Elastic Properties of Induced Pluripotent Stem Cells

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The recent technique of transducing key transcription factors into unipotent cells (fibroblasts) to generate pluripotent stem cells (induced pluripotent stem cells [iPSCs]) has significantly changed the stem cell field. These cells have great promise for many clinical applications, including that of regenerative medicine. Our findings show that iPSCs can be derived from human adipose-derived stromal cells (hASCs), a notable advancement in the clinical applicability of these cells. To investigate differences between two iPS cell lines (fibroblast-iPSC and hASC-iPSC), and also the gold standard human embryonic stem cell, we looked at cell stiffness as a possible indicator of cell differentiation-potential differences. We used atomic force microscopy as a tool to determine stem cell stiffness, and hence differences in material properties between cells. Human fibroblast and hASC stiffness was also ascertained for comparison. Interestingly, cells exhibited a noticeable difference in stiffness. From least to most stiff, the order of cell stiffness was as follows: hASC-iPSC, human embryonic stem cell, fibroblast-iPSC, fibroblasts, and, lastly, as the stiffest cell, hASC. In comparing hASC-iPSCs to their origin cell, the hASC, the reprogrammed cell is significantly less stiff, indicating that greater differentiation potentials may correlate with a lower cellular modulus. The stiffness differences are not dependent on cell culture density; hence, material differences between cells cannot be attributed solely to cell-cell constraints. The change in mechanical properties of the cells in response to reprogramming offers insight into how the cell interacts with its environment and might lend clues to how to efficiently reprogram cell populations as well as how to maintain their pluripotent state.

Introduction

ADULT STEM CELLS have been targeted for many applications—the most important of those being therapeutic strategies for regenerative medicine.^{1–3} The reprogramming of fibroblasts into embryonic stem (ES)-like cells opens the door to even more possibilities in how cells are used therapeutically.⁴ We have recently shown that human adult adipose-derived stem cells (hASCs) in addition to fibroblasts can also be reprogrammed into a pluripotent state,⁵ constituting a significant discovery solving many issues associated with deriving induced pluripotent stem cells (iPSCs) from human fibroblasts⁴ and greatly expanding the source of stem cells. Effective use of iPSCs derived from hASCs requires extensive knowledge of the factors affecting iPSC function and differentiation from biochemical to biomechanical.

The mechanical properties of cells are fundamental to how they sense and respond to their environments. The stress state within the cytoskeleton and cell membrane result from the complex interaction of cell junctions, cell-extracellular matrix adhesions, and the intrinsic material properties of the cell constituents, which in turn dictate the downstream response of mechanosensitive cellular elements, such as stretch-activated ion channels, growth factor receptors, and focal adhesion sites.^{6–8} The cell mechanical modulus is altered by changing substrate stiffness by transmitting stresses from focal adhesion sites through actin filaments and myosin II-driven contraction in the cells.⁹ For instance, mesenchymal stem cells can detect and differentiate in response to differences between collagen-coated gels that mimic various stiffnesses of substrata ranging in values from soft brain to stiff osteoid.¹⁰ Also, recently, researchers have found that less stiff mouse ESCs respond more readily to small, applied

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forces than their more stiff differentiated selves.¹¹ Typically cluster of differentiation markers have allowed researchers to track differentiation by immunophenotyping cells to witness their changing surface marker expression levels.¹² However, additional tools are necessary to characterize cells more thoroughly. The differentiated state of cells may be tracked through cellular biomechanical properties.¹³ The biomechanics of a cell markedly affects its cellular properties and behavior; therefore, we investigated the property of cell stiffness as an indicator of cell phenotype.

The ability of the atomic force microscope (AFM) to function in liquid under physiologic conditions and maintain excellent spatial and force resolution makes it a powerful tool for analyzing living cells. Here, AFM was used as a nanoindenter for determining the elastic modulus of human fibroblasts, hASCs, iPSCs derived from hASCs (hASC-iPSCs), iPSCs derived from fibroblasts (fibroblast-iPSCs), and human embryonic stem cells (hESCs). In aggregate, these cells represent unipotent or fully differentiated cells (fibroblasts), multipotent cells (hASCs), and pluripotent cells (hESCs, hASC-iPSCs, and fibroblast-iPSCs). We found that the differentiation state of the cell inversely correlated with cell stiffness. Cell types in order of increasing cell stiffness were hASC-iPSCs, hESCs, fibroblast-iPSCs, fibroblasts, and, lastly, as the stiffest cell type, hASCs. These results have implications in the use of relative cell stiffness as a unique biomarker, which would create a powerful tool for determining other properties of the cell such as its propensity to differentiate. Moreover, this information may in turn be potentially employed to actively manipulate differentiated cells to exhibit more stem cell-like properties.

