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ENDOTHELIAL CELL MORPHOLOGY AND MIGRATION ARE ALTERED BY CHANGES IN GRAVITATIONAL FIELDS.

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INTRODUCTION: Many of the physiological changes of the cardiovascular system during space flight may originate from the dysfunction of basic biological mechanisms caused by microgravity (1 -4). The weightlessness affects the system when blood and other fluids move to the upper body causing the heart to enlarge to handle the increased blood flow to the upper extremities and decrease circulating volume. Increase arterial pressure triggers baroreceptors which signal the brain to adjust heart rate. Hemodynamic studies indicate that the microgravity-induced headward fluid redistribution results in various cardiovascular changes such as; alteration of vascular permeability resulting in lipid accumulation in the lumen of the vasculature and degeneration of the the vascular wall (5), capillary alteration with extensive endotheilial invagination (6). Achieving a true microgravity environment in ground based studies for prolonged periods is virtually impossible. The application of vector-averaged gravity to mammalian cells using horizontal clinostat produces alterations of cellular behavior similar to those observed in microgravity (7). Similarly, the low shear, horizontally rotating bioreactor (originally designed by NASA) also duplicates several properties of microgravity (8). Additionally, increasing gravity, i.e., hypergravity is easily achieved, Hypergravity has been found to increase the proliferation of several different cell lines (e.g., chick embryo fibroblasts) while decreasing cell motility (9) and slowing liver regeneration following partial hepatectomy (1 O). The effect of altered gravity on cells maybe similar to those of other physical forces, i.e. shear stress. Previous studies examing laminar flow and shear stress on endothelial cells found that the cells elongate, orient with the direction of flow, and reorganize their F-actin structure, with concomitant increase in cell stiffness (11). These studies suggest that alterations in the gravity environment will changethe behavior of most cells, including vascular cells. However, few studies have been directed at assessing the effect of altered gravitational field on vascular cell fiction and metabolism, Using image analysis we examined how bovine aortic endothelial cells altered their morphological characteristics and their response to a denudation injury when cells were subjected to simulated microgravity and hypergravity.

EXPERIMENTAL METHODS: Bovine aorta endothelial cells (BAEC) were obtained from the NIGMS/Coriell Cell Repository. Cells cultured on collagen coated flasks and collagen coated microcarier beads and maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum and 1X antibiotics.

Morphological Studies: Post confluent, Confluent and sparse BAEC were cultured in flask, confluent culture were then subjected to denudation injury, both were then centrifuged at 6G & 12G (HGrav) or clinostat rotated at 30 rpm (Mgrav). Phase contrast photomicrographs were taken at various times following injury and used to assess the morphology of cells as they moved into the denuded area. Cultures were also rinsed with Hanks Buffered Salt Solution and fixed with 10°/0 buffered formalin/2.5% glutaraldehyde for examination by scanning electron microscopy, Sparse cultures and cultures grown on Cytodex 3 microcarier beads in the horizontally rotating bioreactor were assessed similarly.

Migration and Motility Studies: Confluent, 1 -day and 4-days postconfluent (PC) cultures were subjected to HGrav (6G) and MGrav treatment, in 10% or 0.5% FBS, for 24,48, and 72 hr. At each time, an area of the culture was denuded to assess the migration of cells; cultures were kept under HGrav or MGrav following denudation. The migration of cells into the denuded area was monitored by video recording of random fields along the denuded area; cultures were recorded then returned to HGrav or MGrav conditions in the shortest time possible (5-10 rein). Similar studies were conducted using sparse cultures. We also assessed the influence of increasing hypergravity (6 and 12G) and clnostat rotation on BAEC morphology and migration. Control cultures were treated similarly, except they weremaintained under standard cell culture conditions and normal gravity, the clinostat control cultures were vertically rotated.

