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Microrobotics for Spatiotemporally Controlled Human-Induced Pluripotent Stem Cell (hiPSC) Differentiation

Precision control in spatiotemporal cell state-based gene expression and differentiation is essential to engineer native-like multicellular structures. Current differentiation programs often rely on externally provided inputs (e.g., chemical growth factors) whose effects are not targeted to distinct cells in the appropriate state and cannot form sophisticated tissue structures. Our work introduces a magnetically controlled microrobot (MR) platform for guiding mammalian cells to desired locations that, combined with synthetic biology, delivers biological signals at precise locations, enabling spatiotemporal control of cell-fate decision-making.

We use synNotch, a cell-cell contact-based biological signaling that induces relevant gene expression in receivers when the receiver cells contact sender cells through ligand-receptor binding. From synNotch activation dynamics of engineered Chinese hamster ovary (CHO) cells, we observed that a minimum of one sender cell in contact with a receiver cell is sufficient to induce synNotch activation. Additionally, the average time delay in observing receiver cell activation is 7 ± 4 hours after contact with senders. Magnetically driven MRs are then allowed to be internalized by sender cells, resulting in magnetized sender cellbots. Using a 3-pair orthogonal Helmholtz coil system, we guided magnetized sender cellbots to precise locations in a receiver cell culture, allowing activation of a desired fluorescent protein in target receiver cells.
Next, we engineered hiPSC receiver cells that can differentiate into endothelial cells (ECs) by overexpressing ETV2 (ETS variant transcription factor 2), a master transcriptional regulator of endothelial cell development. In co-culture with senders, hiPSC receivers

expressed ETV2, which we validated by co-expression of a fluorescent protein. Using RT-qPCR, we observed significantly higher expression of ETV2 and EC-specific markers, such as VE-Cadherin, CD31, and CD34, in sender- receiver co-culture triggered by synNotch activation. Using immunofluorescence, we also confirmed VE-Cadherin expression in ETV2-expressing hiPSC receivers but not in a co-culture without sender cells.

We finally guided multiple magnetized sender cellbots to different locations on hiPSC receiver colonies using our magnetic-guiding platform and observed synNotch activation in the hiPSC receivers within 24 to 30 hours. Our approach provides a foundation for the engineering of spatial patterns by activating conditional triggers based on MR location and cell state at multiple time points, which may be helpful for several applications, such as control of organoid architecture.