Report #1 for Sonic Medical, From Gray Box Biology
Mount Sinai School of Medicine

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Report #1: Effect of A Lift device on Madin-Darby Canine Kidney cells

As a starting point to investigate the properties of the A-lift device on a cellular behavior, the well established Madin-Darby canine kidney (MDCK) cell line was used. This cell line is the "gold standard" for studying epithelial cells, and is also indispensable in the study of inþuenza growth. The first set of experiments was conducted to determine if the A LIft device had a measurable impact on the normal metabolism of MDCK cells. In a typical cell culture system, a relatively small amount of cells is placed on polystyrene or glass substratum where they attach and begin to multiply. During this period of growth, the cells are highly metabolically active, and do not fully exhibit the properties of epithelial cells. After a few days, the entire surface of the substratum is covered with cells. Approximately 250,000 cells will fit in an area of 1 cm^2. At this point, there is no additional space for the MDCK cells to expand, and they cease to multiply. The cells are less metabolically active, and exhibit the properties of epithelial cells.

To measure the metabolic activity of plated MDCK cells, the chemical MTT ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) is added directly to cells. After approximately 90 minutes, the metabolic machinery of the cells will convert the light yellow MTT substrate into a dark blue formazan product. Solubilization the the cells and the formazan product gives a homogenous blue solution whose color density (absorbance) is proportional to the total metabolic activity of the cells. The absorbance is quantified using a spectrophotometer set to measure the absorbance of light with 595

nm wavelength. An example of a typical result obtained with the MTT assay is shown in figure 1, where the metabolic activity (asborbance at 595 nM) of varying numbers of MDCK cells is plotted. The assay is performed approximately 16 hours after the cells are plated giving time for nearly one full round of cellular replication. Cells that were plated at a density between 3% and 25% have a roughly linear increase in their metabolic activity, proportional to the number of cells. However, even though cells plated at 100% density have the maximum number of cells, their overall total metabolic activity is lower than cells plated at 50% density. This is due to their conversion into a less metabolically active state.

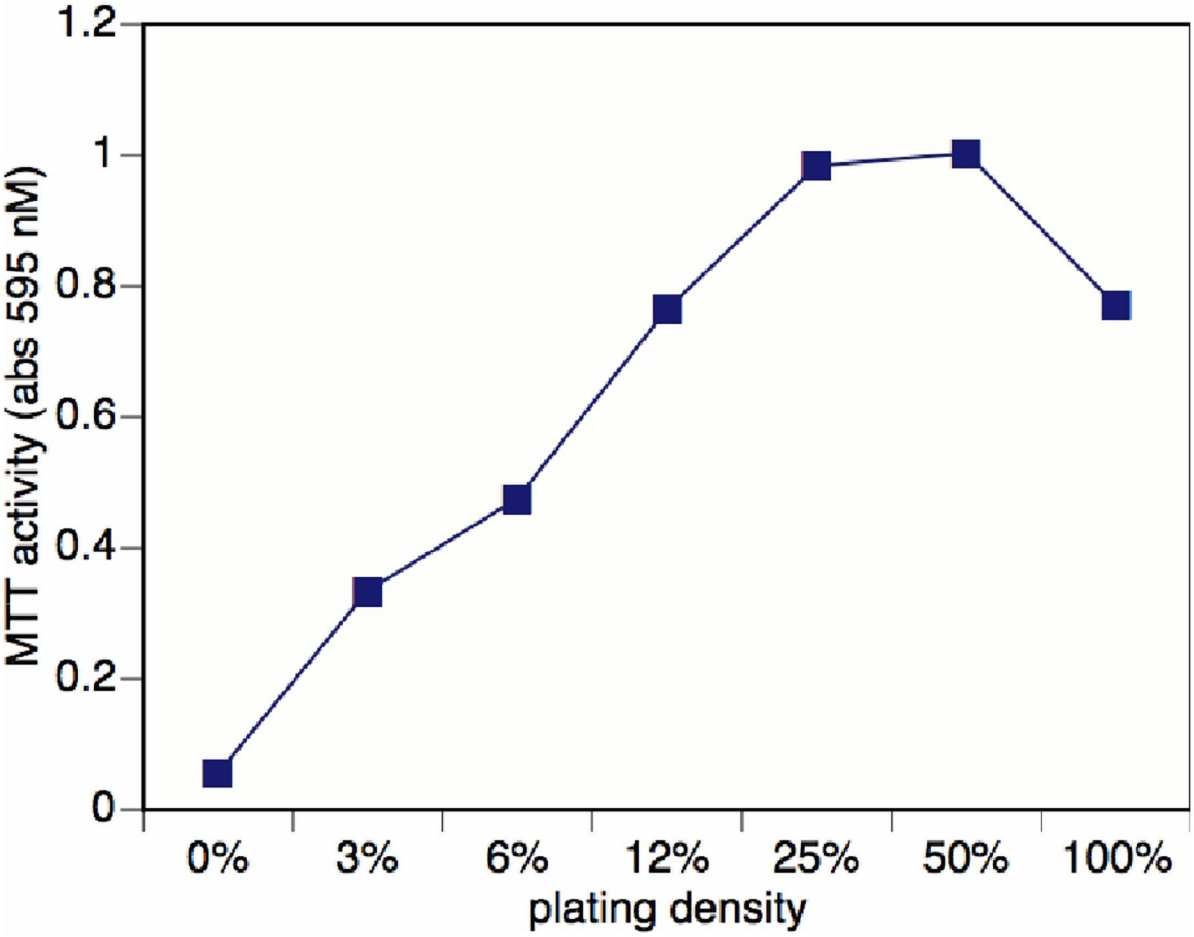
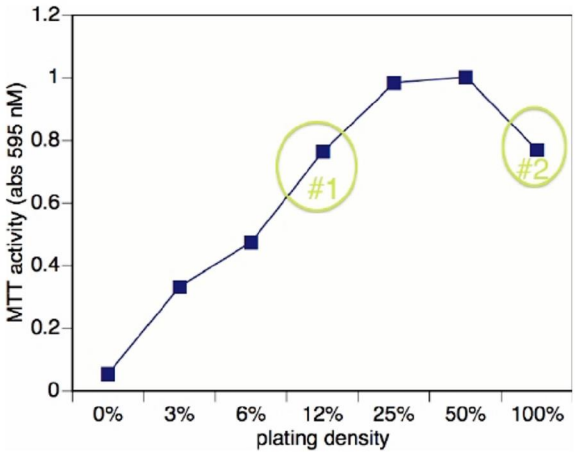


