Generation Of Human Blastocyst-like Structures From Somatic Reprogramming

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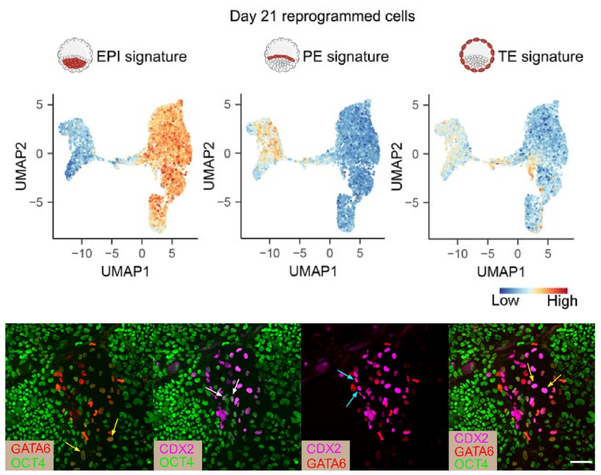
Abstract

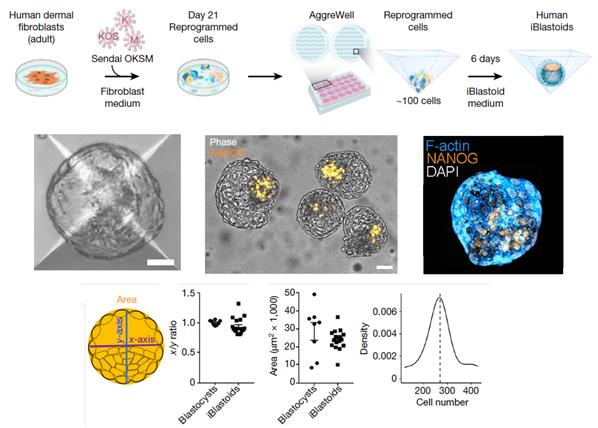
Human pluripotent and trophoblast stem cells have been essential alternatives to blastocysts for understanding early human development. However, these simple culture systems lack the complexity to adequately model the spatiotemporal cellular and molecular dynamics that occur during early embryonic development. Here we describe the reprogramming of fibroblasts into in vitro three-dimensional models of the human blastocyst, termed iBlastoids. Characterization of iBlastoids shows that they model the overall architecture of blastocysts, presenting an inner cell mass-like structure, with epiblast- and primitive endoderm-like cells, a blastocoel-like cavity and a trophectoderm-like outer layer of cells. Single-cell transcriptomics further confirmed the presence of epiblast-, primitive endoderm-, and trophectoderm-like cells. Moreover, iBlastoids can give rise to pluripotent and trophoblast stem cells and are capable of modelling, in vitro, several aspects of the early stage of implantation. In summary, we have developed a scalable and tractable system to model human blastocyst biology; we envision that this will facilitate the study of early human development and the effects of gene mutations and toxins during early embryogenesis, as well as aiding in the development of new therapies associated with in vitro fertilization.

1. Early embryonic signatures at day 21 of reprogramming



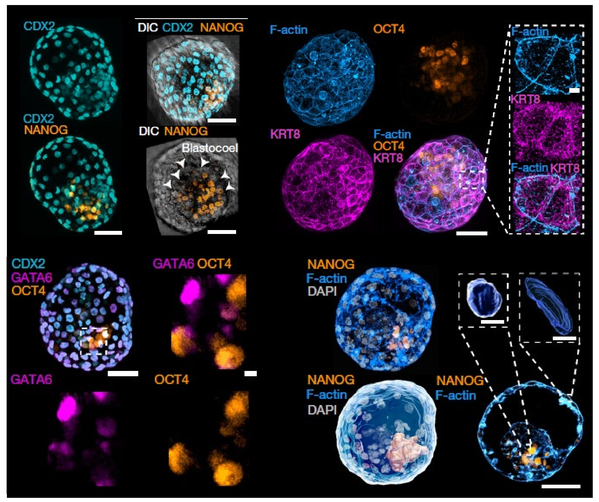
When the fibroblasts are reprogrammed under fibroblast culture condition only without additional signaling cues, the epiblast (EPI), primitive endoderm (PE) and trophectoderm (TE) signatures defined using scRNA-seq dataset from human blastocysts emerge by day 21 of reprogramming. Immunostaining analysis with CDX2 (TE marker), GATA6 (PE marker) and OCT4 (EPI marker) also showed the heterogeneity of day 21 reprogrammed cells.

2. Generation of human iBlastoids



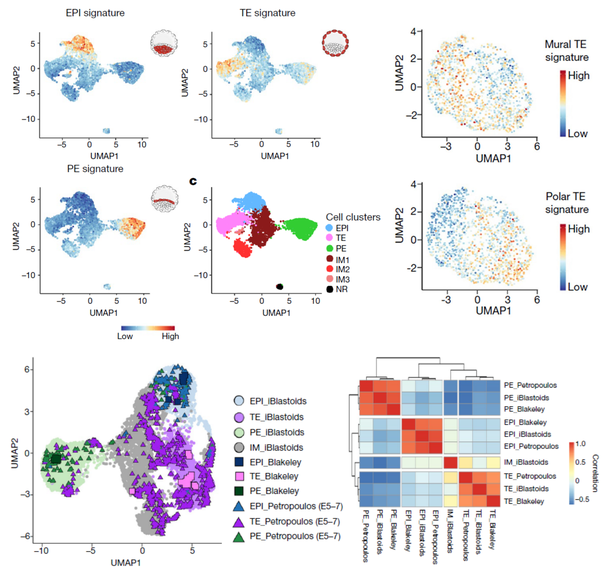
When allowed to aggregate under a 3D culture system (AggreWell), the day 21 reprogramed cells progressively develop into 3D cellular structures, with some of them (iBlastoids) presenting blastocyst-like cavitation. The iBlastoids contain NANOG positive inner cell mass (ICM)-like cellular compartment surrounded by an outer later of NANOG negative cells. The diameters and cross-sectional areas of the iBlastoids were comparable to previously published measurements of E5-7 human blastocysts, and contain between 100-400 cells.

