Electric field induced lineage commitment of mesenchymal stem cells towards neural-like cells on conducting polyaniline substrates

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Statement of Purpose: With so many advantages like plasticity and multilineage potency of stem cells, there is an increased need for the regulation of stem cell fate processes, when it comes to treatment and therapy (Guilak F. Cell stem cell. 2009;5(1):17-26). Stem cells are known to be extremely sensitive to a diverse array of physical factors (substrate topography, matrix stiffness, surface roughness, etc.) and hence altering the microenvironment with such physical attributes can tune the lineage commitment and differentiation of stem cells for specific therapeutic outcomes (Thrivikraman G. Biomater. 2014;35(24):6219-35). In this light, the present work demonstrates the ability of human mesenchymal stem cells (hMSCs) to sense the differences in substrate conductivity by responding to it by adopting extended neural-like morphology in electric field (EF) stimulated culture conditions. Polyaniline (PANI) was selected as the model system, as the electrical conductivity of the polymeric substrates can be systematically tailored over a broad range (10^{-9} to 10 S/cm) from highly insulating to conducting by doping with varying concentrations of HCl. A time dependent morphological change in hMSCs with dramatic filopodial elongation, expressing early-neural like phenotype markers (nestin, βIII tubulin) confirmed the switching of lineage commitment to neural-like cells.

Methods: The synthesis of PANI was carried out by the oxidation of aniline by ammonium peroxydisulfate. Drop casted PANI films were doped with HCl in concentrations ranging from 10^{-5}M to 10M. The molecular, structural, microscale mechanical properties, surface as well as electrical conductivity properties of both doped and undoped PANI synthesized were studied by various techniques such as FTIR and NMR, XRD, Nanoindentation, XPS and four point probe method, respectively. A host of biochemical assays were employed to probe the cellular response. Notably, flow cytometry analysis was used to assess the effect of EF in intracellular reactive oxygen species (ROS) generation and apoptosis induction. EF mediated morphological alterations were observed by fluorescence microscopy and the analysis of the cellular differentiation was done using Reverse Transcription Polymerase Chain Reaction (RT-PCR) and immunocytochemistry.

Results: Based on the FTIR, NMR and XPS analysis, it was confirmed that dopant HCl has effectively protonated PANI base to form conducting PANI salt. The conductivities of PANI films (represented as $\log_{10}(\sigma$ (S/cm))) doped with varying molar concentrations of HCl, were determined from the sheet resistance, derived from the van der Pauw method (Fig.1a). Overall, the combination of physical property measurements indicated that with doping, PANI films with conductivity varying over 10 orders of magnitude along with similar elastic stiffness were obtained. MTT assay revealed a decrease in proliferative capacity of hMSC on conductive substrates after 5 days of EF treatment. Further by flow cytometric analysis, it was confirmed that the observed reduction in proliferation was due to differentiation induction but not due to apoptosis/necrosis of cells. ROS assay showed no alteration in the intracellular redox state under EF stimulated culture conditions. This demonstrates that dopant concentration and electric stimuli, alone or in combination do not have any significant role in inducing cell death.

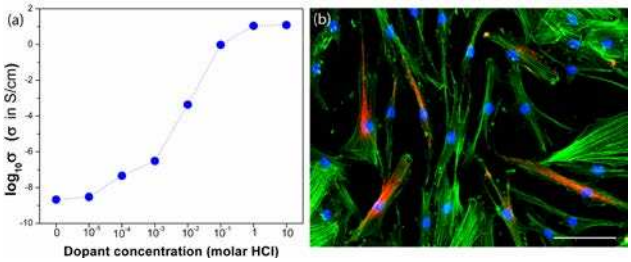

Figure 1: (a) Variation of the conductivities of PANI film doped with varying concentrations of HCl
(b) Fluorescence image of EF stimulated hMSCs grown on conducting substrates, showing βIII tubulin (red) positive cells, suggesting that electric stimuli can induce hMSCs to differentiate into neural-like cells in a conductivity dependent manner. F-actin was labelled green and DNA was labelled blue. Scale bar:100 µm

Fluorescence microscopic examination revealed that the EF-directed distinctive morphological change as well as enhanced cytoskeletal elongation was found to be solely determined by underlying substrate conductivity. The prominent feature that was noticed is the long cytoskeletal extension emerging from the cell bodies of hMSCs after 7 days of EF treatment on the higher conducting substrates. Likewise, the protrusion length also increased with conductivity. It subsequently triggered the expression of early neural phenotypic markers, nestin and βIII tubulin in a conductivity dependent manner, thereby transforming hMSCs to neural-like cells (Fig.1b). In summary, these results showcase a fundamental understanding that under EF stimulated conditions, MSCs differentiation to neural-like cells is achievable, when grown on conducting substrates that allow transmission of electric cues.

Conclusions: All these combined results highlight the usefulness of introducing conductivity as an important physical cue while designing functional tissue engineered constructs, to guide the growth and commitment of stem cells. In view of the synergistic interaction, the substrate conductivity in combination with external electric stimuli can serve as an integrative electrophysical cue to promote early neural-like differentiation of stem cells.