Figure 1: MTT assay showing effect of plating density (~cell number) on activity.

Thus, one caveat to interpreting the results of this type of analysis is that the conversion of the MTT to formazan is a function of both the total number of cells, and their individual metabolic activities. A large population of cells with low metabolic activity can have the same formazan producing ability as a smaller population of cells possessing a high metabolic activity. When conducting an experiment to determine if the A Lift alters the metabolic activity of cells compared to cells not receiving treatment, it is important that both conditions begin with the same number of cells.

Since it was hypothesized that the A Lift device might have an effect on the metabolic rate of cells, our first experiments focused on using the MTT assay on cells at a particular density. The first experiment was performed using cells plated at approximately 12% density (still actively growing), while the second experiment was conducted using cells plated at 100% (see figure 2).

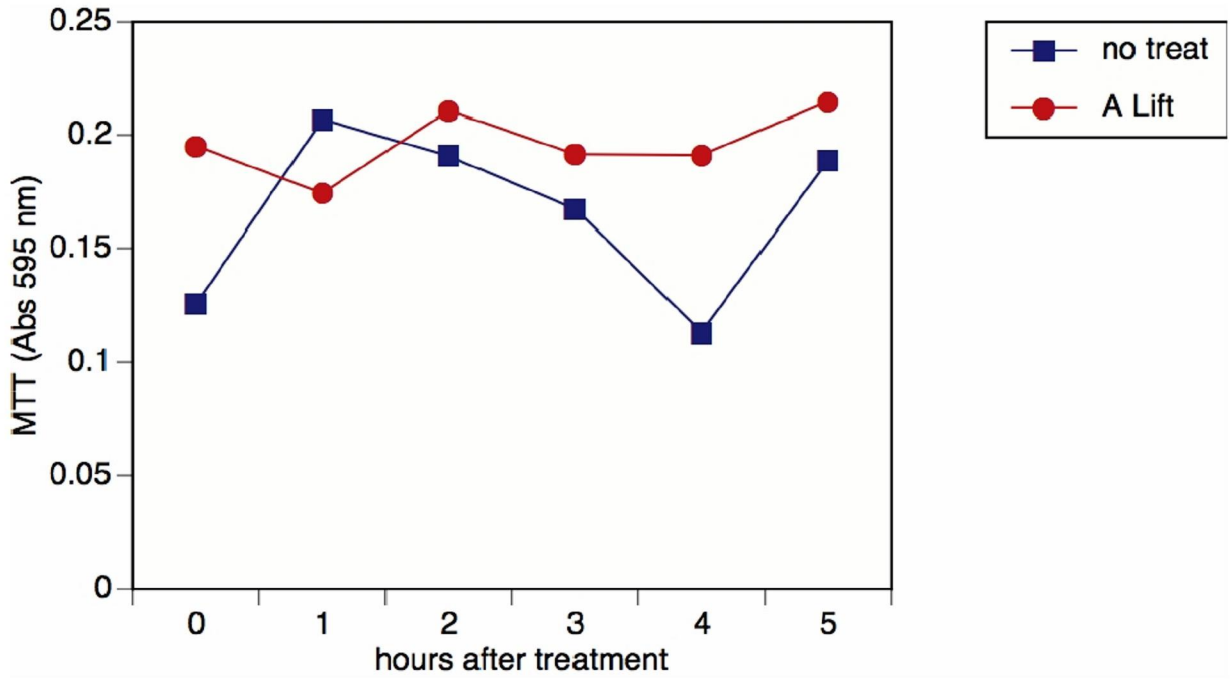


Experiment #1: Effect of A Lift on rapidly growing cells i

For the initial experiments, MDCK cells were grown on 1 cm^2 glass coverslips and transferred into a custom built chamber connected to the A LIft device. The chambers were filled with phosphate-buffered saline (PBS) containing 1 mM calcium chloride and 1 mM magnesium chloride which provides a suitable medium for applying the electrical field to the cells. Each cell containing coverslip (referred to hereafter as "coverslip") was exposed to a single treatment at room temperature. For an experimental control, cov-