RESULTS AND DISCUSSION:

Endothelial Cell Migration: 1 -D post confluent and confluent BAEC maintained in DMEM containing 10% FBS did not show any appreciable differences in total distance migrated under control conditions, HGrav or MGrav , The migration of BAEC was affected by changes in the gravity environment when cultured in low FBS (0.5%). The migration of confluent and 1 -day PC BAEC were retarded by MGrav treatment but stimulated by HGrav when compared to controls (fig. 1). The migration of 4-day PC BAEC was retarded by both Mgrav and HGrav, with HGrav producing the greatest decrease in total migration. Both 12G and 6G Hgrav resulted in a 30-50% retarded migration during the early response period between 2-6hr, between 16 and 48 hr this was slightly reversedby 5- 10% .Increasing Hgrav from 6G to 12G showed no significant difference in migratory response for 0.5%FBS cultures, however there wa a 20% difference observed in the 10% FBS cultures (fig. 2 &6). The 30 rpm horizontally rotated clinostat MGrav simulation resulted in a 30°/0 enhanced migration for the 0.5% FBS cultures tduring the early reponse period of 0-6hr , contrarily the 10'% FBS cultures had a 30% retarded migratory response. MGrav retarded the migratory response by 50% for both the O. 5°/0 and 10 '/0 FBS treated cultures (fig. 3 & 7)

Morphological Assessment: Confluent BAEC subjected to HGrav and examined by scanning electron microscopy had less surface area, fewer membrane-bound vesicles, smaller and more flattened nuclei, and membrane ruffling around the edges when compared to control cells. MGrav treated BAEC cultures were less elongated and had a more cobblestone appearance than controls. Examination of BAEC microcarrier cultures sujected to prolonged horizontal bioreactor rotation, by scanning electron microscopy showed a loss of surface vesicles changes in the appearance of these cells under MGrav compared to controls (fig. 5)

Changes in Endothelial Cell Area: The results of the image analysis of endothelial cell motility are shown in Fig. 4. The 4-day PC BAEC have a 50% smaller mean cell area than confluent cell; this is seen for cell in 10% or 0.5% FBS. Increasing the serum from O. 5% to 10% resulted in a three-fold increase in cell area for confluent cell; PC BAEC cell area also increased but only by 40%. Analysis of cell area alteration in response to denudation under HGrav conditions, 4-day PC BAEC are nonresponsive to serum-induced increase cell area (or spreading). There a dramatic decrease in responsiness to serum-induced increase in cell area for confluent BAEC; cell area incrcascd only 75% compared to 300% for control cells. MGrav inhibited the responsiness of PC BAEC to serum-induced increase in cell area but resulted in an actual reversal of the serum effect with confluent BAEC. In the latter case, cells area decreased by 40% when serum levels increased from 0.5% to 10% (fig.5). Thc sparse cultures subjected to 24,48 hr Hgrav simulations showed a differences in cell shape (circularity and rectangularity) ,cells speared flatened and elongated (fig. 8)

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REFERENCES

1. Rijken PJ, et al. (199 1). Aviat. Space Environ. Med. 62:32-36.
2. Kumei Y, et al. (1989). J. Cell Sci. 93:221-226.
3. Cogoli A, Tschogg A and Fuchs-Bislin P (1984). Science 225:228-230.
4. Gruener R and Hoeger G (1990). Am. J. Physiol. 258: C489-C494.
5. Doty S, E Holton, G Durnova and A Kaplansky. (1 990), FASEB J. 4:16.
6. Philpott D, I Popova, K Kate, et al. (1 990). FASEB J. 4:73.
7. Nixon J, R Murray, C Byrant, et al. (1979). J. Appl. Physiol. 46:541.
8. Nilsson J, Volk-Jovinge S, Svensson J, Landou C, DeFaire U and Hamsten A (1 992). J. Intern. Med. 232:397-404.
9. Rosen EM, Meromsky L, Setter E, Vinter DW and Goldberg ID (1 990). Exp. Cell Res. 186:22-31.
10. Kropacova K, et al. (1988), The Physiologists 31, S75-S76.
11. Girard PR and Nerem RM (1995). J. Cell Physiol. 163:179-193.

