

Measuring cell adhesion forces as a function of the cell cycle by force spectroscopy

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INTRODUCTION: The study of cell-surface interactions is important for several different applications, notably that of medical implants. In the context of bone implants, CSEM is studying the effect of surface structure on the behaviour of osteoblasts. While the effects of surfaces on cell proliferation, alignment, structure and growth are accessible via traditional microscopy and labelling techniques, the determination of cell adhesion remains a major challenge. In the European project Newbone, CSEM will study osteosarcoma (SaOs-2) cell adhesion forces on structured surfaces of fibre-reinforced composite-based non-metallic bone implant materials. Atomic force microscopy, and particularly force spectroscopy will be used to directly measure the adhesion of the cell on the surface.

The adhesion of cells on surfaces depends very strongly on the phase of the cell cycle (G_0 , G_1 , S, G_2 or M). In order to obtain meaningful results, it is necessary to study cell/surface adhesion for each phase separately. For this reason, initial work has focussed on obtaining synchronised osteoblasts to compare cell adhesion by force spectroscopy in the different phases of the cell cycle.

METHODS: The Saos-2 cell line was from the ATCC collection (LGC Promochem, France) and cultured in modified McCoy's 5A Medium with L-glutamine supplemented with 10% heat-inactivated FCS. Saos-2 cells were synchronized using a protocol adapted from Galgano *et al.* [1] with the plant amino acid mimosine as a G_0/G_1 synchronizing agent and with nocodazole as a G_2/M synchronizing agent. Saos-2 cells were seeded into tissue culture flasks 24 hours before beginning the presynchronization with FCS-free medium during 10 hours. Osteoblasts were exposed to 0.4mM of mimosine or to 0.6 μ g/ml of nocodazole for 48 hours. The S phase is reached by releasing cells from mimosine arrest by removing mimosine-containing medium and adding complete growth medium for about 18 hours. DNA content was measured using an EPICS[®] XL-MCL[™] flow cytometer (BeckmanCoulter, Germany) with propidium iodide labelling.

RESULTS: The Saos-2 cells show a synchronization of 86% in the G_0/G_1 phase and of 70% in the G_2/M phase. A broader peak is obtained

on S phase synchronization reflecting the dissipation of cell synchronization 18 hours after mimosine release.

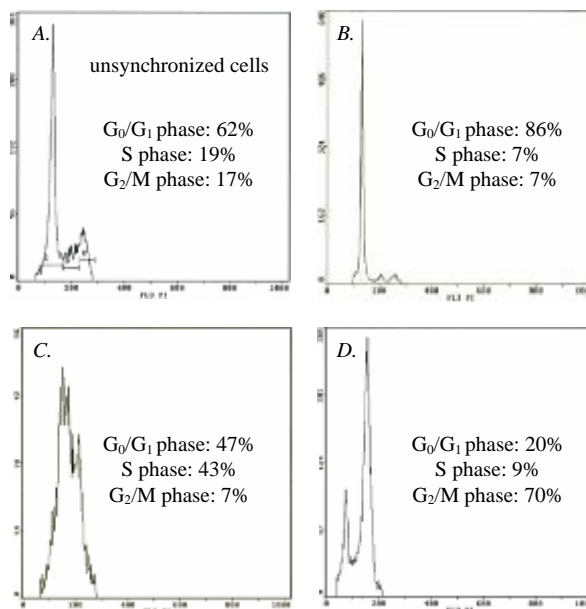


Fig. 1: Human osteoblasts (Saos-2) synchronized at 3 points of the cell cycle by cell cycle inhibitors: mimosine for G_0/G_1 (B) and S with mimosine release (C) and nocodazole for G_2/M (D). Comparison with unsynchronized cells (A).

DISCUSSION & CONCLUSIONS: Good synchronization has been obtained in the G_0/G_1 and G_2/M phases. However, we need still to optimize the releasing time after a reversible mimosine arrest to reach S phase. These will constitute the 3 checkpoints in the cell cycle to perform cell adhesion measurements by force spectroscopy via atomic force microscopy and allow an analysis of the influence of surface structure on cell adhesion.

REFERENCES: ¹P. J. Galgano *et al.* (2006) *CSH Protocols* doi:10.1101/pdb.prot4488.

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