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Optogenetics in 3D combining genetic engineering with tissue engineering for future cell applications

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CRISPR-associated catalytically inactive dCas9 fused to an effector domain (dCas9-E) has developed recently as a powerful tool to modulate endogenous transcription and to interrogate mechanisms in cell signaling. The latest evolution of dCas9-E, called dLITE (dCas9 Light Inducible Transcriptional Effectors), is an optogenetic two hybrid system modified in Keele, integrating the genetic engineering capacity of CRISPR with transcriptional modulation to switch a gene 'ON' or 'OFF', only in response to non-invasive blue light. In our experiments, we tested the efficiency of this system with an eGFP (enhanced Green Fluorescence Protein) reporter to prove sensitivity of the light (470 nm wavelength, pulsed at 0.7 Hz frequency) to modulate GFP fluorescence in HEK293 cells (human embryonic kidney cells). Furthermore, using this reporter, we assessed the spatiotemporal characteristics of light induction using a custom-designed high throughput (96 well) LED-based optogenetic platform and observed that 24 hours exposure to pulsed light was sufficient to produce 5 fold induction in GFP fluorescence, compared to unexposed cells. The combination of CRISPR genetic engineering with tissue engineering is a relatively novel concept for biomedical research and we proceeded to test this concept by using 3D transfection of HEK cells in combination with gelatine. This was achieved by adding the modular components of the dLITE system to HEK cells, sequentially in a gelatine biomatrix. While encapsulation of cells with dLITE in gelatine (also called as reverse transfection) or with cells being pre-seeded and then transfected (invasion/forward transfection) produced modest fluorescence in a range of gelatine concentrations (0.1-2.0%) post 24 hours light exposure, it was the combination of encapsulation with invasion strategy in 0.1% gelatine that produced highest transfection efficiency as determined by imaging. This result, for the first time, gives support to the theory of sequential gene induction, which currently relies on growth factors/small molecules but that can now be potentially replaced by CRISPR optogenetics, making this technique economical and attractive for stem cell and biomedical applications.

Biography

Mohammed Algahtani is pursuing his Medical studies from Al-Imam Muhammad Ibn Saud Islamic University, College of Medicine, with a high interest in optogentics.

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