

Gelatin degradation assay – Buccione Lab.

Preparation of fluorophore-conjugated gelatin

This preparation is performed according to (Mueller and Chen, 1991), with the following modifications. Two¹ mg/ml of gelatin are dissolved in a buffer containing 61 mM NaCl and 50mM Na₂B₄O₇ (pH 9.3) and then incubated at 37°C for 1 hour. After this incubation time, 2 mg/ml of FITC² (or rhodamine ITC) are added and mixed for 2 hours in complete darkness. This mixture is then dialysed o.n. at RT in PBS in complete darkness. Dialysis is usually repeated for 2 days with 2-3 buffer changes per day. After a quick spin to remove insoluble material and addition of 2% (w/v) sucrose and 0.01% thimerosal, small aliquots are stored in the dark at 4°C.

Preparation of fluorophore-conjugated gelatin coated coverslips

Fluorophore-conjugated gelatin coated coverslips are prepared and the assay carried out as described (Baldassarre et al., 2003; Bowden et al., 2001; Mueller and Chen, 1991). Briefly, glass coverslips are sterilized in 70% ethanol for 15 minutes at RT. Air-dried coverslips are then coated with fluorophore-conjugated gelatin, using enough to cover the surface. Each coverslip 1 drop of 0.5% ice-cold glutaraldehyde in PBS and is inverted onto a 200 incubated at 4°C for 15 minutes. Each coverslip is then transferred to a well of a standard 12-well plate with the coated side up, gently washed three times with PBS and finally incubated with sodium borohydride (5 mg/ml) in PBS for 3 minutes at RT. Washed coverslips are sterilized again in 70% ethanol for 1 minute, dried for 8 minutes under a sterile hood and then quenched in complete medium for 1 hour at 37°C. Coverslips are now ready for seeding cells.

Procedure

Cells are cultured on gelatin-coated coverslips for variable lengths of time (1-16 hours) and then fixed and processed for immunofluorescence as described above. To determine the number of degrading cells for each experiment we consider random fields (containing at least 100 cells) at a 63X magnification as previously described (Baldassarre et al., 2003). Most experiments, however are performed by incubating cells in the presence of a broad spectrum metalloprotease inhibitor (e.g. GM6001 from Chemicon at 25-50 micromolar) o.n. or longer, to allow cells to adhere and spread whilst blocking the formation of invadopodia. The inhibitor is then washed out to allow for synchronous invadopodia formation and ECM degradation, which can thus be effectively uncoupled from the former processes. This protocol is also useful for long drug or siRNA treatments.

¹Cells may display different behaviours towards the substrate, for instance some are more aggressive and/or generate more tension. Thus depending on the cell type, the final concentration of gelatin might need to be tweaked to ensure that the experimental readout is within an acceptable (i.e. measurable) range.

²In our experience, there is no need (if not for specific technical reasons) to conjugate gelatin (or other ECM substrate) with the more costly, modern dyes or the ready-made conjugates. Fluorescein and rhodamine work just fine and seem to behave well in terms of photobleaching.