Materials and Methods

Chemicals and media

Stem cell maintenance medium, mTeSR™ 1, was obtained from StemCell Technologies (Vancouver, BC, Canada). Human ASCs and fibroblasts were cultured in the standard growth medium consisting of Dulbecco's modified Eagle's medium (DMEM), 10% fetal bovine serum, and 1% penicillin/streptomycin. Reprogrammed hASCs and fibroblasts were cultured in mTeSR 1 basal medium with the 5× supplement. H7 hESCs were cultured in WiCell hESC culture medium consisting of DMEM-F12 with 20% knockout serum replacer (Invitrogen, Carlsbad, CA), 1% nonessential amino acids, 1 mM L-Glutamine, 4 ng/mL basic fibroblast growth factor, and 0.1 mM 2-mercaptoethanol. Culture dishes were coated with hESC qualified Matrigel (Cat No. 354277; BD Biosciences, San Jose, CA) diluted in DMEM/F-12 (Cat. No. 10565-018; Invitrogen) per manufacturer's instructions depending on Matrigel activity, typically ~70-fold dilution.

Cell isolation and culture

Human lung fibroblasts (IMR90) and hESCs (H7) were obtained as cell lines. hASCs were isolated from human lipoaspirates as described below. iPSCs were transduced from fibroblasts and hASCs via the following procedures.

All lipoaspiration specimens were obtained after acquiring informed consent from patients, in accordance with Stanford University human IRB guidelines. All lipoaspiration procedures were performed using the VASER Lipo

System (Sound Surgical Technologies, Louisville, CO). ASCs were harvested from the adipose tissue of male or female patients between the ages of 18 and 65 undergoing elective lipoaspiration of the abdomen, flank, and/or thigh region. Participating patients had no prior knowledge or evidence of ongoing systemic disease at the time of operation. All specimens were immediately placed on ice and processed after harvest.

Immediately after lipoaspiration, adipose specimens were washed sequentially in serial dilutions of Betadine, followed by two phosphate-buffered saline washes. Tissues were subsequently digested with an equal volume of 0.075% (w/v) type II collagenase in Hank's Balanced Salt Solution at 37°C in a water bath with agitation at 125 rpm for 30 min. The collagenase digest was then inactivated by adding an equal volume of the standard cell culture growth medium (DMEM + Glutamax/10% fetal bovine serum/1% Penicillin/Streptomycin). The stromal vascular fraction was pelleted via centrifugation at 1200 g for 5 min. The supernatant was then discarded, and the cell pellet resuspended and filtered through a 100 µm cell strainer to remove undigested tissue fragments. The cells were pelleted and resuspended in the standard cell culture growth medium at 37°C in an atmosphere of 5% CO₂. Cells were grown to subconfluence and passaged with 0.025% trypsin. The medium was changed every 3 days. Cells were used up to passage four for all experiments.

Reprogramming hASCs and fibroblasts with individual lentiviruses

hASCs and fibroblasts were reprogrammed either on mouse embryonic fibroblast feeder cells or on Matrigel-coated feeder-free surfaces. For reprogramming on feeder cells, 2×10^5 hASCs were seeded in six-well tissue culture dish and maintained with the hASC growth medium. On the second day, cells were transduced with individual lentiviruses containing human Oct4, Sox2, Klf4, and c-MYC at a 1:1:1:1 ratio plus 5 mg/mL polybrene (Sigma, St. Louis, MO). The day of this first time transduction was considered as day 0. Transduction was repeated on day 2 using the same batch of all four lentiviruses. On day 3, cells were digested off the culture dish with 0.05% trypsin-EDTA (Gibco, Carlsbad, CA) and counted with a hemocytometer. Approximately 50,000 cells were then transferred onto a mouse embryonic fibroblast feeder layer in a gelatin-coated 10 cm culture dish and cultured with the hESC growth medium mTeSR-1. The old medium was aspirated and the cells were refreshed with new mTeSR-1 medium every day. Background non-ESC-like colonies usually appeared from day 5 to 6, whereas ESC-like colonies with distinct light refractive property appeared as early as on day 12–13. On days 16–20, the living ESC-like colonies were immunostained with TRA-1-60 mAb (Millipore, Billerica, MA) and Alexafluor488 secondary antibody (Invitrogen). Positive colonies with ES morphologies were picked out with a glass needle and seeded on Matrigel surface in a new culture dish. Each single picked colony was then maintained and expanded following routine ES cell passaging and culturing protocols and established as one individual hASC-iPS cell line. For feeder-free reprogramming, hASCs were seeded at a density of 2×10^5 per well in a six-well tissue culture dish

