Differentiation of Human Bone Marrow Mesenchymal Stem Cells towards a Neural Lineage: -*in Vitro* Study

, Issam Hussain1 ;Emma Bownes1; Carol Rea1
1Department of Life Sciences, School of Life and Environmental Sciences, University of Lincoln, Lincoln LN6 7TS, United Kingdom
ihussain@lincoln.ac.uk

INTRODUCTION

The neural differentiation of mesenchymal stem cells (MSCs) is a promising cell-based therapeutic intervention of neurodegenerative diseases, such as Alzheimer's disease. The cultivation of MSCs isolated from various sources has demonstrated differentiation into cells of neural lineage, under exposure to neural differential media. MSCs can express characteristic neural markers including neuroectodermal stem cell marker (Nestin) and microtubule associated protein 2 (MAP2) when cultured in specific culture condition. Nestin is essential for the survival, self-renewal and proliferation of stem cells, and is also a regulator of cell differentiation and migration. MAP2 is expressed in mature neurons and involved in the formation and growth of microtubules and is required for the formation of neurites in neurons. The purpose of this study is to determine if MSCs can successfully transdifferentiate into neuron-like cells, evidenced by the expression of neural markers and changes in cell morphology towards a neuronal phenotype.

METHODOLOGY

Bone-marrow-derived MSCs were cultured in a 5% CO2 incubator at 37°C in growth media, and the cell morphology of undifferentiated MSCs was observed. In addition, a proliferation assay was performed, and a trypan-blue dye-exclusion assay determined cell viability. MSCs were later induced with neurogenic differentiation media and incubated in a 5% CO2 incubator at 37°C over 24-,48-, 72- and 96-hours. Quantitative and qualitative immunophenotyping of cells for specific neural markers was achieved using immunofluorescent staining of Nestin and MAP2 sandwich ELISA, respectively.

RESULTS

MSCs demonstrated a high proliferative capacity and high degree of cell viability in culture, with a doubling time of 35.08 hours. MSCs adhered to plastic culture vessels in vitro as a monolayer, and adopted a elongated, fibroblast-like cell morphology predominantly elongated. Under exposure to neurogenic differentiation media, cells demonstrated positive expression for Nestin and MAP2 and cells adopted a neuronal phenotype with significantly higher at 72 hours.

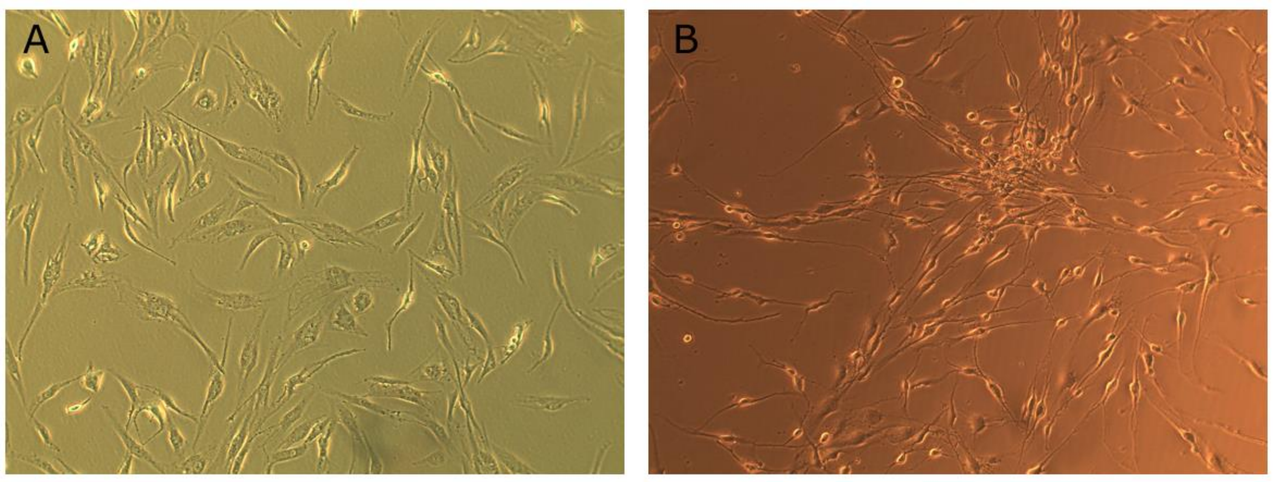

Figure 1. A) Undifferentiated BM-MSCs resembled elongated, spindle-shaped fibroblasts. B) Under exposure to neurogenic differentiation media, MSCs appeared spindle-shaped, with a large neuronal network and pronounced nucleus, suggesting cells have undergone neural differentiation.

