NEUT[1,2], JIM R. VAN HORN[1], HENNY C. VAN DER MEI[2], WILLEM SCHENK[1] & HENK J. BUSSCHER[2]
[1]*Department of Orthopedic Surgery, University Hospital Groningen, Hanzeplein 1, 9713 GZ Groningen, The Netherlands*
[2]*Department of Biomedical Engineering, University of Groningen, Bloemsingel 10, 9712 KZ Groningen, The Netherlands*

1. Bax, R.P. *et al.* Antibiotic resistance–what can we do? *Nature Med.* **4**, 545–546 (1998).
2. Blower, S.M., Small, P. & Hopewell, P. Control strategies for tuberculosis epidemics: new models for old problems. *Science* **273**, 497–500 (1996).
3. Blower, S.M. & Gerberding, J.L. Understanding, predicting and controlling the emergence of drug-resistant tuberculosis: a theoretical framework. *J. Mol. Med.* **76**, 624–636 (1998).
4. Blower, S.M., Porco, T. & Lietman, T. in *Mathematical Models in Medical and Health Sciences.* (Eds Horn, M.A., Simonett, G., Webb, G.) (Vanderbilt University Press, 1999).
5. Neu, H.C. The crisis in antibiotic resistance. *Science* **257**, 1064–1073 (1992).
6. Heck, D. *et al.* Use of antibiotic-impregnated cement during hip and knee arthroplasty in the United States. *J. Arthroplasty* **10**, 470–475 (1995).
7. Törholm, C. *et al.* Total hip arthroplasty with gentamicin-impregnated cement: A clinical study of gentamicin excretion kinetics. *Clin. Orthop.* **181**, 99–106 (1983).

Gene expression in space

To the editor—Many scientific disciplines, from drop combustion to protein crystal growth through suspension culture of cell aggregates, are influenced substantially by gravity. In these disciplines most gravitational effects can be controlled for and some must be counterbalanced, but all are important.

Culture of three-dimensional cell aggregates in suspension is an important gravity-limited phenomenon, as many cell types regain some differentiated features in these conditions. Although a diverse array of spinner flasks and mixers has been designed in the attempt to counterbalance gravity with equal and opposite forces, the tradeoff is that all the balancing forces induce shear and create turbulence. NASA engineers designed and built a cell culture vessel, known as the rotating wall vessel (RWV), which provides the minimum possible shear on a suspension culture, but even this is very gravity-limited: as the cell aggregates grow in size, their paths distort from the ideal circular path. This induces residual fluid dynamic stresses and wall impacts that degrade the mechanical culture environment, destroying the aggregates.

Differentiation of cells in culture may depend on three simultaneous conditions: three-dimensionality, low shear and turbulence, and co-spatial arrangement of different cell types and substrates (Kaysen, J.H. *et al. J. Membr. Biol.* **168**, 77–89; 1999). But the gravity limitation of suspension culture has impeded analysis of the molecular mechanisms of differentiation. To investigate this, we grew primary human renal cell cultures in various conditions. During microgravity culture in space, shear and turbulence approximate zero, whereas co-spatial relations of cells and three-dimensionality are almost perfect. Cell culture in true microgravity elucidates the mechanisms of cell differentiation and dedifferentiation as they relate to the mechanical culture conditions. Culturing in a centrifuge, either conventionally or in rotating vessels, further elucidates the specific mechanical parameters responsible for the genetic and morphological changes. This will allow optimization of culture parameters for specific goals in a mechanical region heretofore unobtainable.

In order to minimize the effects of shuttle launch on microgravity cultures, we chose prolonged 6-day growth in a steady state environment. To avoid changes due to reentry and landing, microgravity cultures were fixed during space flight. Gene expression analysis using microarray technology was used to monitor gene expression in various culture conditions, with quantitative comparison of the nature, grouping and extent of changes.

More than 1,632 genes changed at steady state during microgravity culture on space transportation system flight STS-90 "Neurolab". These were not known shear stress response elements and heat shock proteins (green dots). Specific transcription factors (red dots) underwent large changes in microgravity, including the Wilm's tumor zinc finger protein and the vitamin D receptor (full data set available at http://www.tmc.tulane.edu/astrobiology/microarray.html). In the RWV, 914 genes changed expression relative to the static, non-adherent, bag culture (control) grown in parallel. The genes, which changed most on the microgravity array, are randomly spread throughout the RWV array, demonstrating that the changes in gene expression observed in microgravity are unique and not just an extension of the RWV. Only five genes changed more than 300% during 3*g* centrifugation (not shown).

Escaping the tradeoff of balancing forces by examining the gravity-limited phenomenon of suspension culture in true microgravity demonstrates a considerable degree of steady-state change in gene expression. Microgravity studies indicate there are unique mechanisms of tissue differentiation, which are distinct from the best ground-based simulations.

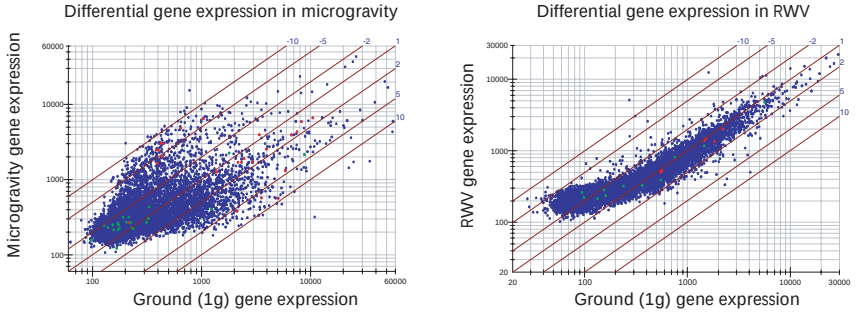

Fig. Gene expression in steady-state cell culture. Human renal cortical cells were cultured for 6d in DMEM/F12, 10% fetal calf serum, on Cytodex-3 beads, then samples were fixed and stored at 7 ºC. mRNA was reverse-transcribed with fluorescent bases and analyzed on DNA microarrays (Incyte, Fremont, California). Microgravity (left) and rotating wall vessel (right) were compared with a static non-adherent bag culture grown in parallel; 10,000 genes are represented by individual dots: green dots, shear stress proteins and heat shock proteins; red dots, transcription factors. Genes with similar expression lie on a line from the origin to the top right corner (labeled 1); expression level indicated by distance from the origin. Scale on each axis is $\log_2$.

T.G. HAMMOND[1,3,4], F.C. LEWIS[1,2,3], T.J. GOODWIN[3,5], R.M. LINNEHAN[3,5], D.A. WOLF[3,5],K.P. HIRE[6], W.C. CAMPBELL[1,3], E. BENES[1,3], K.C. O'REILLY[1,3], R.K. GLOBUS[6] & J.H. KAYSEN[1,3]
[1]*Nephrology Section, Dept. of Medicine and*
[2]*Dept. of Surgery, Tulane Univ. Medical Center*
[3]*Tulane Environmental Astrobiology Center, Center for Bioenvironmental Research*
[4]*VA Medical Center, New Orleans Louisiana,*
[5]*NASA, Johnson Space Center, Houston Texas*
[6]*NASA, Ames Research Center, Moffett Field, California*