The effect of combined hypergravity and microgrooved surface topography on the behavior of fibroblasts

WA Loesberg, XF Walboomers, JJWA van Loon, JA Jansen.
Radboud University Nijmegen Medical Centre, Dept. Periodontology & Biomaterials, PO BOX 9101, 6500HB Nijmegen, The Netherlands.

Statement of Purpose: Cell behavior guided by texture is well known. It seems that the underlying principles are similar to the processes occurring when mechanical forces are applied to cells. This mechanotransduction of stationary (texture) or dynamic (mechanical strain) forces has led to research in a new topic: interaction between different forces. One of such forces, whether it is present or absent, is gravity. In a laboratory environment, it is very well possible to simulate conditions of hypergravity by using centrifuges. The aim of this study is to evaluate *in vitro* the differences in morphological behavior between fibroblast cells cultured on substrates, both smooth and microgrooved, placed in a hypergravity environment. Our hypothesis is that cellular shape and orientation is predominantly determined by the topographical clues on the substrates. As controls fibroblast cells will be cultured on similar substrates which will remain at normal (Earth) gravity.

Methods: Smooth and microgrooved polystyrene substrates (groove depth: 1 μm, width: 1, 2, 5, 10 μm) where solvent cast, using silicon wafers as templates, and treated with a radio frequency glow-discharge shortly before cell seeding. Rat dermal fibroblasts where cultured onto all substrates. Substrates were placed inside boxes which undergo simulated hypergravity by centrifugation (10, 25, and 50 g) or 1g control (Fig. 1).

Fig. 1 Medium Size Centrifuge for Acceleration Research used to simulate hypergravity.

Morphological characteristics were compared using scanning electron microscopy and fluorescence microscopy to obtain qualitative information on cell spreading and alignment. Confocal laser scanning microscopy visualized distribution of actin filaments and vinculin anchoring points through immuno-staining. Finally, expression of collagen type I, fibronectin, and α1- and β1-integrin were investigated by PCR.

Results / Discussion: Microscopy and image analysis showed that the fibroblasts aligned along the groove direction on all grooved surfaces. On the smooth substrata (control) cells were spread out in a random fashion. The alignment of cells cultured on grooved surfaces increased with higher g-forces until a peak value at 25g. It was seen that cells on the 10 and 5 micron wide grooved parts were able to reach the bottom of the 1μm deep grooves, whereas on the parts with 2 and 1 micron wide grooves the cells lost contact with the bottom of the grooves. The cellular extensions probing the substrate surface only find the top ridge, resulting in extension of the cellular body along these small ridges, and always parallel to the grooves (Fig. 2).

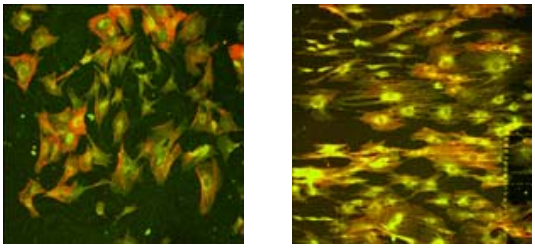

Fig.2 CLSM micrographs of smooth (left) and grooved (right) substrates. Notice aligned of cells on grooved substrate and random fashion on smooth surfaces. Both 24hours/10g samples.

From our RT-PCR results it became clear that the production of these components was vital for cells, so they have a mechanically resistant attachment support, indicated by the fact that cells under gravitational stimulation show decreased β1-integrin levels. Collagen type 1 and fibronectin are seemingly unaffected by time or force. The 25 g sample groups are the ones with the most profound orientation, yet with lowered values of β1-integrin. RDFs can apparently rely on another anchor protein member of the β-integrin family in order to help them align. ANOVA was performed on the data, for all main parameters: topography, gravity force, and time. In this analysis, all parameters proved significant. 89% of the cells were aligned along the grooved substrates compared to 12% of the smooth substrates. The amount of gravity influences cell alignment along the grooved topography. Although the effect of gravity from 1 to 10 g is not significant the increase from 10 to 25g and from 25 to 50g is significant. Time effects on alignment are low, but significant. 24 hour samples show a lower alignment (63%) compared to their 4 hour counterparts (76%) on grooved substrata. It is suggested that although actin cytoskeleton is closely involved in the orientation process, yet when the orientation is completed, the cells no longer dependent upon that system to maintain their position. However, hypergravity is still present and exerts a continuous influence making the cytoskeleton respond and provide the cell with the means to maintain equilibrium with its environment.

Conclusions: From our data it is concluded that the fibroblasts primarily adjust their shape according to morphological environmental cues like substrate surface, whilst a secondary, but significant, role is played by hypergravity forces.