Controlling hiPSC-derived Neural Stem Cells Behavior Using Electrical Stimulation

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Statement of Purpose: Central nervous system (CNS) problems affected nearly 100 million Americans causing high mortality/morbidity rate, lifelong disabilities, social and economic burdens.[1] There have been innovative approaches relying on regrowth of disrupted neuronal axons, replacement of lost neural cells, and recovery of lost neural function to address CNS problems. Among them, electrical stimulation (ES) of exogeneous NSC, which can be derived from other stem cell types (i.e. induced pluripotent stem cells) and are able to differentiate into major cell types of CNS (i.e. neurons, astrocytes, and oligodendrocytes), can be used as an alternative approach to control differentiation, transplantation and integration into CNS to enhance regeneration, repair and recovery.[2-4] However, there is still limited knowledge about controlling NSC differentiation and associated physiochemical mechanisms.[3,4] In addition, NSC transplantation is challenging due to limited proliferation, migration, differentiation, and viability.[3,4] We hypothesized that mimicking the physiological electrical fields present in the body or during the embryonic development along with providing natural extracellular matrix via a biointerface enables a favorable environment to control and elucidate the NSC differentiation. Therefore, the objective of this study is to understand human induced pluripotent stem cells (hiPSC)-derived NSC's differentiation behavior directed through in situ ES via biodegradable and implantable flexible electronic biointerfaces and develop a mechanistic approach. To prove our hypothesis and reach out objectives we performed the following studies.

Methods: A 6-well plate lid with conductive graphene electrodes was custom designed and fabricated using 3D printing to apply different ES conditions simultaneously. hiPSC-NSC obtained from XCell Science were cultured and grown by following manufacturer's protocols. Upon seeding to the wells ($1x10^5$ cell per well), hiPSC-NSC were exposed to different DC voltages (0-200 mV) for varying durations (0-30 min) every day for two weeks to determine the ES conditions providing neuron, astrocyte, and/or oligodendrocyte-like phenotypes. To evaluate the synergistic effect of 3D microstructural/mechanical properties and ES, the cells were seeded in gelatin scaffolds (obtained via phase inversion, transparent, in the form of flat sheet and possessing different microstructural mechanical properties) placed in each well and same ES conditions were applied. Upon ES, the cells were harvested, immunolabelled with neuron (TuJ1, MAP2ab), astrocyte (GFAP), and oligodendrocyte (RIP) markers and imaged using high-content imager. To observe specific genes regulated upon ES, total RNA from the cells was isolated using TRI reagent and PCR experiments were conducted. Based on obtained microstructural/mechanical and ES conditions, a gelatin/whey protein isolate composite, biodegradable and implantable flexible electronic biointerface with graphene microcircuits was designed and fabricated using our patented method (US Patent 11,066,296). The circuit geometry was optimized to mimic the electrical fields using COMSOL simulation. hiPSC-NSC differentiation into specific phenotypes were conducted using the designed implantable biointerfaces.

Results: Our initial screening of ES parameter space using our well plate set up indicated that varying the ES conditions within physiological range enables expression of different cell markers. Our results showed that 100 mV favors glial marker expression while the voltage increased to 150 mV expression of neuronal markers dominates. These results are also supported by PCR. Based on this observation, a graphene and gelatin/whey protein isolate-based composite biointerface was successfully fabricated. The biointerface provided good conductivity (sheet resistance ~0.2 kΩ/sq) and stability after multiple washing and bending cycles (**Fig. 1a)**. The mechanical stiffness of substrate (complex modulus) was around 4 KPa. COMSOL simulation showed that the electric field created on the surface was local **(Fig. 1b)**. The cells attached and proliferated on biointerface for at least 15 days without any viability issue. The application of ES via biointerfaces resulted in expression of early and late neuronal markers as well as glial markers. The degree of immunolabeling for each marker was determined around 85% **(Fig. 1c)**.

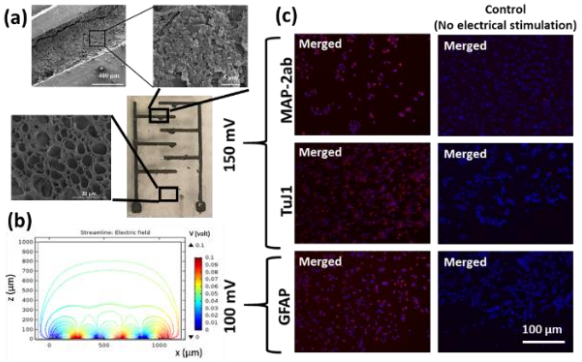

Fig. 1. (a) Designed biointerface and SEM images for graphene circuit and gelatin substrate. **(b)** COMSOL simulation of electrical field created. **(c)** Immunocytochemical analysis of electrically stimulated hiPSC-NSC on biointerfaces for different markers.

Conclusions and Future Work: These findings support our hypothesis of controlling hiPSC-NSC differentiation behavior using sole ES and paves the way for in situ and *in vivo* control of transplanted NSC population using local ES via developed biointerfaces. However, more mechanistic data is being produced to obtain mature cell lineages.

References:

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