INVESTIGATING ROLE OF INTRACELLULAR MESSENGERS AND ENERGY METABOLITES DURING STEM CELLS TRANSDIFFERENTIATION ON BIOENGINEERED PLATFORMS

Thesis submitted in partial fulfillment

for the Award of Degree

Doctor of Philosophy

by

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7.1 Conclusions

The present study provides insight into material structural and functional parameters to dictate stem differentiation into the specific lineage. The study discusses about the detailed fabrication of platforms having metallic and polymeric surfaces highlighting the role of physical cues in forms of nanostructures and providing electrical stimulation as an efficient engineering approach to guide stem cell fate. The different properties of the materials were well characterized to understand material-cell interactions. The study also included understanding different cellular mechanisms inducing the transformation of stem cells to specialized cells. In this perspective, mitochondrial dynamics in terms of its membrane potential and intracellular messengers, namely ROS and Ca^{2+} were studied.

Various strategies by tailoring material properties were adopted for tuning stem cell response. Particularly, hMSCs were considered for the study, which were commercially procured to ensure high purity and stability as a control model system. Additionally, these cells are advantageous due to their multilineage differentiation capability, easy availability from several tissue sources (bone marrow, adipose, umbilical cord, dental pulp), high yield capacity, and remarkable differentiation plasticity compared to other adult stem cell types. Moreover, these cells also have advantages compared to neural stem cells (NSCs), as they lack the ability of teratoma formation as being derived from adult tissues, the potentiality to migrate towards inflammatory foci through the expression of chemokine receptors and stimulate potent immunomodulatory and anti-inflammatory effects which could elude toxicity of the immunosuppressive regimens used with NCS transplantation.

Firstly, the effect of non-conducting polymer, chitosan and its conjugate with charged gold nanoparticles having acidic and basic pH were explored for fabricating platforms to guide differentiation process. The fabricated platforms were biocompatible however, no lineage-specific response was observed. Therefore, further use of this was excluded in a detail investigation of cellular responses.

Further, the use of metal-oxide based platforms, particularly transparent TiO_2 coating for quantifying their potential in lineage-specific differentiation with the objective without changing its crystalline structure and surface morphology, chemical characteristics and static surface charge were modulated by doping with transition metal ions and the whole purpose for this was to make the metal-based platform electroactive material for inducing neurodifferentiation of hMSCs. The neural cells are well known to be electroactive and electrogenerative, capable of generating action potential, thereby emulating the natural physiological electrical niche of neural tissues through electroactive biomaterials may provide a biomimetic strategy to amplify the neurogenesis of stem cells. The La doped TiO_2 platform showed enhanced hMSCs proliferation rate and modulated commitment towards neuronal lineage after 24 h of seeding, confirmed by expression of nestin. It was observed that the La-doped TiO_2 coating provides a relatively more effective approach for initiating neuronal-specific differentiation from hMSCs. However, the yield of differentiation remained poor. Therefore, this approach has also been dropped.

Considering the characteristics of La, which is a slightly better electroactive material compared to other dopants (Ni, Au) used in the study, provided us a clue about the differentiation towards neuronal lineage. Thus, considering the response of electroactive characteristics, a unique strategy was adopted by synthesizing nanostructures of TiO_2 on conducting substrate and their utilization for stem cell differentiation. This study confirmed

that the conducting nature of substrate plays an essential role in regulating stem cell responses to a particular lineage under the influence of simultaneous local stimulation mediated by continuous charge passivation of the cellular system through the conducting nature of substrate used for cell seeding. Therefore, further to confirm whether the electroactive characteristic or nature of the material (metallic or polymeric) is essential for tuning stem cell's response, organic synthesis of glycopolymers with electroactive nature and changing their relative characteristics were evaluated. It was found glycopolymers holding non-polar characteristics showed a positive response for neurogenic differentiation. Subsequently, to validate that electrostimulation are more vital than material-induced approach, a new strategy has been developed to specifically provide localized and homogenous stimulation to the cells in a controlled way and dictate their neurogenic differentiation. The overall findings confirmed that electrostimulation is more efficient for achieving selective differentiation of stem cells than passive stimulus through metallic or polymeric materials. This approach has demonstrated its superiority over the standard protocol of providing stimulations through chemical routes, physical cues, or other strategies explored in this field.

The study also made an important conclusion about the change in mitochondrial membrane potential during neurodifferentiation of hMSCs. The mitochondrial membrane gets hyperpolarized as hMSCs become specialized towards the neuron, which modulates the production of intracellular ROS. The ROS acted as a secondary messenger, which up-regulated signaling pathways activating transcription of neuron-specific genes. A positive correlation between ROS production and high $\Delta\psi m$ during neurogenic differentiation was observed. The findings shifted the paradigm from considering mitochondria as mere powerhouses of the cell to realizing them as regulators for stem cell fate. Thus, studying mitochondrial metabolism advances the comprehensive understanding of how intracellular

metabolic signaling molecules coordinate to modulate the lineage-specific differentiation of stem cells and facilitate differentiation efficiency with metabolic cues.

7.2 Future Scope

Stem cell therapy is being touted as a crucial treatment modality for treating many diseases, including neurodegenerative disorders. The increasing burden of mortality led by death and atrophy of specific neurons in the brain due to various pathological conditions has paved the development of stratagems to direct selective differentiation of stem cells towards neuronal lineage. The proposed approaches can become a potential tool for neural stem cell therapy by inducing rapid differentiation of hMSCs. The study shed light on the essential requirement for a fine-tuned regulation of mitochondrial dynamics for dictating stem cell fate. Elucidating mitochondrial biology can lay a strong foundation in the rapidly evolving field of mitochondrial medicine and may advance therapeutic outcomes of stem cell therapy in the future.