Polyethylene Glycol Hydrogels Functionalized with a Continuous Ile-Lys-Val-Ala-Val Concentration Gradient for Optimizing Neural Differentiation of Murine Embryonic Stem Cells in 2D

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Statement of Purpose: Spinal cord injuries (SCI) which result in neurological impairments affect approximately 270,000 persons in the U.S. and cost the health care system over $10 billion per year in direct medical costs and disability support[1, 2]. Pluripotent stem cells (PSCs), which are capable of giving rise to all the cell types in the body, represent a potential therapeutic candidate for SCI repair. Due to inconsistency amongst currently available protocols for directed differentiation of PSCs, however, confounding outcomes have been identified. There is a need to standardize culture parameters by replacing constituents that suffer from batch variability with consistent synthetic materials in order to maximize the desired lineage choice of differentiating cells derived from PSCs. Laminin is a major component in many of the substrate coatings used in these protocols. Replacement of Laminin with a bioactive peptide, such as Ile-Lys-Val-Ala-Val (IKVAV) that has similar biological effect could greatly improve consistency of these differentiation protocols[3]. The objective of this study was to develop a novel polyethylene glycol dimethacrylate (PEGDM) hydrogel system that possesses continuous concentration gradient of IKVAV, a bioactive peptide fragment from laminin, in order to determine an optimal range of peptide concentrations for two-dimensional (2D) induction of murine embryonic stem cells (mESC) neural differentiation.

Methods: 50 mm × 10 mm × 1 mm hydrogel gradients were fabricated by dispensing 12% 10 kDa PEGDM solutions with and without 1.9 mM IKVAV through two syringe pumps running in inverse linear ramping profiles ranging from 0 mL/h to 52 mL/h, respectively, over 75s into a custom mold, followed by photopolymerization with 2.3 mJ/cm2 for 6 min. After swelling in media, one 9.6-mm disc was punched out every 10 mm along each gradient, resulting in six discs per gradient. Discs were then evaluated for IKVAV concentration (CIKVAV), swelling ratio, mesh size and mechanical properties. For cellular experiments, the cored discs were placed in the wells of 48-well plates, seeded with D3 mESCs and cultured with serum-free neural media for 6 days with medium exchanges every other day. Cell-seeded constructs were stained for neuron-specific β3-tubulin at day 3 and length of neurite extension was measured. Cultured cells were harvested at days 3 and 6 for analyses of gene expression, alkaline phosphatase (ALP) and apoptotic activities. Statistical significance was determined using one-way or two-way ANOVA in conjunction with the Bonferroni post-test with a p-value of < 0.05.

Results: Fabricated PEGDM hydrogels presented a linear C_{IKVAV} gradient ranging from 125 µM to 920 µM with an R squared value of .998 while other material properties

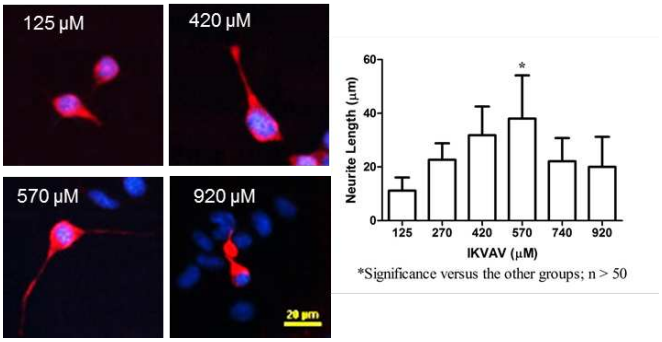

Figure 1: Neural differentiation of mouse embryonic stem cells after 3 days of direct neural differentiation. A) β3 tubulin (red) and nuclear (blue) staining B) quantification of neurite extension from immuno-fluorescence images.

remained similar throughout the gradient. Compared with the starting ESC population, cells cultivated with IKVAV at any levels exhibited inferior ES-related mRNA expression and secreted a lower content of ALP, a molecule highly expressed in ESCs, suggesting that the cells were undergoing differentiation. TUJ1 mRNA expression, an immature neuron marker, increased under all of the C_{IKVAV} conditions except for 920 µM at day 3 while the peak appeared in 420 µM and 570 µM groups at day 6. Similarly, the highest mRNA expression of MAP2, a mature neuron marker, was detected in both 420 µM and 570 µM samples at day 6. A significantly longer neurite extension was identified in the cells cultured with 570 µM IKVAV as shown in the figure 1. Noteworthy, ESCs in the 920 µM group experienced a greater level of apoptosis during the cultivation which may account for their compromised neural differentiation.

Conclusions: Through development of a hydrogel gradient system to maximize neural differentiation of ESCs in 2D, our results suggest that better differentiation is achieved at a C_{IKVAV} between 420 µM and 570 µM whereas an excessive C_{IKVAV} can induce apoptosis in differentiating ESCs. Collectively, the present work demonstrates that hydrogels functionalized with bioactive peptides provide a defined and tunable platform and thus can be utilized to characterize culture conditions suitable for cell growth and differentiation.

References:

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Acknowledgements: Our research was supported by start-up funds from Department of Neurosurgery and William Stamps Farish Fund.