CELL BEHAVIOR IN SIMULATED MICROGRAVITY: A COMPARISON OF RESULTS OBTAINED WITH RWV AND RPM

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INTRODUCTION

Some pathological conditions described in astronauts, such as cardiovascular deconditioning, represent the adaptive response to the absence of gravity and are partially due to the effects exerted by microgravity (\(\mu g\)) at the cellular level. Since endothelial cells are crucial in the maintenance of the functional integrity of the vascular wall, it is noteworthy that we described endothelial dysfunction in response to simulated \(\mu g\). In particular, we have shown that cultured Human Umbilical Vein Endothelial Cells (HUVEC) in the Rotating Wall Vessel (RWV) grow faster than controls, rapidly remodel their cytoskeleton and, after a few days, markedly down-regulate actin (Carlsson et al., 2002, Carlsson et al., 2003). The RWV system requires cells suspended or seeded on microcarriers. This is an obstacle to evaluate other endothelial functions, among which cell migration, a crucial event in vasculogenesis and angiogenesis. We therefore decided to simulate \(\mu g\) using the Random Positioning Machine (RPM) that allows to culture cells in standard plates. RPM has been demonstrated to be a good \(\mu g\) simulator for plants, osteoblasts and T lymphocytes. Up to now, there are only few data about the behaviour of adherent cell cultures in RPM.

To optimize the RPM operative parameters, we chose a model human monocytoid U937 cells that have been studied in simulated and real \(\mu g\) (Hatton et al., 1999; Maier 2004). On the basis of previous results, our aim was: a) to evaluate the best operative experimental conditions of RPM, b) to compare the results with those obtained in simulated (RWV) and real (spaceflight) \(\mu g\) conditions; c) to use RPM to grow HUVEC in order to confirm and, possibly, broaden the RWV results.

Rotating Wall Vessel

The RWV is a suspension culture vessel optimized to produce laminar flow and minimize mechanical stress on cell aggregates in culture. It provides fluid dynamic operating principles characterized by 1) solid body rotation about a horizontal axis that is characterized by colocalization of cells and aggregates of different sedimentation rates, optimally reduced fluid shear and turbulence, and three-dimensional spatial freedom; and 2) oxygenation by diffusion, excluding undissolved gases from the bioreactor (Hammond et al., 2001). The cylindrical culture vessel is filled with culture fluid and the cells or tissue particles are added. All air bubbles are removed from the culture vessel. Oxygenation is achieved through a gas permeable silicone rubber membrane. Since the Rotary Cell Culture System\(^{\text{TM}}\) has no impellers, airlifts, bubbles, or agitators, tissue damage from impact and turbulence is decreased with shear stress and damage essentially insignificant.

Random Positioning Machine

The RPM is essentially a 3-axis clinostat, which creates a condition in which the weight vector is continually reoriented as in traditional clinorotation, but with increased directional randomization (Klaus, 2001). The instrument operates inside an incubator with controlled temperature and atmosphere (CO\(_2\) percentage, relative humidity...). Standard consumable experiment hardware can be used, such as 6 wells multidishes sealed with a gas permeable membrane, in order to achieve a suitable gas exchange. More sophisticated hardware, derived from spaceflight experience, will enable automatic medium exchange, fixation, etc. Primary applications are cell and developmental biology and tissue engineering. Simulation of \(\mu g\) by the means of continuous random change of orientation of objects relative to the gravity's vector can generate effects comparable to those of true \(\mu g\) when the changes are faster than the object's response time to gravity. Thus, slow responsive living objects, are excellent candidates to be studied on RPM.

MATERIALS AND METHODS

HUVEC were obtained from the American Type Culture Collection (ATCC) and cultured in M199 containing 10% fetal calf serum, Endothelial Cell Growth Factor (150 mg/ml) and heparin (5 U/ml). The cell culture system was based on 2% gelatin-coated 6 wells multidishes. In order to achieve a suitable gas exchange, every dish was sealed with a gas permeable membrane (Breathe-Easy, Sigma) avoiding air bubbles formation. To be used in the RWV, HUVEC were seeded on beads (Cytodex 3, Sigma). Cells were subcultured using 0.05% trypsin 0.02% EDTA solution. All culture reagents were from Sigma. U937 cells have been cultured in RPMI medium containing 10% calf serum. In order to assess the viability, cells have been counted every 48h after staining with Trypan Blue, using a Burker chamber. To simulate \(\mu g\), we used the RWV bioreactor (Cellon) with 10 and 50 ml disposable vessels and the RPM facility at the Dutch Experiment Support Center (DESC, Amsterdam, NL) accommodated in a dedicated temperature controlled incubator capable of supplying a 5% CO\(_2\)/air mixture. To observe the cytoskeleton, immuno-fluorescence/confocal microscopy of cells fixed with paraformaldehyde and stained with fluorescein isothiocyanate (FITC)- phalloidine has been performed.

RESULTS AND DISCUSSION

Cell proliferation

It is reported that \(\mu g\) affects cell growth. Here we evaluated the proliferation of U937 and HUVEC grown in
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the RWV and RPM systems. Figure 1 reports the duplication time of U937 and HUVEC.
As expected on the basis of in flight experiments performed on the Shuttle, U937 in the RWV and in the RPM grew 40% slower than controls. On the contrary, HUVEC proliferated 50% faster than controls. These effects are reversible upon return to normal growth conditions. We conclude that µg simulated either by RWV or RPM leads to comparable results.

![Figure 1](image)

**Figure 1.** Duplication time of U937 and HUVEC cultured in RWV and RPM vs. static gravity controls. Standard Errors are <10%.

**Cytoskeleton reorganization**
Different cell types cultured in µg show cytoskeleton reorganization. We therefore stained HUVEC grown in simulated µg in the RPM and their ground controls with fluorescent phalloidin to visualize the cytoskeleton. Ground controls (A, C) show a well-organized cytoskeleton with abundant stress fibers organized into bundles. HUVEC grown in RPM (B) for 96 h show major modifications of cell shape, as previously observed in RWV experiments, with elongated extended podia (D), disorganized actin fibers and clusters. Cytoskeleton modifications are reversible upon return to normal growth conditions.

**CONCLUSION**
The similar U937 behavior in space-flight (Hatton et al., 1999) and simulated µg conditions supports RWV and RPM as good tools to simulate µg on earth. In addition, RWV and RPM exert similar effects on HUVEC behaviour. Our results indicate that both the systems work at similar simulation levels, thus allowing a wider spread of experiments.

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REFERENCES


