A Novel Assay for Cell Invasion using Cytostar-T Scintillating Microplates

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INTRODUCTION

Cell invasion is typically measured using micro-porous filters coated with extra cellular matrix (ECM), which forms a barrier between an upper chamber containing cells and media, and a lower chamber containing media plus chemo-attractant. Counting the cells which invade through the filter to the lower chamber can be difficult and time consuming. We have developed a scintillation proximity assay using 96-well Cytostar-T™ scintillating microplates, to measure the invasion of [^{14}C] and [^{35}S] labeled cells through ECM gel. In the test wells, a lower layer of ECM gel is added to form a barrier preventing the labeled cells from reaching the scintillant containing baseplate. The labeled cells are then added in an upper layer of ECM gel, and the microplate is incubated overnight. Only cells invading the lower layer of ECM gel and gaining proximity to the scintillant generate a signal in the assay. A variety of cell lines with invasive and non-invasive phenotypes have been examined, including the effects of inhibitor compounds.

METHOD

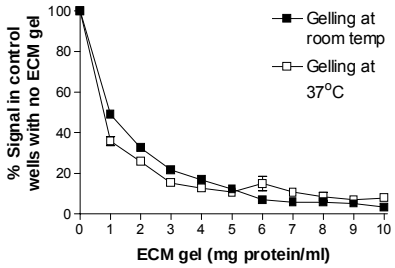

***Figure 1**: Testing the gelling properties of ECM gel by measuring suspension of dense [^{3}H]labelled particles. The Cytostar-T microplate was counted in a Wallac MicroBeta™ scintillation counter after 5 min incubation.*

The properties of ECM gel (Sigma, E1270, 8-12mg protein/ml) were tested using streptavidin coated 3µM particles of yttrium oxide (density >4g/cm^3), labelled with [^{3}H]biotin and mixed with DMEM diluted ECM gel (30µl/well). A detectable signal in the MicroBeta was generated when these labelled particles touched the base of the Cytostar-T microplate wells. At 37°C, gel at >3mg protein/ml efficiently suspended these particles.

For assays with cell lines, the following protocol was developed. Each well received 30µl 4mg/ml ECM gel. Control wells contained either 30µl 100% ECM gel or 30µl medium. ECM gel was diluted with complete medium on ice, then added to the plate and incubated at 37°C for 2 hours to gel. To the wells containing a lower layer of gel, cells (50,000/well) were added on top as a suspension in 4mg/ml ECM diluted in complete culture medium. To the control wells without gel, the cells were added in culture medium. The microplate was incubated overnight and counted on the MicroBeta.

RESULTS

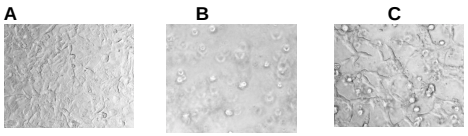

***Figure 2:** Photomicrographs of PC-3 cells (invasive prostate adenocarcinoma) after o/n incubation in (A) medium only (F-12K/7% FBS), in (B) 4mg/ml ECM gel over a lower layer of undiluted gel, or in (C) 4mg/ml gel over a similar dilution of gel in the lower layer. The microscope was focused on the base of the well.*

In order to quantify the number of cells reaching the baseplate, the cells were added to the microplate in an upper layer of 4mg/ml ECM gel containing 25nCi per well [^{14}C]leucine, methionine or [^{35}S] methionine.

