Gold nanoparticles actuates human mesenchymal stem cell differentiation under electric field stimulation

Greeshma Thrivikraman[1,2], Giridhar Madras[2] and Bikramjit Basu[1]
[1]Materials Research Centre, Indian Institute of Science, Bangalore, India-560012
[2]Centre for Nanoscience & Engineering, Indian Institute of Science, Bangalore, India-560012

Statement of Purpose: It is widely recognized that stem cells can sense, react and adapt themselves to physical cues. In line with this, the aim of this study was to induce human mesenchymal stem cell (hMSC) differentiation by directing electroactive nanoparticles (within and outside the cells) that are controlled by external electric fields (EF). In order to investigate the effect, uniform layer of gold nanoparticles (AuNPs), electrostatically embedded onto thin polyaniline (PANI) films was employed as the extracellular actuators for the adhered cells. Furthermore, hMSCs were internalized with monodispersed poly-L-lysine capped AuNPs to trigger cellular uptake of these particles, thus rendering them to actuate from inside the cells, in response to EF. Hence, by the simultaneous application of EF, it is inferred that a combination of nanoscale forces generated through internalized AuNPs (intracellular actuation) and through substrate embedded AuNPs (extracellular actuation) stimulate the adherent cell via cytoskeletally mediated traction forces.

Methods: AuNPs were synthesized by citrate reduction method. Ultrathin films of AuNP coated PANI substrates for stem cell growth was assembled by Layer by Layer (LBL) technique and were characterized by Atomic force microscopy (AFM), cyclic voltammetry and UV–vis spectrophotometry. AuNP uptake by hMSCs was visualized by dark field optical microscopy as well as by confocal microscopy. hMSCs were pre-induced transiently with a 1:1 combination of retinoic acid (RA) and 5-Azacytidine (Aza), before the stimulation experiment. The changes in the morphology were assessed using fluorescence microscopic imaging. Any change in the gene/protein expression profile was determined using semi-quantitative Reverse Transcription Polymerase Chain Reaction (RT-PCR) and Immuno-cytochemistry. Cell cycle alterations, Ca^{2+} level and Reactive oxygen species (ROS) generation were examined by flow cytometry.

Results: In order to investigate whether varying the type of EF stimuli can elicit different lineage commitment of stem cells, the study was bifurcated so as to understand the effect of steady direct current (D.C) stimuli (100 mV/cm) and pulsed D.C stimuli (1 Hz, 100 ms pulse width) on hMSC differentiation. From the altered cellular morphology, genotypic and phenotypic expression profile it was elucidated that steady D.C stimuli favoured neural-like differentiation. In contrast, the pulsatile stimuli evoked cardiomyogenic differentiation of hMSCs. In particular, upon stimulation with steady D.C stimuli, majority of hMSCs acquired longer outgrowth/filopodial extension with multiple branch-points and were positively stained for nestin and βIII tubulin. Such observations in cellular shape change were marked with higher mRNA expression level for nestin, Neurofilament, MAP2 and βIII tubulin.

Following pulsatile stimuli, hMSCs exhibited stick-like morphology with higher expression of cardiomyogenic markers such as cardiac troponin T, desmin, GATA-4 and α-cardiac actinin as confirmed by semi-quantitative RT-PCR. Moreover, the study unfolded the role of molecular events such as transient ROS production, G0/G1 phase cell cycle arrest and intracellular Ca^{2+} levels in inducing differential response in hMSCs, as validated using a combination of flow cytometry and time lapse fluorescence imaging techniques. Taken together, these comprehensive results support the concept of stem cell differentiation to neural/myogenic cells (Figure 1) by inducing biophysical stresses through external means.

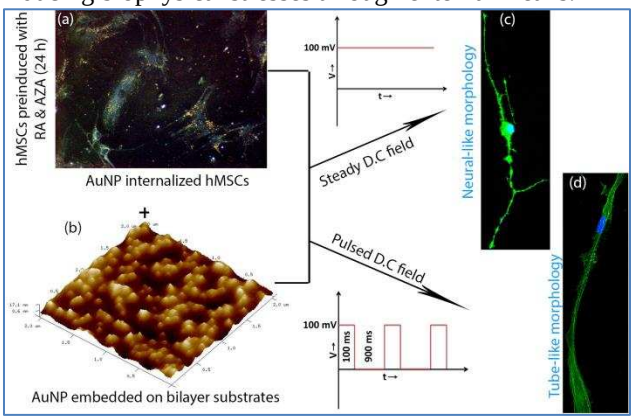

Figure 1: hMSC differentiation induced by electric field driven AuNP actuation. (a) Dark field image showing AuNP internalized hMSCs. (b)AFM showing LBL assembled conducting substrates. (c) and (d) Fluorescence image showing cytoskeletal actin (green) and nuclei (blue).

Conclusions: On the basis of these examinations, our findings demonstrate that interplay of extra/intra cellular physical stresses along with electric stimuli efficiently promote the differentiation of hMSCs towards cardiomyotube and neural-like cells. The application of steady D.C EF (superposition of high frequency D.C pulses) similar to the physiological rate of nerve impulse transmission has led to the higher degree of neural transcript expression in hMSCs. On the contrary, the application of low frequency pulsed D.C, similar to the action potential duration and frequency of cardiac impulses has promoted cardiomyogenic expression in hMSCs. In both the cases, AuNPs largely mediated the impulse transmission to the cells. Therefore, this comprehensive study provides better insight into the utility of electroactive nanoparticles and EF stimulated culture methodologies as an instructive cue for orchestrating lineage commitment of stem cells. Moreover, mechanical intervention through electric actuation can be considered as an effective strategy to regulate stem cell functionality for tissue engineering and regenerative medicine applications.