How to Plate Cells for Microbeam Experiments

Preparing Dishes

As most mammalian cells plate poorly on polypropylene, the polypropylene film on the bottom of microbeam dish wells is treated with Cell-Tak (BD Biosciences) to enhance cell attachment. For best results, Cell-Tak is coated at a concentration of about 3.5 μg/cm^2 onto the polypropylene film. Note that the Cell-Tak precipitates out of solution almost *immediately*. Mixing the Cell-Tak and the sodium bicarbonate in a separate vessel before applying to the cell dishes will result in the Cell-Tak precipitating in the vessel, not on the dishes, so the following protocol should be follwed without modification:

- Dilute the stock solution to 1 mg/ml with sterile water.
- Dilute an appropriate amount of the stock solution to 100 μg/ml to make a working solution.
- Add 10 μl of the working solution to each well.
- Add 10 μl of 0.2 M sodium bicarbonate solution and mix thoroughly, making sure that the entire polypropylene surface is covered. This step will precipitate the Cell-Tak from solution allowing it to coat the polypropylene surface.
- Incubate at 37° C for 30 min. (We have been using the dry incubator in the microbeam lab for this purpose).
- After incubation, rinse each dish three times with 5 ml of sterile distilled water and return to the incubator until ready for use. Note: If dishes are to be used on some other day, it is best store them in the refrigerator once they are dry. For maximum performance, dishes should be used within 15 days of preparation.

Plating Cells

To enable the microbeam image analysis system to locate cells, they must all be plated in the center of the dish. This is accomplished as follows:

- Determine the number of cells to be plated (100-300 cells per well works best).
- Trypsinize cells and bring them up at a concentration that will enable you to plate the desired number of cells in a total volume of 2 μl, *e.g.*: a concentration of 10^5 cells/ml will allow you to plate 200 cells in a 2 μl droplet.
- Using a micropipetter place the droplet as close as possible to the center of the dish and cover the well with a sterile 18-22 mm square or 18-22 mm D coverslip (Note: coverslips can easily be sterilized by soaking them in 70% alcohol and flaming or by autoclave.). Place the dishes in an incubator and incubate until cells attach to the polypropylene. Depending on the cell line this will take between 5 and 30 min. A small amount of medium may be added to the dish adjacent to the well if attachment does not occur quickly.
- After cell attachment, carefully add 5 ml of medium to the dishes. This should be done gently to avoid removing cells from the polypropylene. At this time coverslips may be removed. Cells will typically flatten out within 1 to 3 hours.