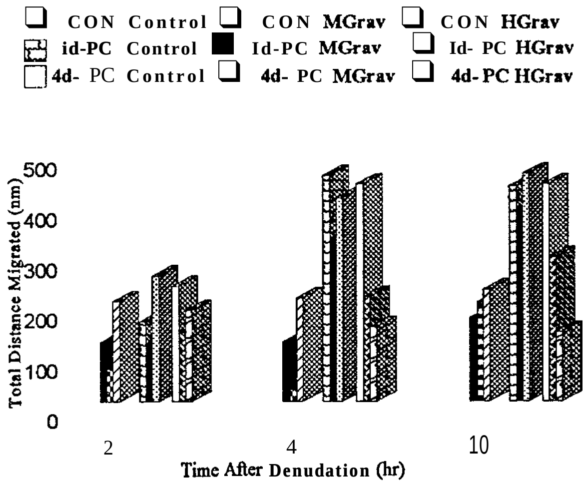


Figure 1. Alterations in BAEC migration induced by culture under HGrav and Mgrav conditons and 0.5% FBS: Dependency of BAEC migration on the degree of confluency.

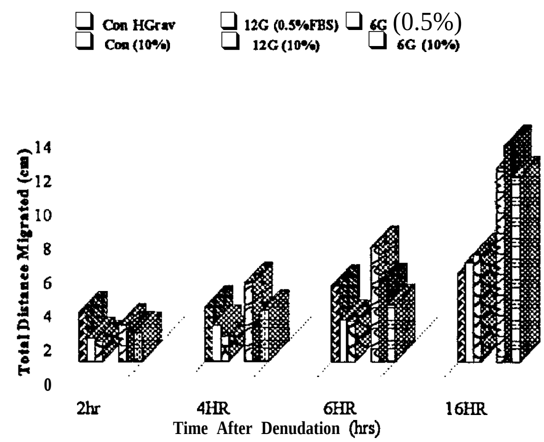


Figure 2. Alters in BAEC migration under 6 and 12G HGrav in 10% and 0.5% FBS.

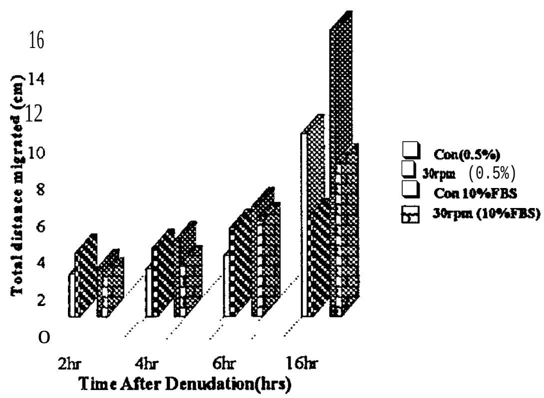


Figure 3. Alterations in BAEC migration under clinostat rotation in 10% and 0.5% FBS.

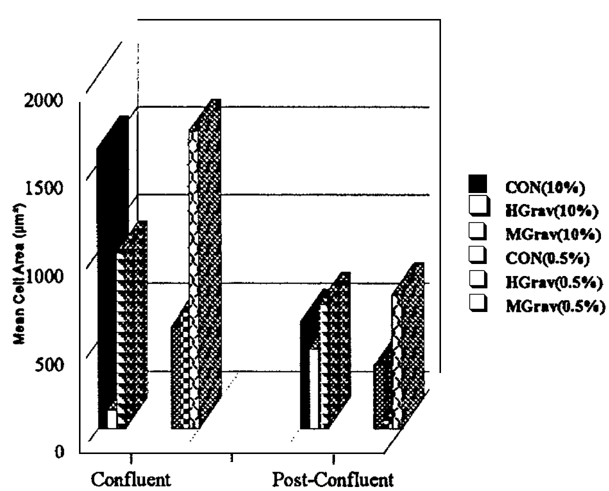
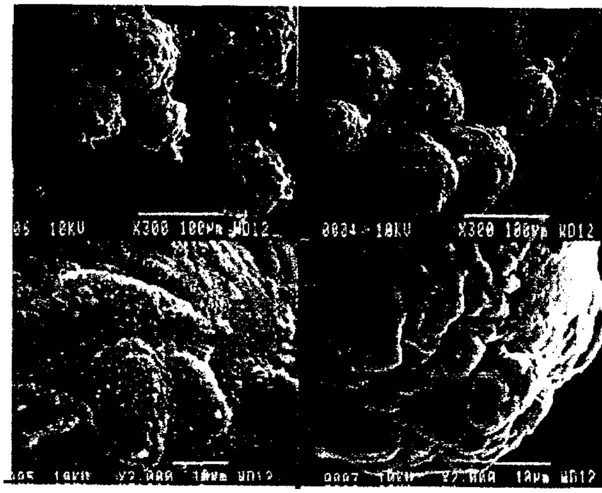