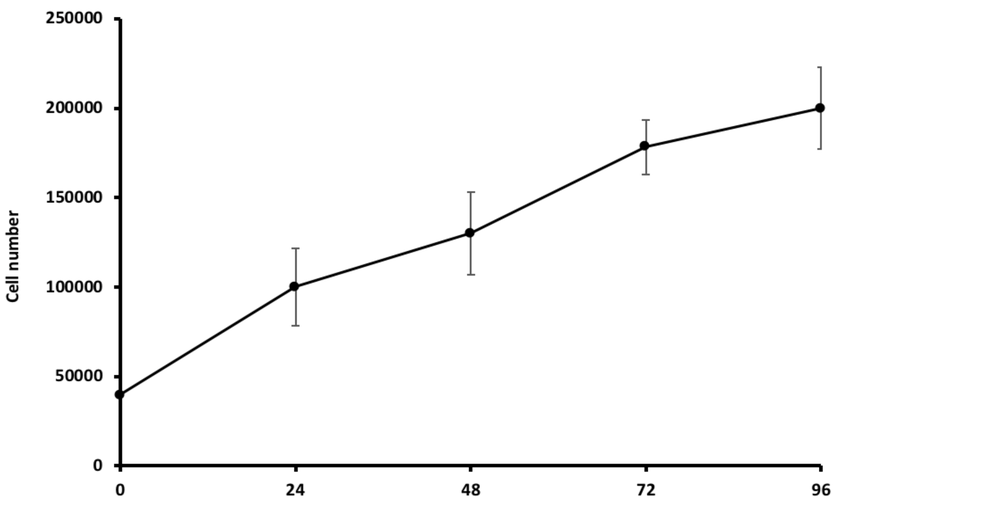

Figure 2. Cells expanded and proliferated in culture, and maintained a high degree of viability.

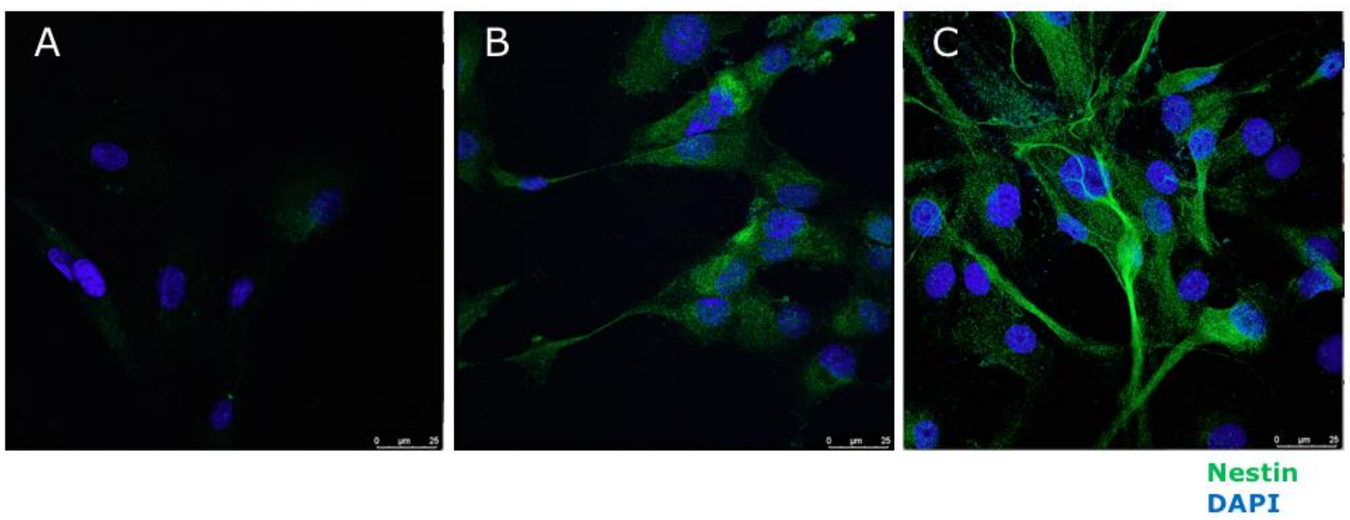

Figure 3. Representative immunofluorescence images of Nestin in differentiated BM-MSCs at time points of A) 24-, B) 48- and C) 72-hours. Images show that Nestin (green) is expressed at all time points and cell nuclei are counterstained with DAPI (blue).

