Matrix microenvironment regulates neural stem cell differentiation into neural and glial lineages

Kurt W. Farrell, Chandra Kothapalli*
Department of Chemical & Biomedical Engineering, Cleveland State University, Cleveland, OH 44115

Statement of Purpose: The inability of the adult mammalian central nervous system (CNS) for self-repair has prompted increased efforts within the field of regenerative medicine to re-establish and reconnect diseased and damaged neural networks. The prevalence of neurological disorders is widespread; every year thousands of new patients are diagnosed with various neurological related injuries and diseases in the United States, and a majority of them succumb to this condition within 3-5 years post-diagnosis [1]. Neural stem cells typically assume a quiescent phenotype, unless provoked by environmental signals, at which point they begin to proliferate, migrate, or differentiate into a specific neural or glial lineage [2]. In this study, we developed and evaluated the utility of four different compositions of 3D biomimetic ECM scaffolds on NSC homing, survival, proliferation and differentiation into neural and glial lineages. These scaffolds consist of HA, collagens and proteoglycans in varying proportions. The scaffolds used in this study have not been crosslinked to minimize toxicity emanating from crosslinking agents.

Methods: Murine neural stem cells were acquired from the brain cortex of neonatal mice (Neuracell, Rensselaer, NY) and seeded on T-25 flasks for a 6 day neurosphere proliferation period. Cells were then detached and seeded in the aforementioned 3D collagen-HA gels, as well as in the individual components of the aforementioned hydrogels. Additionally, bFGF was removed from the media to induce differentiation. Cells were incubated for a period of 10 days, fixed and stained for the following primary antibodies: TUJ1 (pre-neural marker) GFAP (astrocytes) and MBP (oligodendrocytes). Experimental conditions were repeated keeping the scaffold conditions the same, but supplementing the media with other signaling molecules as described previously [3]. The scaffolds were characterized to understand the effect of the material properties on cellular differentiation. Ethanol displacement was used to measure porosity, compression testing was used to measure material stiffness, and water absorption to understand diffusive properties. Additionally, DSC and TGA analysis was performed to understand the thermal properties of the materials. Lastly, the scaffolds were imaged using a scanning electron microscope.

Results: Data from material analysis revealed several trends. Scaffold porosity was found to be in the range 60-80%. All tested materials had a high affinity for water absorbance, approximately a 5-fold increase in weight after 15 sec of water immersion. Collagen had the highest Young's modulus at approximately 528 ± 25 Pa, and 1% HA had the lowest modulus of 101 ± 25 Pa. Other hydrogels fell within this range depending on their respective collagen-HA ratios.

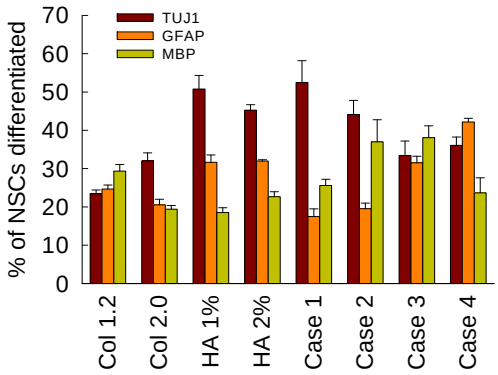

Figure 1. Percentage of neural stem cells differentiating into neural and glial lineages within different hydrogel compositions.

The percentage of cells stained positively for a given neural marker (Fig. 1) was obtained by quantifying stained cells using fluorescent imaging. (at least 300 cells were counted per case, n = 3/case). Cases 1 & 2 produced significantly higher amounts of NSCs expressing TUJ1, whereas Cases 3 & 4 along with Col 1.2 and 2.0 mg/mL expressed similar amounts of all three markers, which may indicate HA concentration is playing a strong role in TUJ1 differentiation. In general, the softer modulus of the gel scaffolds (E < 700 Pa) was found to elicit higher amounts of cells expressing the TUJ1 marker, as well as variations of comparable amounts of GFAP and MBP which is likely dependent on scaffold composition. The data strongly suggests that scaffold composition plays a major role in differentiation, specifically pre-neural, astrocyte and oligodendrocytes linages.

In conclusion, we observed that injectable non-crosslinked hydrogels with low mechanical moduli provide favorable neuronal cell culture micro-environments. These hydrogels elicit spatio-temporal cues similar to those found in the naturally occurring mammalian neural ECM, which have been identified through a variety of characterization tools. Lastly, our experimental design aims to understand which combination of signaling molecules and ECM components synergistically create biomimetic microenvironments for differentiation of specialized neuronal population.

References:

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3) Kothapalli, C.R. Roger D. Kamm, 3D matrix microenvironment for targeted differentiation of embryonic stem cells into neural and glial lineages, Biomaterials, 2013, 34 (25): 5995–6007.