3. Spatial cellular localization of iBlastoids



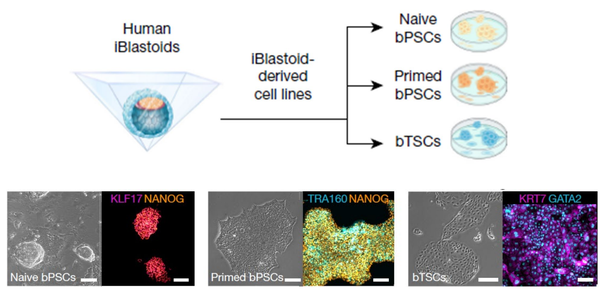
Further analysis reveals that iBlastoid contains an outer layer of TE-like cells that express TE markers (CDX2 and KRT8), envelopping the OCT4 and NANOG expressing EPI-like cells. Closer examination also identifies GATA6 positive PE-like cells neighbouring the OCT4-poisitive EPI-like cells in some of the iBlastoids. In addition, iBlastoids also recapitulate the cellular architecture differences between EPI and TE cells, with the TE-like cells of the iBlastoids having a flattened appearance and EPI-like cells being more rounded and columnar-shaped.

4. Transcriptomic similarity of iBlastoids to blastocysts



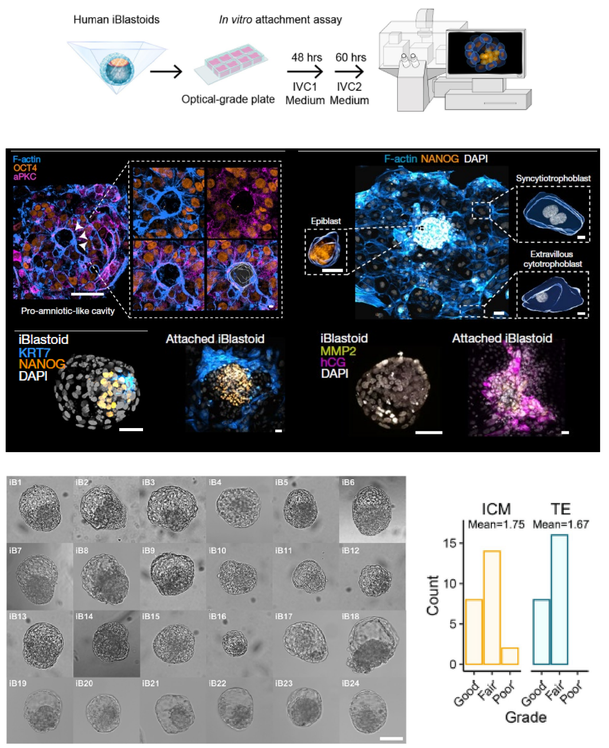
Transcriptomic profiling of the iBlastoids using scRNA-seq confirms the presence of EPI-, PE, and TE-like lineages using gene signatures defined for each lineage in human blastocysts. Further integrative analysis with two scRNA-seq datasets obtained from human blastocysts reveals a high concordance between cells of the iBlastoids with the blastocysts, which is also similarly suggested by the correlation analysis of iBlastoid EPI, PE and TE cell clusters with the annotated counterparts in blastocysts. Moreover, further examination also reveals two distinct populations of TE-like cells, each respectively upregulate the polar or mural TE signatures.

4. Derivation of stem cells from iBlastoids



Similar to the human blastocysts, iBlastoids are also capable of giving rise to naïve pluripotent stem cells (naïve bPSCs), primed pluripotent stem cells (primed bPSCs) and trophoblast stem cells (bTSCs). These iBlastoid-derived stem cells could be maintained in long-term and demonstrate respective functional properties.

5. iBlastoids model implantation in vitro



When adapting an assay established on the human blastocysts to model in vitro the implantation, iBlastoids are capable of modelling several aspects of this developmental event such as the growth and expansion of EPI-like cells, polarization of EPI-like cells with formation of pro-amniotic cavity, emergence of syncytiotrophoblasts-like and extravillous cytotrophoblast-like cells, together with secretion of human chorionic gonadotropin (hCG). Further characterization of iBlastoids using the in vitro fertilization blastocysts quality criteria score indicated that iBlastoids were graded as good or fair with an average score of 1.75 for ICM and 1.67 for TE.

Conclusion

In summary, by studying the behaviour of reprogramming intermediates, we identified an unanticipated approach for generating an integrated model of human blastocyst, which is generated via somatic reprogramming rather than assembly of previously obtained stem cell lines. The iBlastoids demonstrate several molecular and functional properties similar to the human blastocysts, representing an accessible and scalable model system to model in vitro the pre-implantation and peri-implantation stages of human embyrogenesis that will facilitate many applications in basic research and translational approaches