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# Sustained Cell Differentiation of 2D H9 Human Embryonic Stem Cells into Mesenchymal Stem Cells

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## Introduction

This experiment consisted of the controlled differentiation of H9 embryonic stem cells to mesenchymal stem cells. Though this experiment was repeated twice and improvement was seen in these repetitions, the cells were only able to be partially differentiated. However, the morphology of the differentiated cells is similar to those of healthy adult mesenchymal stem cells.

## Method

When the H9 embryonic stem cells reached 50% confluency in E8 full medium, the differentiation process began. The H9 human embryonic stem cells were differentiated into mesenchymal stem cells by changing the well medium of the cells from E8 full medium to DMEM + 10% FBS + P/S. The medium was replaced every day during the entire differentiation process. When the cells in each well plate reached 90% confluency (Day 5), the cells were passaged by treating the cells with a 0.25% trypsin and 0.1% EDTA solution, transferring the cells to a new well plate without Matrigel, and returning to the DMEM + 10% FBS + P/S solution medium. Cell growth continued until the wells reached 50% confluency, or at Day 17 where the cells were passaged for a second time. For the protocol for mesenchymal stem cell differentiation, the cells are to undergo four passages until the completion of the differentiation.

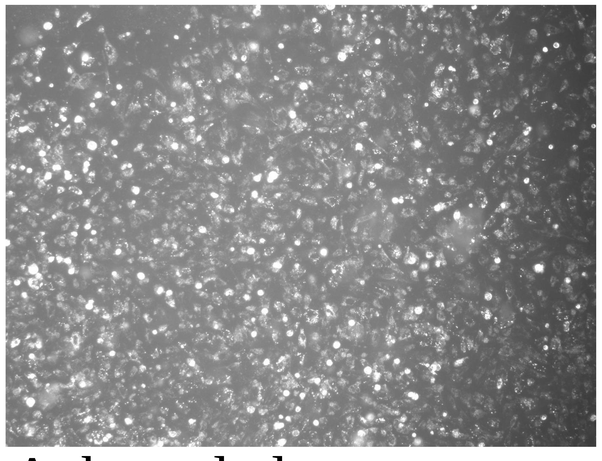
## Results

The progression of the differentiation can be seen in the microscope slide photos below. Throughout the differentiation, there occurred a decrease in cell survival and reduction of cell growth, but an increase in mesenchymal stem cell morphology. Throughout the last week of the differentiation, very little cell growth was seen and the cells were fixed on Day 20. With this, the cells were unable to sustain survival and growth to complete the four passages required for complete mesenchymal stem cell differentiation.

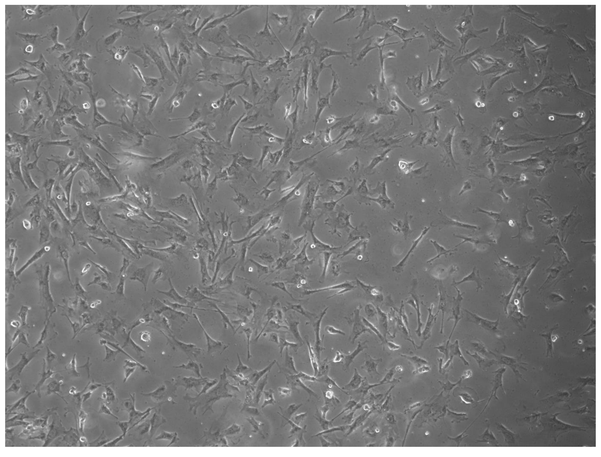
## Discussion

As stated above, the mesenchymal stem cell differentiation was unable to go to completion as cell growth only allowed two passages. This protocol was performed twice, and the results discussed are of the second trial. The second trial yielded greater progress than the first, so more trials may yield better results or result in a complete differentiation. There are no targetable sources of error, but the medium was changed at different times during the day, so this inconsistency may result in cell agitation and death. Due to the similarity in morphology of the differentiating cells to the proper morphology of mesenchymal stem cells, there is supporting evidence that this protocol has the potential to produce healthy and proliferating mesenchymal stem cells.

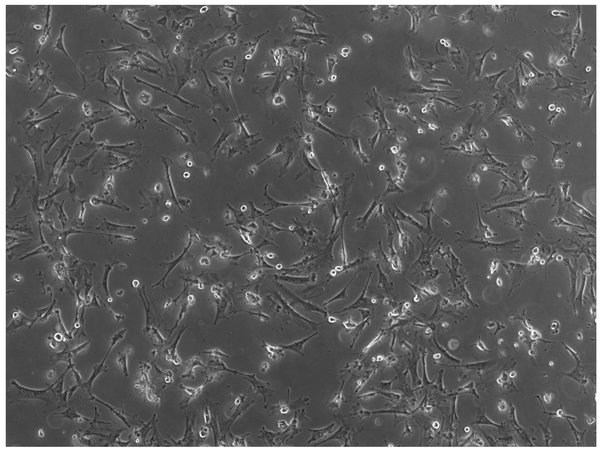
Beginning of Differentiation (Day 5, before first passage)



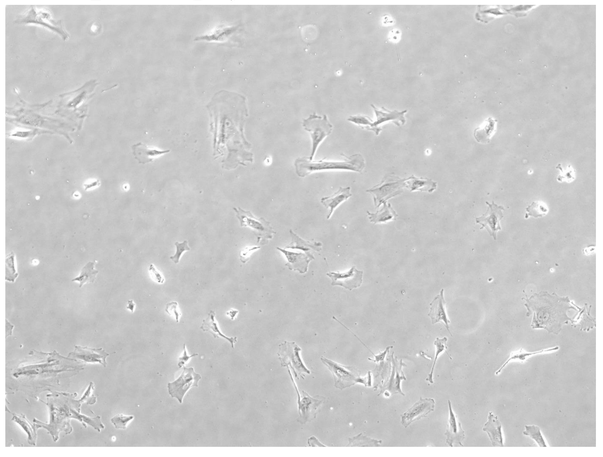
Middle of Differentiation (Day 8, after first passage)



Middle of Differentiation (Day 11)



End of Differentiation (Day 19, after second passage)



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