl

ABSTRACT

Within the frame of a project supported by the Microgravity Application Program of ESA we have developed a module for the growth of cartilage tissue starting from primary chondrocytes. The module is based on the scaffold-free chamber “Denovo” of Centerpulse. The long-term goal of the project is the development of a modular bioreactor for tissue engineering on the International Space Station. The two objectives of the experiment on MASER 9 were: first, to test the module and its service unit, second, to investigate whether short exposure to microgravity may alter the structure of the cytoskeleton and genetic expression of 10 selected genes. Post-flight analyses showed that the apparatus worked nominally. No changes were detected in the cytoskeleton. The genetic expression of biglycan was slightly depressed at 0 g.

1. INTRODUCTION

Twenty years of experimentation in space laboratories, sounding rockets and satellites have shown that mammalian cells undergo profound alterations at low-gravity conditions. Such findings nourished hypotheses and speculations on possible commercial and medical applications. Bioprocessing in space is now one of the priority topics of the exploitation of the International Space Station ISS.

It is still too early to give a credible judgement on the potential benefits of biotechnology in space. Too little experiments were carried out so far and there is only a very limited database on the subject. For this reason ESA has started a “Microgravity Application Program”, MAP to support application-oriented projects with participation of non-aerospace industries. ESA is granting to universities funds of the same amount as that the associated companies are investing in the project. The work reported here has been conducted in the frame of a MAP project dedicated to the design and development of a modular bioreactor for tissue engineering on the ISS.

In fact, ESA is presently financing studies dedicated to the definition of a biotechnology mammalian tissue culture facility, BMTC, for the ISS. Parallel activities are in progress at NASA.

It is believed that low-g may contribute in two aspects to progress in this field. First as a useful and non invasive tool to study important and still obscure biological events like signal transduction, gene expression, and cell proliferation. Second, low-g may favour smooth cell-cell aggregation and three-dimensional organogenesis in the absence of sedimentation and shear forces. Experiments in the random positioning machine, RPM, a device that provides modelled low-gravity on Earth shall contribute to define the future projects in space.

The purpose of this paper is to present the results of the experiment conducted on the sounding rocket MASER 9 in March 2002 with pig chondrocytes in a module developed in collaboration with Dutch Space, Leiden. Preparatory experiments carried out in the RPM have been described previously [1].

2. MATERIALS AND METHODS

2.1 Flight Instruments

The chondrocytes were sealed in so called “Codi” modules. Eight Codi modules can be accommodated in one service unit. The Codi modules and the service units were manufactured by Dutch Space. The Codi modules (Fig. 1) are cell culture devices for the cultivation of chondrocytes, and have been developed for MASER 9 and as a precursor of a future long-term cultivation bioreactor. The actual culture compartment is the Denovo chamber (Fig. 2)
developed for scaffold-free chondrocyte cultivation and patented by Centerpulse, Winterthur, Switzerland. Two membranes, on the top and at the bottom of the insert, allow for nutrient diffusion from the surrounding culture medium to the cells enclosed in the insert. The service unit (Fig. 3) consists of two liquid supply systems (LSS) enabling independent injection of two different solutions, and two valve blocks (one for each solution) ensuring a closed Codi unit system after assembly. The two LSS are independently operated via a preprogrammed code.

Each LSS consists of a double reservoir emptied by means of two motor-activated pistons (Fig. 4). The service unit works automatically at pre-set times under microprocessor control, also responsible for keeping the temperature at 37°C [2].

2.2 Cell Cultures

Chondrocytes were isolated from knee cartilage from healthy pigs in the laboratory in Zurich, Switzerland. The cells were then frozen in liquid nitrogen at passage P0 according to procedures developed by Centerpulse and transported on commercial airlines to Kiruna, Sweden. 12 modules, 8 for in-flight experiments and 4 for the ground control were filled each with 12.5x10^6 chondrocytes (cell conc. 50x10^6/ml). In-flight chondrocytes were lysed with Trizol (Invitrogen, Basel, Switzerland) for genetic expression analysis immediately before onset of the 0 g phase (2 modules) and at the end of the 0 g phase lasting 360 sec (2 modules), respectively. Similarly, the cells in the other 4 modules were chemically fixed with paraformaldehyde, PFA, for cytofluorimetric analysis. In parallel with the in-flight samples, two ground samples were lysed and two chemically fixed. The flight samples were recovered 60 min after landing. The apparatus worked nominally. The cell samples were prepared for transportation and brought back to the home laboratories one day after flight. The PFA fixed samples were stored at 4°C, the lysed samples were stored frozen in liquid nitrogen.

