COMMENTARY

Scalable culture of human pluripotent stem cells in 3D

Todd C. McDevitt[a,b,1]

[a]The Wallace H. Coulter Department of Biomedical Engineering at Georgia Tech and Emory University, and [b]The Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA 30332

The promise of human pluripotent stem cell (hPSC) therapies is ever-increasing, with the rapid proliferation of reports over the past several years demonstrating homogeneous and efficient differentiation of hPSCs to clinically relevant cell types (1–3). Such discoveries have renewed the enthusiasm and motivation for the acceleration of clinical trials using hPSC-derived cells to treat nervous system injuries and ocular degeneration, as well as diabetes and cardiac diseases in the near future. On the path to clinical implementation of hPSC therapies, the need for scalable culture technologies capable of producing sufficient quantities of cells becomes of paramount importance, as the proposed therapies demonstrate efficacy in human patients. However, despite several significant advances in recent years establishing defined, xeno-free conditions for hPSC culture (4–6), numerous challenges still remain to be overcome, including advanced 3D platforms capable of efficient, facile, and robust expansion, differentiation, and retrieval of hPSCs.

In PNAS, Lei and Schaffer address many of these technical challenges with their report of a unique 3D culture system for scalable and efficient hPSC expansion and differentiation that is completely defined and capable of being compatible with good manufacturing practices (7). An important trait of the synthetic polymer hydrogel system chosen by Lei and Schaffer is that it is thermoresponsive, thereby enabling simple encapsulation and rapid retrieval of hPSCs at any time by switching the temperature between 4 °C and 37 °C, based on the phase transition behavior of the poly(N-isopropylacrylamide)-co-poly(ethylene glycol) (PNIPAAm-PEG) hydrogel going from a liquid to a solid gel as the temperature is increased. In addition, because the polymeric materials are synthetic, they can be combined with recombinant molecules necessary for self-renewal or differentiation to offer a completely defined system for hPSC culture. These advantages make the described technique an attractive alternative to conventional adherent culture methods that have traditionally been used by most hPSC researchers to produce the cells for basic in vitro experiments all the way up to preclinical animal studies.

3D Environments

Moving from 2D adherent culture to 3D suspension culture adds significant space for cells to grow without detrimental agglomeration in the third ("Z") dimension, and therefore offers the opportunity to significantly scale-up the expansion of hPSCs and differentiated progeny. By systematically screening

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different culture media and cell-seeding densities within the 3D hydrogel, Lei and Schaffer (7) found that they could attain an ∼20-fold expansion of hPSCs per passage after only 4–5 d of culture, and obtain 10^{72}-fold expansion total after 60 passages (280 d). Three-dimensional culture in suspension is inherently capable of yielding a higher cell density than 2D adherent formats, even with comparable amounts of culture media and exchange intervals. This finding implies that the same concentrations of nutrients and critical factors can increase the growth of hPSCs within a similar period simply by transitioning the cells from 2D to 3D culture. This physical change alone is sufficient to yield an increase in cell number by more than an order of magnitude within the time of a single passage; moreover, with repeated passages, hPSC expansion can increase exponentially without increasing the cost of culture media, which typically accounts for >50% of the cost-of-goods of most cell products. Thus, the described method potentially offers a cost-effective way of enhancing hPSC production.

In addition to the significantly increased expansion of the hPSCs, growth of the cells from a single-cell dispersion throughout the PNIPAAm-PEG hydrogel matrix also yielded a more homogeneous overall population of cells. The single cells grew in a clonal manner as small aggregates that remained evenly distributed throughout the gel and retained their pluripotent phenotype (>95% $Oct4^+$). Passaging of hPSCs as single cells onto 2D substrates is commonly avoided because of the poor adhesion of the cells to the surface and reduced survival that together limits overall expansion resulting from high cell loss at every passage. Small molecules, such as Rho kinase inhibitor, are commonly added during hPSC passaging in an attempt to inhibit cell death, and were used by the authors in their final optimized protocol because it similarly improved single-cell survival in the 3D hydrogels. Seeding of the single cells dispersed evenly throughout the hydrogel matrix enabled growth of cells under nearly identical biochemical and biophysical conditions, and different cell lines behaved similarly in the 3D environments (7). The similarities in the size and appearance of the hPSC clusters in the hydrogels suggested better synchronized growth of the overall culture.

Manufacturing Considerations

The biomanufacturing industry is increasingly recognizing that new good manufacturing practice-compatible platforms are sorely needed to enhance the scalability of current cell manufacturing practices and facilitate the development and translation of novel hPSC therapies. Almost all hPSCs have often been originally derived and expanded in the presence of xeno- or allogenic cells (i.e., fibroblast feeder layers of mouse or human

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[1]E-mail: todd.mcdevitt@bme.gatech.edu.

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origin) or soluble media supplements (i.e., FBS, Matrigel, BSA) that might compromise their survival and efficacy in vivo as a result of potential immunogenicity concerns. Since the original descriptions of human embryonic and induced PSCs (8–10), advanced culture conditions for 2D adherent culture of hPSCs using defined materials have been developed, but they don't typically increase the growth kinetics of the cells or enable higher cell density culture to be achieved (11–13), as Lei and Schaffer demonstrate here for their 3D hydrogel culture system (7). Synthetic polymers like PNIPAAm-PEG, analogous to the polystyrene dishes that cells are currently cultured on for manufacturing of most clinical cell therapies, can be well-defined, mass-produced, and easily formed into various physical conformations while containing hPSCs. In an era where the mantra "the process is the product" applies to the regulatory approval of cell therapy products, it is critical to devise and implement completely defined culture systems to safely and robustly produce well-defined hPSC products for clinical applications.

Currently, most materials used to encapsulate cells, and especially those used previously for hPSC culture, require degradation of the material in some manner to liberate the cells from the gels before they can be subsequently used, such as for transplantation studies. The necessity to physically degrade the gels can introduce additional processing steps to adequately remove enzymes or other materials used to break down the hydrogels from the final population of cells. In this instance, the use of thermoreversible gel properties enables Lei and Schaffer (7) to simply change the temperature to quickly and efficiently extract cells from the gels without adversely affecting cell viability. The reversible use of the 3D hydrogel for hPSC growth is similar to the thermoreversible hydrogel surface-coating technologies pioneered by Okano and colleagues that are now being used to produce intact cell-sheet products for tissue-engineering applications (14).

Several intriguing possibilities remain for the potential use of this unique 3D culture system for human pluripotent cell biology and biomanufacturing. For example, because the materials facilitate the rapid growth of hPSCs, they might be coupled with reprogramming technologies to enhance the efficiency and yield of obtaining pluripotent cells from somatic sources in a manner similar to what has been reported for other suspension culture systems (15, 16). Comparing the differentiation of various human induced PSC lines to neural (and other) lineages within the context of the 3D hydrogels might also be interesting in light of reports about the reduced efficiency and variability of differentiation of induced PSCs compared with human embryonic stem cells (17). Moreover, the 3D hydrogel environments might also be conducive to enhance strategies for direct reprogramming of somatic cells to neural lineages and functional cell types (18–20). Only time will tell how much 3D scalable suspension culture techniques will ultimately impact hPSC growth and differentiation protocols, but it appears likely that their use for such purposes will "expand" in the near future based on the encouraging results of this study reported in PNAS (7).

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