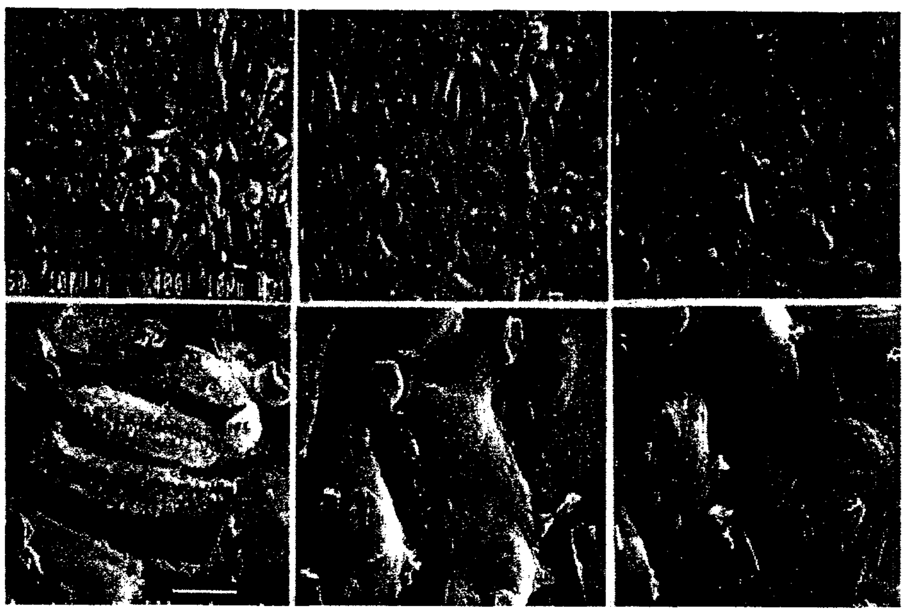
Figure 4. Changes in migrating BAEC area following culture under Hgrav and Mgrav conditions: dependency on the degree of confluence and serum levels.

A



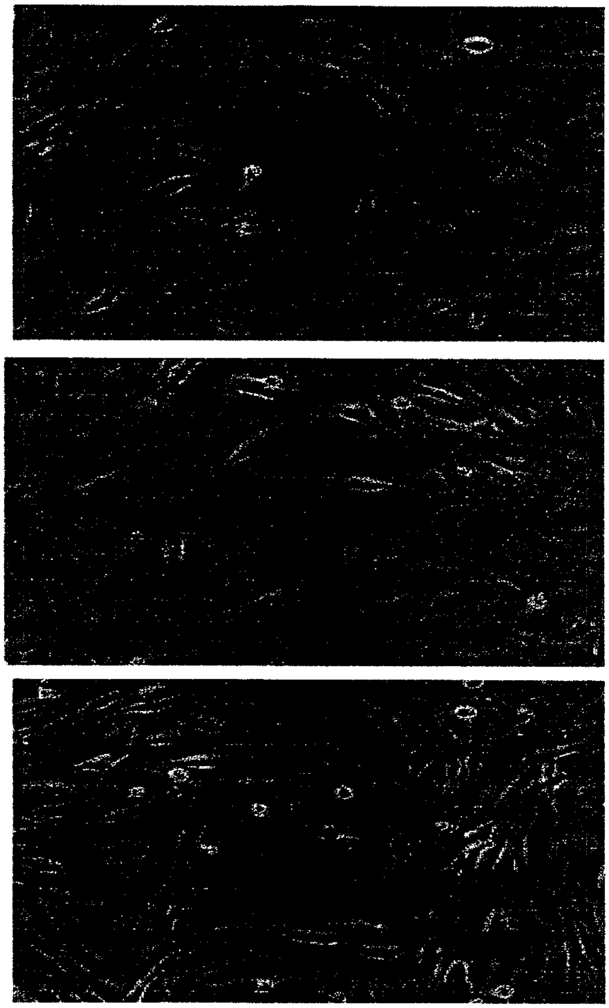
Upper: 3d rotation, 300x | Upper: 2wk rotation,300x
Lower: 3d rotation, 2000x | Lower: 2wk rotation, 2000x

B



Normogravity | Clinostat (30rpm) | Centrifuge (6G)

Figure 5. Scanning electron micrograph of BAEC cultured on Cytodex 3 microcarrier beads in the horizontally rotating bioreactor (A), and in flasks (B).



