Microwells for Controlled and Uniform Embryoid Bodies

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Summary: a new micro-technology that captures embryonic stem cells (ESCs) into microwells for the induction of uniform EBs from single cell suspension by controlling the seeding concentration.

Many cell types have been shown to engraft in areas of myocardial damage and partially repair cardiac function, suggesting cardiac/stem cell transplantation to be a promising treatment for cardiomyopathy. However, full cardiac tissue regeneration will only become possible when technologies allow the generation of a sustainable, purified source of mature functional cardiac progenitors. ESCs are one exciting potential cell source for cardiac repair due their proliferative potential, pluripotency, and scale-up capabilities. Embryoid body (EB) formation for initial induction of human ESCs (hESCs) closely recapitulates early embryonic development with respect to lineage commitment. Traditional suspension culture often gives rise to EB populations which are heterogeneous in morphology and, thus, differentiation capacity. The hanging drop can yield relatively uniform mouse EBs, but this method is very labor intensive. In order to address this problem, we employ a new micro-technology that captures ESCs into microwells by gravity for the induction of uniform EBs from single cell suspension. Both microwell and hanging drop can control the mouse EB sizes by adjusting cell numbers. But mouse EBs formed in microwells are more circular and contained significantly more viable cells compared with the mouse EBs form via Hanging Drop. Unfortunately, the hanging drop technique has not been possible for human EB formation due to the apoptotic nature of hESCs while in single cell suspension. By controlling the seeding concentration of the single cell suspension and adding Rho-associated kinase (ROCK) inhibitor we can generate uniform human EBs with controlled sizes, thus influencing lineage commitment.