**Cell Block: Cell Migration using Scratch Assay**

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**Overview**:

* Used to determine cell migration. This is different from cell proliferation.

**Pedagogical and implementation notes:**

* Works best after drug concentration assays. Students generally do this as follow up experiments after the MTT assay. You must determine appropriate drug treatment concentrations before you can do this assay
* Works well with exosome and drug treatments
* Students can also do cell migration invasion/migration assay with Boyden chambers. However, the scratch assay is cheaper and can be done with limited supplies.
* While 250,000 cells per well (6-well plate) is recommended. You can simply plate cells and allow them to grow to 70-80% confluency. Do not let cells overgrow.
* It may help to put the pipette tip on a micropipette and use it as if it were a pencil

**Estimated Time**

 3-4 days.

**Special Material/reagents**

* Low serum media. Low serum reduces cell growth. If cells are growing too fast it is hard to determine cell migration from cell proliferation
* Caspase inhibitors may be added to prevent apoptosis

**Assessment: (**notes and recommendations**)**

Students are required to present the rationale for choosing this assay. At OBU, I require students to present a research plan 4 weeks into the semester. Part of this plan is the presentation of their MTT/drug concentration data. Students must present information from the literature to justify this assay.

 **Pre-Lab** - Before the experiment can begin student research teams must have a tentative plan outlining the timing of their experiment. The plan must include cell plating dates, drug concentration, supplies needed, how many days they will take pictures, and who is responsible for each step.

 **Post-lab –** Data analysis. Graph or images. Notebook and in final lab presentation. Data is sometime presented in 5min “lab meeting” format through out the semester.

**Link to Video Protocol**

<https://www.youtube.com/watch?v=qbyUsSgIieU&t=10s>

**Written protocol**

1. Create a dilution of cells at 175,000 cells/ml. You need 14 ml of media in total for one 6 well plates. (you can save some cells for maintaining cells lines). Use appropriate media containing the regular amount of serum. (see notes for more information on plating cells)
2. Using a 6 well plate, plate 2 ml of the media with the cells into each well.
3. Allow the cells to adhere overnight in the incubator.
4. Aspirate off the old media careful not to touch the bottom of the well.
5. **VERY** gently, place 2 ml of **low serum media** (1% FBS) in each well. Go slowly as to not disturb the cells. If any treatment is being used, it should be used in this media.
6. If using a treatment, allow the cells to incubate overnight or the desired time; if no treatment is being used, you may skip this step. This step is optional and specific timing must be determined by each research group.
7. Starting at the top of the well and going down, carefully take a 200 μl plastic pipette tip on the end of a micropipette and make a single scratch down the middle of the well. Be sure to be as aseptic as possible. It may help to put the pipette tip on a micropipette and use it as if it were a pencil
8. Take pictures of the scratches to act as time 0 for the assay.
9. Incubate the cells for 24 and then 48 hours and take pictures at each time.
10. Record time ti takes cells to move into scratched space. Programs such as ImageJ (<https://imagej.net/Welcome>) can be used to measure gap distances. Data can be graphed.

**Data Interpretation and Analysis**

**Image of scratched cells Day 0.** 

**References and Reources:**

 Image J: <https://imagej.net/Welcome>