previously coated with hES qualified Matrigel (BD Biosciences). Cells were transduced twice with the four individual lentiviruses on day 0 and 2. On day 4–5, the medium was switched from the hASC growth medium to mTeSR-1. Cells were refreshed with mTeSR-1 everyday. ES-like colonies appeared as early as on days 13–14. On days 18–20, positive colonies with ES morphologies and TRA-1-60 expression were picked out and expanded under feeder-free conditions.

Cell mechanical property investigation

Cell mechanical properties comparing fibroblasts, hASCs, hASC-iPSCs, fibroblast-iPSCs, and hESCs were quantified by indentation with an AFM. An Agilent 5500 AFM (Agilent Technologies, Santa Clara, CA) mounted on a Zeiss 200M Axiovert inverted microscope (Carl Zeiss Microimaging, Thornwood, NY) was used to precisely position an AFM tip on a cell. A silicon cantilever (Novascan Technologies, Ames, IA) with a nominal spring constant of 30 mN/m with a 5- μ m-diameter borosilicate glass sphere attached to the tip was mounted on the AFM. The spring constant of each cantilever was calibrated with Sader's method by measuring the thermal noise spectrum of the cantilever deflection signal with a Labview DAQ card (NI 9215; National Instruments Corporation, Austin, TX).¹⁴ Size 22 $\times$ 22 mm No. 2 glass coverslips (VWR International, West Chester, PA) were coated overnight in hESC qualified Matrigel. Human ASCs were enzymatically lifted from pre-confluent 10 cm culture dishes. hASCs were plated in cloning rings at densities of 5×10^5 cells/cm² and allowed to adhere overnight. IMR90 human fibroblasts were plated under similar conditions. Both iPSCs and hESCs were mechanically passaged and plated on Matrigel coated coverslips and allowed to adhere for up to 2 days. The liquid cell was subsequently filled with 0.6 mL CO₂ independent medium (Cat. No. 18045-088; Invitrogen) containing 5 $\times$ mTeSR-1 supplement. The liquid cell was composed of a frame and a rubber gasket that can be mounted with spring tension clips on top of a coverslip on the AFM stage, allowing mounting of samples requiring liquid (i.e., cells). The chamber was then filled with medium such that specimens can be maintained in an aqueous environment while they are examined. The AFM stage and liquid cell were mounted on the AFM, and the cantilever, fitted with a closed-loop piezoelectric scanner, was lowered into the liquid cell. Regions within each colony were chosen for force deflection curves to reflect either a specific location in the colony, for example, edge or center, or to reflect well-formed ESC-like morphology. The AFM probe approached the cell from a free deflection point and indented at a speed of 1 μ m/s for a range of 2.5 μ m. Indentation speeds ranging from 0.25 to 5 μ m/s were tested for their ability to discriminate the elastic from viscoelastic cell response. A speed of 1 μ m/s was chosen because it was the best compromise between exceedingly long probing time periods that may allow cells to change and higher indentation rates that cause viscous drag effects on the cantilever. The maximum indentation depth was controlled by a preset force limit to avoid damaging the cell. Force distance curves were recorded during the indentation and retraction of the probe from these curves. The cell apparent elastic modulus could

be extracted by fitting experiment data with Hertz model for elastic indentation,

$$F = \frac{4}{3} \left(\frac{E}{1-\nu^2} \right) \sqrt{R} \delta^{3/2}$$

where F is the indentation force, E is the Young's modulus, ν is the Poisson ratio (typically between 0.3 and 0.5 for soft materials such as cells; 0.5 was used for this study), R is the radius of the indenting sphere, and δ is the indentation depth.

