Spatially Patterned Differentiation of Human Pluripotent Stem Cells via Morphogen Localization

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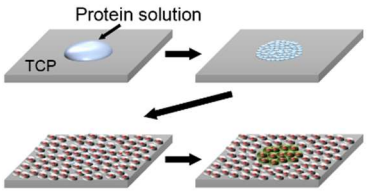

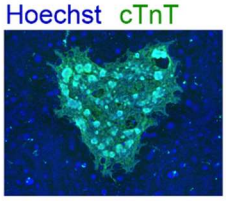

During development, spatiotemporal control of morphogen presentation is critical for proper organ and tissue formation. Despite its importance, studying mechanisms of human developmental events is challenging due to practical and ethical issues related to studying human embryos. While the advent of human pluripotent stem cells (hPSCs) has provided a powerful *in vitro* tool for studying human developmental biology as well as for modeling diseases and screening drugs, conventional *in vitro* cell culture and differentiation system has difficulties in recapitulating spatial pattern formation mainly due to difficulties in localizing morphogens. In this study, we present a simple and versatile method to spatially localize morphogens on a solid substrate including a conventional tissue culture plate to pattern hPSC differentiation in 2-dimensional culture.

Morphogens including BMP4, noggin, activin A, and Wnt3a, as a form of solutions, were simply placed on a tissue culture plate as a droplet, drawn with a pipette tip, or confined with polydimethylsiloxane (PDMS) stencil masks. The morphogens were adsorbed on the surface likely via hydrophobic interaction and remained deposited after washing while maintaining their patterns. hPSCs and hPSC-derived progenitors were seeded on top of the morphogen patterns and assessed for their fate commitment.

Patterned BMP4, activin A, and Wnt3a induced localized mesendoderm, endoderm, cardiomyocyte, and epicardial cell differentiation from hPSCs and hPSC-derived progenitors. Simultaneous localization of BMP4 and activin A and differentiation of hPSCs on these patterns allowed the formation of all three germ layers in a single well. Patterned cardiomyocytes and epicardial cells differentiated from hPSC-derived cardiac progenitors by localized Wnt3a and BMP4 allowed investigation of intercellular interactions between these closely related cell types in a spatially controlled manner. Importantly, cardiomyocytes in the proximity of epicardial cells exhibited longer sarcomere length and increased myofibril alignment, which are important features of cardiomyocytes *in vivo*, compared to the cardiomyocytes away from the pattern boundary.

This approach provides a facile *in vitro* platform not only to study early pattern formation but also to investigate intercellular interaction in a spatial context. Furthermore, it can be used to generate a 2-dimensional patterned hPSC-derived tissue structure for modeling disease and drug interactions.

Reference: Jin G. Adv Healthcare Mater., 2021; 2100995