New and Notable

Fast and Furious: The Mass and Motion of Stem Cells

Sravanti Kusuma[†‡] and Sharon Gerecht[†§*]

[†]Department of Chemical and Biomolecular Engineering, Johns Hopkins Physical Sciences Oncology Center and Institute for NanoBioTechnology, and [‡]Department of Biomedical Engineering, and [§]Department of Materials Science and Engineering, Johns Hopkins University, Baltimore, Maryland

The field of human pluripotent stem cell (hPSC) biology has garnered significant attention ever since the establishment of human embryonic stem cell lines well over a decade ago. Stem cells self-renew almost indefinitely in culture and can differentiate into every cell type, a phenotype defined as pluripotency. The field has experienced another boost more recently with the discovery of human-induced PSCs (hiPSCs), which are derived by genetically reprogramming a patient's somatic or stem cells with key factors, allowing them to become pluripotent. A large body of work on hPSCs has focused on their differentiation potential into various cell lineages to facilitate the development of cell-based therapies for regenerative medicine. Indeed, a handful of these studies are already beginning to progress toward clinical trials (1).

Despite great advances in the field, several fundamental parameters of hPSCs remain elusive, primarily due to the atypical culture conditions of hPSCs compared to somatic cell cultures. Human PSCs are often grown in compact colonies and under specific media conditions, properties that help maintain their pluripotency. Disruption to this environment precludes meaningful studies of hPSCs, including studies pertaining to their physical properties. Upon cellular reprogramming of somatic cells into hiPSCs, even physical properties are reestablished, making these properties an especially intriguing aspect of hPSC biology (2). A previous study demonstrated that the perinuclear actin cytoskeleton is completely absent in both undifferentiated human embryonic stem cell lines and hiPSCs, and forms in conjunction with differentiation, highlighting the complexity of physical changes that occur with differentiation (3). Previous efforts to determine what are otherwise straightforward characteristics of somatic cells, such as cell biomass, proliferation, and motility, have faced technical limitations that deter the precision of their measurements in hPSCs. High-resolution, time-lapse imaging previously uncovered that reprogrammed somatic cells dramatically increase their proliferation and decrease their cellular area before expressing pluripotent markers (4). Yet another study revealed that cells undergo a two-cell intermediate before complete reprogramming toward hiPSCs (5). These methods have relied heavily on sophisticated imaging modalities, but quantitative methods could help reveal cellular properties more robustly and ensure unbiased interpretation.

In the study under discussion here, Zangle et al. (6) seek to resolve some of these fundamental properties of hPSCs, namely cell biomass and motion. To determine these cell properties accurately and robustly, they harness the principles of light scattering in an approach known as live cell interferometry (LCI), which measures the phase shift from a light passed through a cell. The phase shift is caused by the interaction of light with the cellular matter, causing retardation of the light. A Michelson interferometer is then used to measure this phase shift, which is directly proportional to the amount of biomass at that particular location. LCI imaging across an entire hPSC colony, for example, would yield information on the distribution of biomass throughout the whole colony without disrupting its structure. Similar techniques of quantitative microscopy have been used to study cell growth and cell death properties of various cell types but not hPSCs (7,8).

In their study, Zangle et al. use LCI to follow physical changes of hPSCs in culture and at the onset of differentiation. Using LCI, the authors were able to determine that hPSC colonies accumulate mass at a consistent and exponential rate regardless of the starting mass of the colony and with a specific growth rate, defined as the increase in cell mass per unit time, of 0.03 h^{-1}. When colonies were differentiated with retinoic acid (RA), an inducer of trilineage differentiation, the mass accumulation rate of colonies decreased by only ~15%, contradicting the observation obtained via imaging techniques that these cells exhibited noticeably larger projected areas. These seemingly opposing findings lead to the hypothesis that cells regulate their cell mass independently of their cell volume during differentiation.

Zangle et al. also harness LCI to study changes in mass distribution throughout the colonies. They found that pluripotent colonies exhibit greater intracolony mass reorganization (or movement) compared to RA-differentiated colonies. Furthermore, they used LCI to deduce that pluripotent colonies exhibit a greater coordination distance, defined as the distance over which regions increase or decrease in mass together and reflects the cells' ability to coordinate movement with one another. Contrastingly, the coordination distance was very small in RA-treated colonies, indicating that hardly any coordinated cell movement was observed with the onset of differentiation. Altogether, these studies reveal that upon differentiation, the mass

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*Correspondence: gerecht@jhu.edu

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accumulation rate of cells does not change radically, but coordinated cell movement is abrogated.

The use of LCI in hPSC culture and differentiation opens up new (to our knowledge) avenues to study fundamental parameters of these processes to reveal differences in pluripotent versus differentiated phenotypes. However, several limitations of their system exist: for example, LCI is most appropriate for relatively flat entities, preventing the study of cellular activity within three-dimensional embryoid bodies. Furthermore, it is unclear how LCI would account for single cell death or apoptosis within colonies. Despite these challenges, the work of Zangle et al. (6) presents a unique approach to study intrinsic and previously undiscovered physical properties of hPSC growth and differentiation. Using an approach such as LCI, which examines physical properties in particular, opens greater opportunities to understand more complex differentiation features such as cellular patterning, fate, or organization.

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