2.3 Genetic Expression

Genetic expression was analyzed by the reverse transcriptase polymerase chain reaction technology, RT-PCR. Total RNA from the lysed flight samples was isolated with the RNeasy mini kit (Qiagen, Basel, Switzerland). For the semi-quantitative measurement of the mRNA by RT-PCR, the QuantumRNA kit (Ambion, Austin, USA) was used. Using a modification of a published procedure [3], the cDNA
samples were produced by the RT System (Promega), starting with 1 µg of total RNA. The 10-fold diluted cDNA samples were subjected to PCR for the following genes: decorin, vimentin, aggrecan, biglycan, sGAPDH, L-myosin, versican, β-actin, collagen I, and collagen II.

2.3 Cytoskeletal Analysis

The cytoskeleton analysis of six different samples was performed on the second day after flight. Prior to labelling, the cells were solubilized for 5 min at 4°C in 0.5% TritonX-100 in PBS and washed twice in PBS (centrifugation 5 min at 600 g). The cells were then incubated with monoclonal antibodies against vimentin (clone V9, DAKO, Glostrup, Danmark), β-Tubulin (Roche, Mannheim, Germany) for 60 min at 4 °C and washed 3 times in PBS (centrifugation 5 min at 600 g). In case of vimentin and β-tubulin staining, the cells were incubated with a FITC-conjugated secondary sheep-anti-mouse IgG antibody (Sigma, Taufkirchen, Germany) for 60 min at 4 °C and washed 3 times with PBS. Actin filaments were visualized with phallolidin FITC (Sigma, Taufkirchen, Germany). After staining, cytospin preparations of labelled cells were embedded in Fluoromount-G (Southern Biotechnology Associates, Birmingham, Alabama, USA) and analysed with a confocal fluorescence microscope (LSM Nikon PCM 2000).

Fig. 6. Confocal fluorescence microscopy of tubulin before (A) and after (B) onset of weightlessness.

Fig. 5. Genetic expression determined in flight immediately before onset of weightlessness (black bars) and 6 min after exposure to 0 g (void bars) and in ground control (gray bars) by RT-PCR.
3. RESULTS AND DISCUSSION

First, the main objective of the experiments on MASER 9 was to test the instrumentation under real 0 g conditions as well as under the harsh conditions of a rocket launch (vibrations and acceleration). Post-flight inspection revealed that all components (injection devices of fixative and lysing buffer, °T control) worked as expected. This is not trivial as past experience showed that transfer of liquids in automated devices often fails due to leaks or blockage of the pistons of the syringes or of the valves.

Second, the results obtained by RT-PCR showed that all genes analyzed remained unaffected by exposure to 0 g, except for biglycan, whose expression was slightly lower at 0 g (Fig. 5). Also a comparison between the flight samples fixed immediately before onset of 0 g and the samples lysed after 6 min at 0 g shows that weightlessness does not trigger the expression of the genes analyzed. In addition, the data seem to indicate that the launch conditions may selectively depress the expression of biglycan and that, however, the baseline level of expression at 1 g is almost completely restored after 6 min at 0 g.

Third, confocal fluorescence microscopy showed no difference in the cytoskeletal arrangements of tubulin (shown in Fig. 6), vimentin and actin. All three sets of samples, namely the cells fixed in flight before 0 g conditions were achieved, those fixed after exposure for 6 min to 0 g, as well as the cells fixed on ground, showed the same cytoskeletal architecture.

In conclusion, the automatic modular cultivation device for chondrocytes (modules and service unit) performed nominally on the MASER 9 flight. No relevant and consistent changes in the cytoskeletal arrangement nor in the gene expression (except for the changes in biglycan) occurred.

In the long term experiments with the RPM clear morphological differences were obtained [1]. Clear microgravity effects on morphology from the short duration sounding rocket flight, including mechanical stress induced by the harsh launch environment, were not expected. Future experiments in a true long-duration microgravity environment, such as offered by the International Space Station, and with experiment hardware such as successfully tested on MASER 9, are needed for further research.

4. REFERENCES


Acknowledgements: The work has been supported by the ESA MAP programme and by Centerpulse.