

Aggregate formation and suspension culture of human pluripotent stem cells and differentiated progeny.

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Public Summary:

Culture of human pluripotent stem cells (hPSC) as in vitro multicellular aggregates has been increasingly used as a method to model early embryonic development. Three-dimensional assemblies of hPSCs facilitate interactions between cells and their microenvironment to promote morphogenesis, analogous to the multicellular organization that accompanies embryogenesis. In this paper, we describe a method for reproducibly generating and maintaining populations of homogeneous three-dimensional hPSC aggregates using forced aggregation and rotary orbital suspension culture. We propose solutions to several challenges associated with the consistent formation and extended culture of cell spheroids generated from hPSCs and their differentiated progeny. Further, we provide examples to demonstrate how aggregation can be used as a tool to select specific subpopulations of cells to create homotypic spheroids, or as a means to introduce multiple cell types to create heterotypic tissue constructs. Finally, we demonstrate that the aggregation and rotary suspension method can be used to support culture and maintenance of hPSC-derived cell populations representing each of the three germ layers, underscoring the utility of this platform for culturing many different cell types.

Scientific Abstract:

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