erslips were placed in a separate chamber that was not connected to the A Lift device but exposed to the PBS for same amount of time. At the end of the treatment time the coverslips were returned to a culture dish containing normal growth medium and placed in a 37°C incubator and cultured in normal growth medium. The amount of time spent in the incubator was varied from zero to five hours. After the incubation period, the coverslips were subjected to the MTT assay.

Based on the preliminary experiments, it was anticipated that the non-treated coverslips would have a steady increase in MTT activity over time, due to the increasing replication. However, the experiment revealed a slight decrease in activity over time (blue line, figure 3). Coverslips that received A Lift treatment (red line, figure 3) demonstrated the predicted increase in activity over time.



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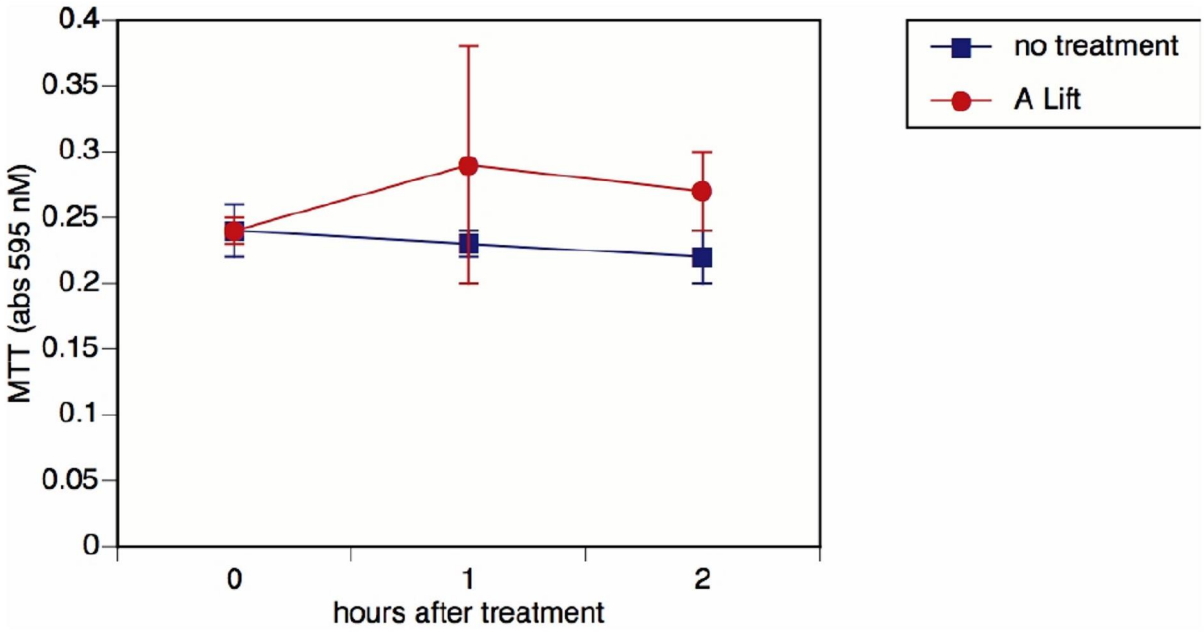
Experiment #2: Effect of A Lift on cell in fully differentiated phase

Cells on coverslips were grown until they reached a fully mature epithelial state, to reduce the variability between samples. With cells in this state, it was anticipated that the metabolic activity would be constant over time in non-treated coverslips. Using three coverslips for each time point, the activity of non-treated coverslips remained the same 1 and 2 hours after incubation at 37°C. In comparison, coverslips that received A LIft treatment exhibited higher MTT activity at 1 and 2 hours after incubation at 37°C. This increase is interesting, but does not appear to be statistically significant. It does suggest that the A LIft has an effect on the cells that have already stopped dividing. Additional experiments will be conducted to determine if this effect is reproducible.



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Conclusions and future directions

These first two experiments demonstrate that the cell culture system and MTT assay are working correctly with the A Lift device.