after a 24 hour incubation. Viability was measured at 490nm using a –1, MAP2, Nestin, GFAP, and O1 (Figure 3).

A migration assay was performed using 96-well Migration Assay:

Viability Assay:

A viability assay was conducted to investigate the effect of (Millipore) was used and the cell lines were tested for five markers: SSEA-, cell types after treatment. A Neural Stem Cell Characterization Kit remain in the media. All cells were maintained at 37°C and 5% CO2.

three cell types may be reacting to different components of the UBM. Therefore, each cell type may be responding to different UBM components as well as different signaling pathways. It has been previously shown that UBM degradation products act as a positive chemotactic agent, and a mitogenic agent for undifferentiated cells only. Therefore, the increased migration in undifferentiated P19 cells, and decreased migration in P19 cells exposed to UBM were expected (Figures 6&7). However, post differentiated P19 cells also demonstrated an increased chemotactic response (Figures 6&7).

This experiment differs from previous studies in that the cells are being identified as differentiated, as opposed to primary, differentiated cell lines. Astrocyte Lineage – - - + -

Pluripotent Cell

Neural Stem Cell

Astrocyte Lineage

Oligodendrite Cells

Materials & Methods

UBM/Elute Preparation: Fresh pig bladder was purchased from the local butcher and immediately stored at -20°C. Bladder was then thawed in a 37°C water bath, cut open and muscle layers were scraped off. The sheet was washed in 0.1% peracetic acid 4% ethanal solution in deionized water for 2 hours. The sheet was then frozen at -40°C, lyophilized and ground to a fine powder with a SPREX Freezer Mill. The powder was mixed at a ratio of 100mg pepsin per gram of powder in 0.01M HCl in order to digest components of the matrix. Eluates were created by centrifugation of the above mixture which removed larger protein components of the extracellular matrix, with growth factors or small fragments of collagen remaining in solution.

Differentiation of P19 Cells: Pluripotent P19 cells were grown to about 80% confluency and treated with complete growth media for 5 days, splitting and replenishing media as necessary (24-48 hours) to avoid spontaneous differentiation. Another group of P19 cells were spontaneously differentiated by replenishing complete growth media only every 5 days, allowing growth factor released by cells to remain in the media. All cells were maintained at 37°C, 5% CO2.

Immunocytochemistry: Cells were tested for specific markers to confirm differentiation. A Neural Stem Cell Characterization Kit (Millipore) was used and the cell lines were tested for five markers: SSEA-1, MAP2, Nestin, GFAP, and O1 (Figure 3).

Viability Assay: A viability assay was conducted to investigate the effect of UBM and eluate on proliferation of the three cell types using CellTiter 96® AlamarOne Solution Cell Proliferation Assay (Promega). Concentrations of UBM from 25μg/mL-100μg/mL were used and the assay was performed after a 24 hour incubation. Viability was measured at 490nm using a microplate reader.

Migration Assay: A migration assay was performed using 96-well Chemotaxis Chambers (NeuroProbe). UBM was prepared at concentrations from 200ng/mL-500ng/mL. Cells were incubated for 24 hours at 37°C and 5% CO2.

References