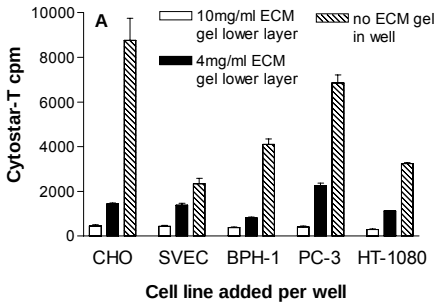

***Figure 3A**: Cytostar-T counts for cell invasion assay using various cell lines. CHO (non-invasive) and PC-3 (invasive) were added to 4mg/ml ECM gel diluted with F-12K/7% FBS. SVEC4-10 (invasive vascular endothelial) and BPH-1 (non invasive benign prostate hyperplasia) were added in ECM diluted with DMEM/10% FBS. HT-1080 (invasive fibrosarcoma) were added in ECM gel diluted with MEM/10%FBS. The microplate was incubated overnight before counting in a MicroBeta. Results are presented as means (n=3).*

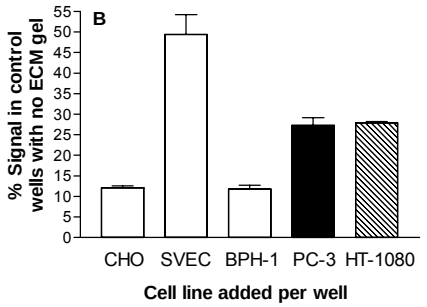

***Figure 3B:** Data from Figure 3A has been normalised by expressing the results as a percentage of the signal obtained in control wells containing cells but no gel.*

Cell lines giving the highest counts in Figure 3 are those with an invasive phenotype, in the order SVEC > PC-3 /HT-1080 > CHO > BPH-1. These responses were investigated further by varying concentration of ECM gel in the lower layer.

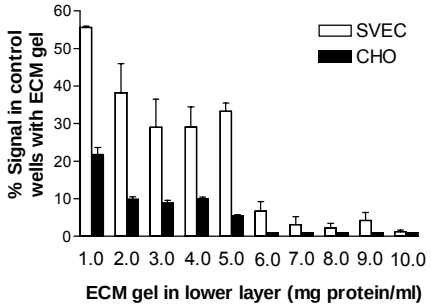

***Figure 4**: Invasion of SVEC and CHO cells through a varying concentration of ECM gel in the lower layer. The cells were added in the top layer as a suspension in 4mg/ml ECM gel. All ECM dilutions were in complete culture medium. Results are normalised using values for control wells with cells but no gel.*

Inhibition of the invasive response was investigated by incubating the cells with Cytochalasin B and Paclitaxel for 5 minutes before addition to the top layer of ECM.

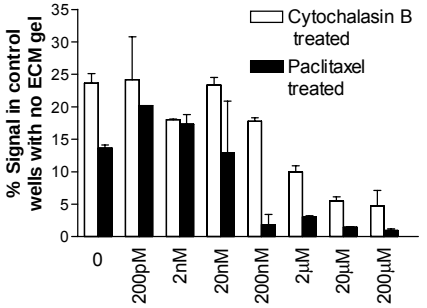

***Figure 5:** Inhibition of PC-3 cell invasion using Cytochalasin B and Paclitaxel*

The invasive cell response was not stimulated by diluting the lower layer of gel with NIH 3T3 conditioned medium instead of normal complete culture medium.

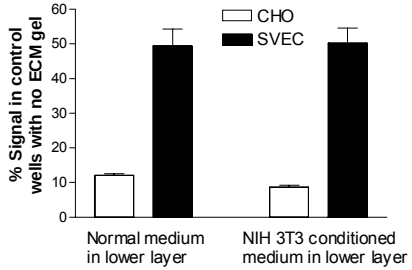

***Figure 6:** Effect of using either complete culture medium or NIH 3T3 conditioned medium to dilute the lower layer of gel.*

CONCLUSIONS

- A simple assay for cell invasion has been developed using Cytostar-T microplates, which uses as a barrier a layer of ECM gel diluted with the culture medium of the test cell line.
- The response of several invasive and non-invasive cell lines has been investigated and the results match the reported invasive potential of these cells. Inhibition of this response was observed using Cytochalasin B and Paclitaxel.
- The assay incorporates in-well amino acid labelling of the cells, using either [^{14}C] or [^{35}S], and the isotope at 25nCi/well.

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