Hold-'Em In Place

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We've used gelled agarose to cover & hold monolayer cells in place when treatment leads to cell lifting. Low-gelling temperature agarose (Type VII) (Sigma A 4018 or A 0701) can be made as a 2% stock solution in "phosphate-buffered saline" (PBS) (boiled then kept at 42° C to remain liquid). The agarose can be stored at room temperature at this time, but must be boiled and melted again before use.

1) Immediately before applying to the cells (with the experimental and/or control treatment), dilute the stock to a final agarose concentration of 0.8% in 2X culture media (37° C) appropriate for the cells.

2) Apply a volume of the agarose solution to the monolayer, incubate approximately 5 minutes at room temperature or briefly at 4° C to gel the agarose.

3) Transfer the slide to the incubator to keep the cells metabolically active.

The slides used for this application are very important. Teflon masked slides from Erie Scientific (Portsmouth, NH 1-800-258-0834) proved to be the most useful as they create wells without a gasket. However, the agarose forms a bubble in each well and prevents quality visualization. To circumvent this issue, we used an optics kit form CSM, Inc. (Phoenix, AZ; http://creative-sci.com). The kit includes a mechanism to suspend a coverslide over the bubble of agarose which eliminates the distortion and does not put pressure on the cells. The optics kit was placed on the agarose just after application and BEFORE it gelled. As a unit, the two separated slides, with the gel in between them, were allowed to sit at room temperature (or placed in the refrigerator very briefly) to allow the agarose to cool and gel. It should also be noted that the temperature of the agarose/media solution cannot drop below 37° C and therefore, temperature must be monitored carefully. ■

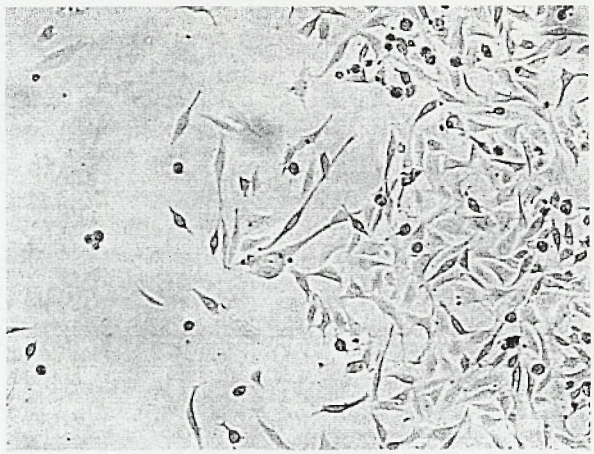

Figure 1. Untreated cells growing in MEM + 10% FBS media

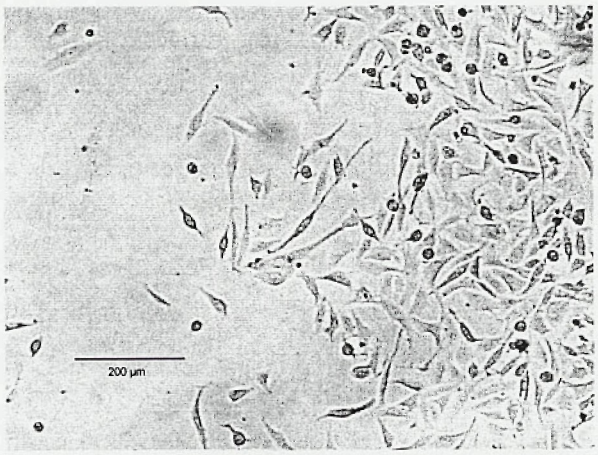

Figure 2. The same cells under 0.5% agarose (Sigma, type VII, cat# A-4018) before C2 ceramide (100ng/mL) has taken effect.

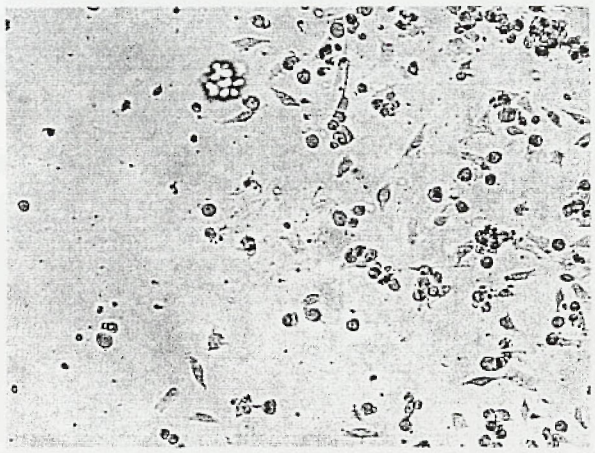

Figure 3. The same cells under 0.5% agarose after 1.5 hours treatment with C2 ceramide. The rounded cells demonstrate loss of cell attachment occurring from cell death. Without the agarose, the cells would have lifted from the slide and floated away.

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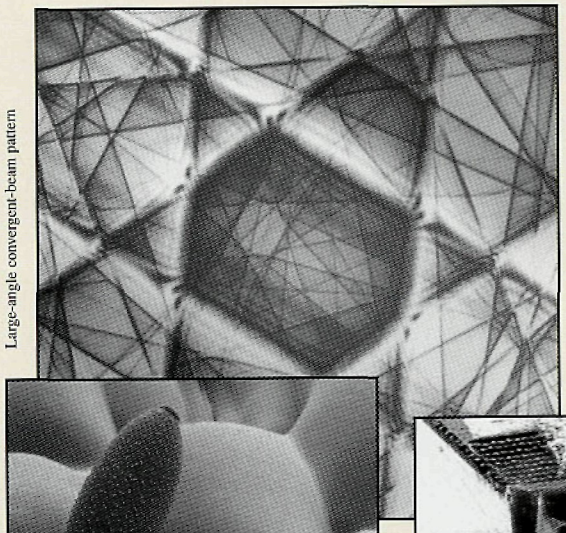
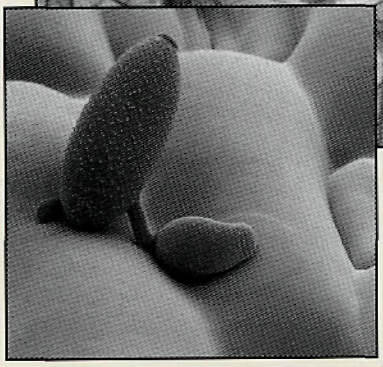
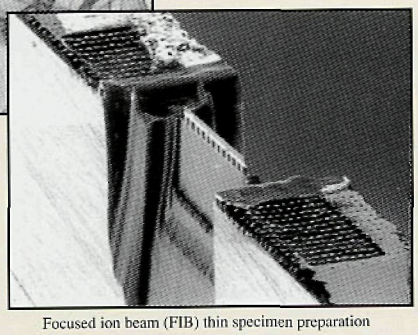


Large-angle convergent-beam pattern



Germinating powdery mildew on a wheat leaf imaged at 5kV and -150°C in a Philips XL30 FEG-SEM (courtesy of Alison Daniels, ArgEvo UK)



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