THE EFFECT OF TEMPERATURE ON THE ADHESION OF CULTURED CHINESE HAMSTER LUNG (CHL) CELLS

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Cell adhesion is a multistep process involving initial contact of the cell with the surface, cell attachment and spreading on the surface, and cell growth (1,2). To determine whether cell adhesion is an active or passive phenomenon, initial cell attachment assays have previously been carried out either at various temperatures (3) or in the presence of metabolic inhibitors (4). However, all of these studies are concerned only about initial cell attachment. In this communication we report the results of a study relating the attachment of cells and their adhesion strength, at various temperatures between 4°C and 37°C.

CHL cells were maintained in minimum essential Eagle Medium with Earls salts supplemented with 20mM HEPES buffer, 10% (v/v) foetal calf serum, 200U penicillin, 200mg streptomycin, 20mM glutamine and 2% non essential amino acids. Cultures were incubated in an atmosphere of 5% CO2 air (5). In all attachment studies CHL cells were subcultured onto 35mm tissue culture grade polystyrene dishes.

All unattached cells were removed with gently washing and any cell not removed by series of these washes was considered as attached. The number of attached and non attached cells were counted in a haemocytometer and the results are presented as percent attached cells.

The strength of cell attachment when it occurred was measured using a converging channel (6).

In this system a laminar flow of medium (minus serum) is passed over the attached cells, after they have grown for 24 hours. The critical shear stress at which they are removed is measured accurately (7).

There is a direct relation between the rate of attachment and the temperature of attachment. That is, after 30 minutes of incubation, there was no cell attachment at 4°C, 9°C, 12°C and 15°C. However, there was 4% and 41% attachment at 20°C and 26°C. While during the same incubation period at 30°C and 37°C, 70% and 72% of cells were attaching respectively. Even after 1 hour of incubation there was still no attachment at 12°C and below, whereas the percentage of attached cells was 6%, 30%, 76%, 90% and 93% at 15°C, 20°C, 26°C, 30°C and 37°C respectively after an hour. Even after 24 hours incubation at 4°C and 9°C cells did not attach, though they remained 90% viable.

The adhesion strength of CHL cells was increased as the temperature increased. Although cells were attaching at 15°C this attachment was too weak to measure. However, the hydrodynamic shear stress which was required to detach cell at 20°C, 26°C, 30°C and 37°C was measured, and the values were, 6.6 ± 0.5 Nm² 8.3 ± 1 Nm², 0.9 ± Nm² respectively.

These show a significant statistical difference in critical shear stress (c.s.s.) of detachment of CHL cells between 37°C and 20°C (P = 0.001) and 37°C and 26°C (P = 0.0073).

These results suggest that initial cell attachment and final adhesion strength are an energy dependent, active phenomena.

REFERENCES