Manipulating Self-Organization of Stem Cells through Micropatterning

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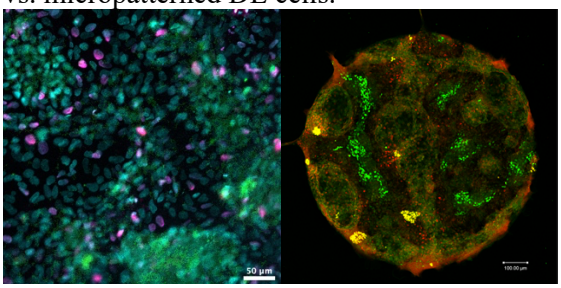
Introduction: Human Stem Cells (hSCs) stimulated by morphogens under standard culture in-vitro show some instances of self-organization and established signaling gradients, but the results are inconsistent [1]. Micropatterns are used to spatially restrict cells, helping cells self-organize and establish internal signaling gradients [1]. By evaluating endoderm development under both standard culture and micropatterns, we can compare the self-organization exhibited, giving insight into spatial restriction and the signaling hierarchy occurring in-vivo during early development.

Methods: For standard culture, hSCs were passaged using Gentle Cell Dissociation Reagent onto coverslips coated with ECM (Cultrex). For micropatterns, hSCs were passaged using Single Cell Accutase Dissociation Reagent onto coverslips stamped with ECM by a PDMS micropattern stamp. MTeSR + ROCK Inhibitor was added to each well for 48 hours to allow adhesion. For unstimulated wells, MTeSR was added from day 2 forward. For BMP-4 Stimulation, ADMEM + BMP-4 was added from day 2 forward. For definitive endoderm (DE), ADMEM + Activin A + CHIR99021 was added for 24 hours, then changed to ADMEM + Activin A + LDN-193189 for 48 hours. Anterior foregut endoderm (AFE) followed the DE process until day 5. Media was then changed to ADMEM + A83-01 + LDN-193189 for 72 hours. Slips were fixed at appropriate times with 4% Paraformaldehyde, permeabilized and blocked with PBS+Triton-X and Bovine Serum Albumin, and stained with fluorescent antibody markers corresponding to their stage of development.

Results: Epi-fluorescent and confocal microscopy showed qualitative evidence of limited self-organization in standard culture cells, while micropatterned cells exhibited clear self-organization in concentric rings. Markers for each stage of development were present in both kinds of culture, but micropatterned samples expressed the self-organization desired.

Figure: Microscopy image of standard DE cells vs. micropatterned DE cells.



Conclusion: In both groups, organ progenitor cell type markers are present, indicating proper development towards the desired organs of the anterior foregut endoderm. However, micropatterned samples display self-organization that indicates different signaling gradients absent in standard culture techniques.

References:

1. Deglincerti A., et al. Nat Protoc. 2016; 11: 2223-2232.

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