AE

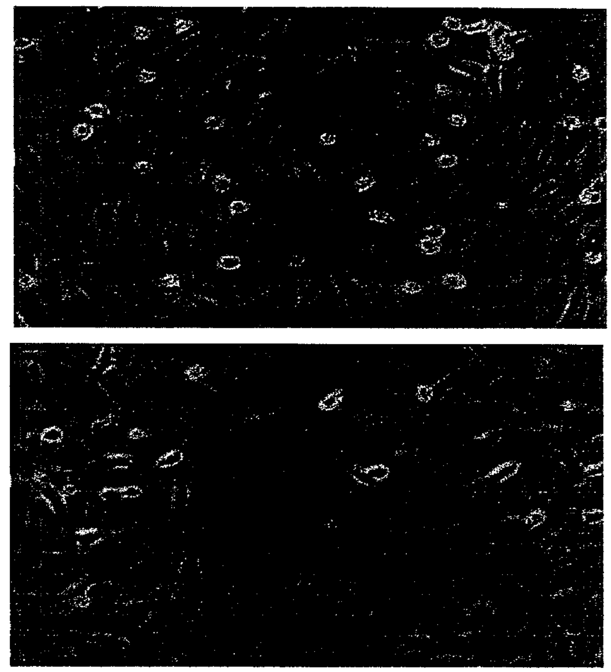


Figure 7. BAEC sheet migration after 16 hr normogravity (upper) and simulated microgravity (lower).

A

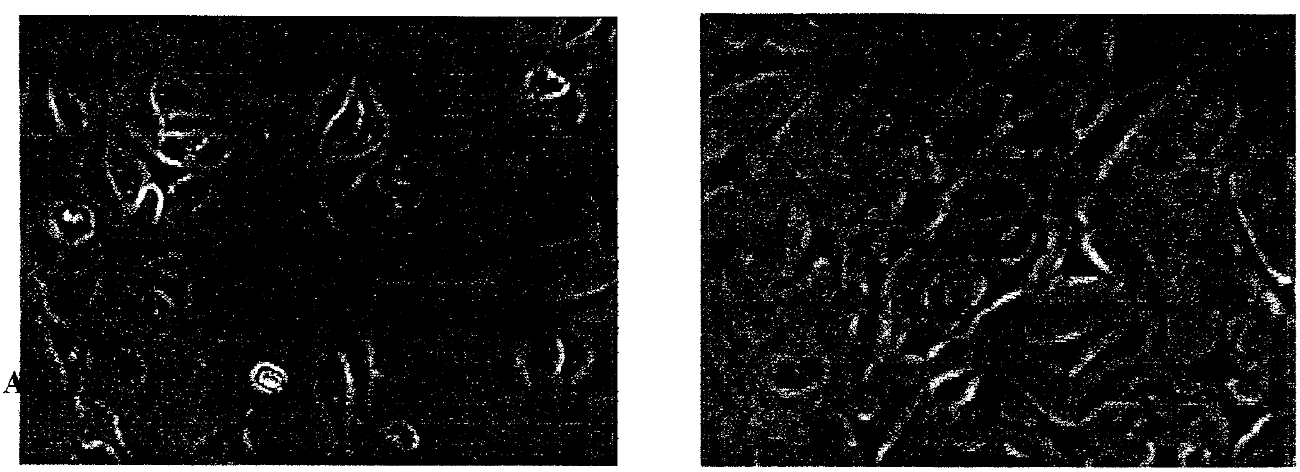


Figure 8. The change in BAEC morphology induced by 48 hr of hypergravity-6G (B) compared to non-centrifuged control (A). Sparse BAEC cultures were photographed under phase at a magnification of 250x.