Biomimetic polyurea for improved differentiation of human Neural Stem Cells into mature motor neurons

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Statement of Purpose: There is currently no effective cure for a spinal cord injury (SCI). In hopes of finding the first restorative treatment, researchers have turned their focus to stem cell therapies due to its potential to repair or completely replace the injured cells and tissue. Although this type of research has shown a great deal of promise, there are still many complications to overcome before stem cell therapies become institution in SCI treatment. A significant obstacle that stands in the way is the lack of an efficient source of naïve spinal motor neurons (sMNs) for research and cell transplantation. So far, several protocols for differentiating neural stem cells (NSCs) into sMNs have been reported; however these methods display a limited percent production (~10%) of sMNs from the initial NSCs population. Therefore, improvement in the percent production of sMN would progress future research and potential cell therapies. For this reason, we aimed to increase the differentiation efficiency of NSCs into mature spinal motor neurons using an RGD functionalized polymer scaffold.

Methods: We began by synthesizing a functionalized RGD polymer scaffold (PSHU-RGD). First, N-Boc Serinol is combined with urea to make the PSHU backbone. The N-Boc serinol groups protect the reactive amine groups on the polymer backbone from reacting with any other chemicals and will later be removed to add RGD-COOH using EDC/NHS chemistry. The RGD conjugation amount can be controlled by varying the molar ratio of RGD/EDC/NHS with the amine groups available. HPLC was used to quantify the RGD conjugation. To analyze the differentiation efficiency of our polymer coating, we began by coating a 24-well plate with varying concentrations of PSHU-RGD (100ug/ml-0.01ug/ml). For a positive control we coated wells with poly-d-lysine and laminin (PDL-Laminin). After this, NSCs were seeded on top of the coatings in N2B27 media with ATRA and SAG factors. N2B27 media and ATRA/SAG factors were changed daily for 7 days. After 7 days, ATRA and SAG were replaced with NT3, BDNF, and GDNF and left for another 7 days or 14 days. At this point, the cells were fixed and immunocytochemistry was conducted to test for the expression of specific motor neuron markers, Islet1 (Isl1) and HB9.

Results: After deprotecting the free amine groups on the PSHU backbone, we used NMR to detect any remaining BOC-protecting groups. We were able to show complete deprotection of the BOC-protecting groups from the PSHU backbone. Next, we used HPLC to determine the rate of conjugation for RGD attachment to the free amine groups on the PSHU backbone. Samples with varying RGD to free amine group molar ratios (1.3, 1, 0.8, 0.6, 0.4) were used and the corresponding HPLC data was analyzed. After seeding the NSCs on the PSHU-RGD and laminin/poly-d-lysine coated plates, images were taken to confirm initial attachment of the NSCs. After the 2-week and 3-week culture period, the cells were fixed and marked with HB9 (red), Islet-1 (red), DAPI (blue), and BIII Tubulin (green). The cells were immunostained and pictures were taken to observe and compare the differentiation efficiency of each coating (Fig 1), with counter staining using DAPI at 14-day and 21-day culture period (Fig 2b). We also determined the number of cells stained with HB9 and Isl1 in order to quantify the difference in differentiation efficiencey between the two types of coating (data for both 14-days and 21-days are shown in Fig 2a).

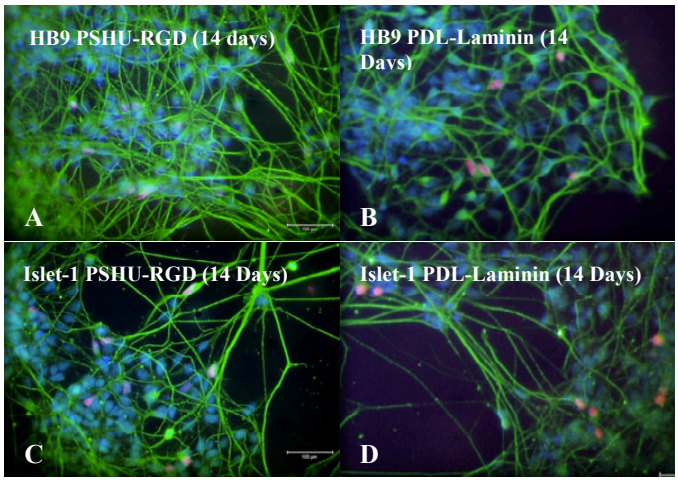

Figure 1

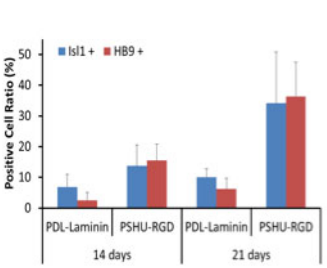

Figure 2A

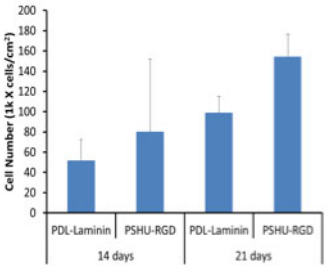

Figure 2B

Conclusions: The images taken after the 14-day culture period (Fig 1) show that the PSHU-RGD polymer coating encouraged more axonal and cell growth than the positive control (PDL-Laminin). We were also able to conclude that the cell number after 14-days and 21-days was greater for the PSHU-RGD coating than for PDL-Laminin (Fig 2B). From figure 2A we can see that the number of cells expressing Isl1 and H89 was greater on the plates coated with PSHU-RGD than for the plates coated with PDL-Laminin.

References:(Rowland et al. Neurosrug Focus. 2008;25:E2.)

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