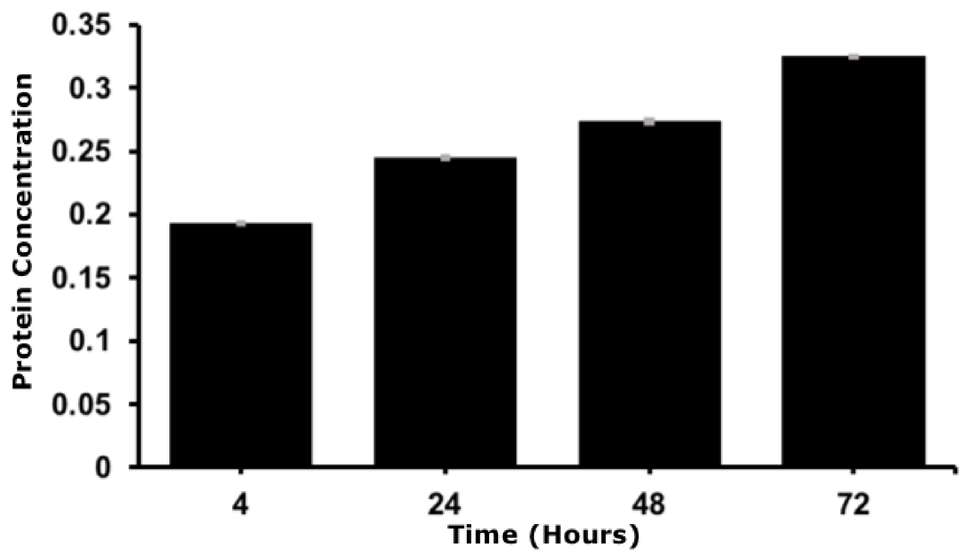

Figure 4. Neural-induced MSCs expressed MAP2, with an increased protein concentration at each time point.

CONCLUSION

BM-MSCs harness neurogenic potential, evidenced by altered cell morphology and expression of neural markers over 72 hours. Previous studies have documented that MSCs are an effective source of neuron-like cells in neurological disease or neuronal injury cases. However, further research is required to validate these findings and the potential to treat neurological diseases, and investigate if BM-MSCs transdifferentiate to functional neurons with signaling capacity.

ACKNOWLEDGEMENTS

This project was supported by the Master of Bioscience (MBio) program at the University of Lincoln.

References

1. Kruminis-Kaszkiel, E., Osowski, A., Bejer-Oleńska, E., Dziekoński, M. and Wojtkiewicz, J., (2020) Differentiation of Human Mesenchymal Stem Cells from Wharton's Jelly Towards Neural Stem Cells Using a Feasible and Repeatable Protocol. *Cells*, 9(3) 739.
2. Mareschi, K., Novara, M., Rustichelli, D., Ferrero, I., Guido, D., Carbone, E., Medico, E., Madon, E., Vercelli, A. and Fagioli, F. (2006) Neural differentiation of human mesenchymal stem cells: evidence for expression of neural markers and eag K+ channel types. *Experimental Hematology*, 34(11) 1563-1572.
3, Reger, R., Tucker A., Wolfe M. (2008) Differentiation and characterization of human MSCs. Methods Mol Biol 449 93-107.
4. Urrutia, D., Caviedes, P., Mardones, R., Minguell, J., Vega-Letter, A. and Jofre, C. (2019) Comparative study of the neural differentiation capacity of mesenchymal stromal cells from different tissue sources: An approach for their use in neural regeneration therapies. *PLOS ONE*, 14(3), 0213032.

UNIVERSITY OF LINCOLN