Reducing background fluorescence reveals adhesions in 3D matrices

Kristopher E. Kubow & Alan Rick Horwitz

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We have been made aware of a manufacturing issue with the rat-tail collagen solution we used in the above recent study. Life Technologies (Invitrogen) informed us in July 2012 that they made a mistake in the measurement of the concentration of multiple lots of rat-tail collagen solution (catalogue #A10483) that they sold in 2010, including lot 759437, which we used. They certified the concentration to be 5 mg ml⁻¹ but now report that it was actually 3 mg ml⁻¹. We used this collagen solution in one supporting experiment (Supplementary Fig. S1i–n) of our paper. Although Life Technologies' error does not change the conclusions of the paper, it should be noted that the collagen gel concentration used in this one experiment was actually 1.2 mg ml⁻¹ instead of 2 mg ml⁻¹. No other experiments were affected.

We repeated the experiment shown in Supplementary Fig. S1i–n in 2 mg ml⁻¹ collagen gels and obtained similar results (Fig. 1a–d below). In these experiments we used rat-tail collagen from BD Biosciences (Bedford, Massachusetts, catalogue #354249) because it was not possible to make 2 mg ml⁻¹ gels with the 3 mg ml⁻¹ solution provided by Life Technologies. Experiments using rat-tail collagen from Life Technologies (catalogue #A10483) at the maximum possible concentration (1.8 mg ml⁻¹) gave similar results (Fig. 1e–f).

Figure 1 HT-1080 cells expressing promoter-truncated EGFP-paxillin in collagen gels. HT-1080 cells, expressing promoter-truncated EGFP-paxillin, were cultured in 2 mg ml⁻¹ rat-tail collagen gels (BD Biosciences) and imaged at 18–24 h to determine the percentage of cells with visible adhesions (see original paper for full experimental details). Results were compiled from three independent experiments. (a,b) Representative fluorescence z-projections of HT-1080 cells with visible adhesions (arrows). Bar: main images, 10 μm; insets, 5 μm. (c,d) Percentage of fluorescent HT-1080 cells with at least one visible adhesion, binned according to depth in the gel or to the relative intensity of the protrusion background fluorescence ($n = 27$ cells; see original paper for details). (e,f) The same quantifications were performed on cells in gels made from rat-tail collagen from Life Technologies ($n = 23$). Cells with visible adhesions were observed at depths up to the limit of the working distance of our objective (~300 μm).