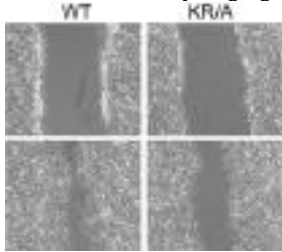


Wound Assay Protocol

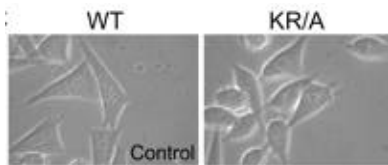
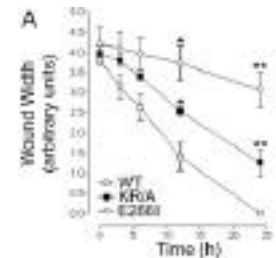


Introduction: Tissue wounds undergo a complex and ordered series of events to repair tissue. These events may include infiltration of inflammatory immune cells as part of the process to remove and destroy necrotic tissue, increased vascularization by angiogenic factors, and increased cell proliferation and extracellular matrix deposition. Wound healing assays have been carried out in tissue culture for many years to estimate the migration and proliferation rates of different cells and culture conditions. These assays generally involve first growing a confluent cell monolayer. A small area is then disrupted and a group of cells destroyed or displaced by scratching a line through the layer. The open gap is then inspected microscopically over time as the cells move in and fill the damaged area. This “healing” can take from several hours to over a day depending on the cell type, conditions, and the extent of



the “wounded” region. For detailed background on cell motility see the handout that Dr Provost has.

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The cells must be nearly confluent (~90%) for this to work. For successful wound assays it is critical that everything you do to the cells is done aseptically. Simply put that means you must use agonist, inhibitors, activators or other additives that are filter sterilized. Tips used for scratching must be sterilized. Handling of the cell dishes must be done carefully. Otherwise by the time you get to the most important late time point you will get to take nice pictures of contamination. Also

remember to keep the cells in the incubator while waiting in between pictures. It is important at the 6 and 12 hour time points to take a couple of good higher mag pictures 40X or greater. The changes in morphology at the leading and trailing edge of the cell can tell us quite a bit about the process of cell migration. The figure above is taken from the Denker and Barber paper. Note the manner in which data are quantitated, the overall framing and details of the wound assay and the “I am ready for my close-up Mr. Demille” shots of the cells. KR/A are NHE mutant cells. Read the following paper for details on method and interpretation: Sheryl P. Denker and Diane L. Barber. The Journal of Cell Biology, Volume 159, Number 6, 1087-1096.

Protocol

Prior to the assay:

- 1) Cells should be cultured to confluence or near (>90%) confluence in either 6 well dishes or 35 mM dishes.
- 2) Depending on the conditions, cell should be rinsed with PBS and starved in low serum media (1.5 ml; 0.5% - 0.1% serum in DMEM) overnight.

On the day of the assay:

- 3) Prepare 10ml of base media with any additives. Sterile filter the mixtures and place in sterile 15 ml falcon tubes. Store at 4°C. *Warm up before using.*
- 4) Draw a line with a marker on the bottom of the dish.
- 5) Using a sterile 200 µl pipet tip, scratch three separate wounds through the cells moving perpendicular to the line drawn in the step above. See the figure for arraignment of the scratches.
- 6) Rinse the cells (very gently as sheets of the cells may lift off if you are not careful) with PBS and replace with 1.5 ml of media containing any additives (agonist, inhibitors, activators, ect...).
- 7) Take a picture using phase contrast and 10X. Do this just above and just below each line. This will help orient your measurements. Make certain the line just appears in each picture. Name each picture 6hr2LA - This indicates the 6th hour, dish 2, Left scratch (vs right or center) above the line (vs B for below).
- 8) Take another picture at 6, 12 and 24 hours. After each measurement, replace the old media with the media/additives that you prepared in step 3.