Colonies were chosen for morphology representative of undifferentiated cells in locations around the colony. Regions of 90 $\times$ 90 μ m, the limit of the AFM scanhead, were probed. A custom script was used to automatically sample a 16 $\times$ 16 grid array within this region with a total acquisition time of 30 min. These points were averaged and the overall population modulus is expressed as the geometric mean $\pm$ standard error. Statistically significant differences in cell stiffness between cell types were assessed with a paired Student's t -test. Human ASCs were treated with Cytochalasin D (CytoD) as a positive control to demonstrate that actin filaments as elastic components of the cytoskeleton are the major contributor to the elastic modulus. Human ASCs were treated with 1 μ M CytoD for 1 h before probing and maintained in the same concentration of CytoD during the AFM probing.

Results

Cell colony formation

A result of successful reprogramming of hASCs is that the cells form compact, dense colonies resembling ESC colonies. One of the challenges of assessing the mechanical properties of cells is to separate the cell properties as generated from the intrinsic biology from those that are merely a byproduct of cellular constraints such as culture density. To approach this problem, iPSC morphology was recapitulated by culturing typically noncolony forming fibroblasts and hASCs at very high densities, creating comparable pseudo-colonies. Human ASCs and human fibroblasts cultured to confluence normally approach densities of $\sim 5 \times 10^4$ cells/cm² (Fig. 1A, B), whereas hESC and iPSCs have colonies with local densities often exceeding 7×10^5 cells/cm². After plating hASCs and IMR90 at very high densities under the local confinement of a cloning ring, cell densities were closer to 4×10^5 cells/cm² (Fig. 1C, D), approximating that of natural colony forming hASC-iPSCs, fibroblast-iPSCs, and hESCs (Fig. 1E–G).

Force displacement

To measure the elastic properties of the entire iPSC colony, a Labview program was made to control the AFM probe to take force distance curves at 16 $\times$ 16 point array over regions of 90 $\times$ 90 μ m. The elastic modulus was extracted by fitting experimental data with Hertz model and averaging within each region and between different regions. To study the elasticity distribution within the iPSC colony force, distance curves were taken at 3 $\times$ 3 point array over regions of 20 $\times$ 20 μ m at the center and the edge of the colony.

Three different regions were probed within the cell colonies: center, mid-point between the center and edge, and

FIG. 1. Light microscope images of the different cell types in culture. **(A)** Confluent culture of hASCs; **(B)** confluent culture of IMR90 human lung fibroblast pseudo-colony, high-density culture of **(C)** hASCs and **(D)** IMR90 cells; **(E)** hASC-iPSC colony; **(F)** fibroblast-iPSC colony; and **(G)** H7 hESC colony. The black scale bar is 500 μm . hASCs, human adipose-derived stromal cells; hESC, human embryonic stem cell; iPSCs, induced pluripotent stem cells. Color images available online at www.liebertonline.com/ten.

edge. Within those regions, a 16×16 grid of points was sampled, resulting in 256 total points per region (Fig. 2A). It appeared that there was some variability in the cell-regional modulus within the colony itself. Cell regions that lacked constraints on all four sides due to their presence on the edge of the colony had a lower modulus than regions confined by cells on all sides within the inner region and at the center of colonies for both the hASC pseudo-colonies and the naturally formed hASC-iPSC colonies (Fig. 2B–D). This does suggest some contribution to the modulus from simple geometric considerations; however, overall the differences between the iPSCs and hASCs were far greater than the intracolony variability. Typically, the greatest degree of spontaneous cell differentiation (Fig. 2E) was seen at the colony periphery and this may be related to the changing stress states there.

Mechanical properties of fibroblasts, hASCs, hASC-iPSCs, fibroblast-iPSCs, and hESCs

Elastic moduli of fibroblasts, hASCs, hASC-iPSCs, fibroblast-iPSCs, and hESCs were acquired using an AFM. The moduli displayed a lognormal probability distribution (Fig. 3A). Cell types in order of increasing elastic modulus were hASC-iPSCs ($E = 0.9 \text{ kPa}$), hESCs ($E = 1.2 \text{ kPa}$), fibroblast-iPSCs ($E = 1.3 \text{ kPa}$), fibroblasts ($E = 3.5 \text{ kPa}$), and lastly hASCs ($E = 5.2 \text{ kPa}$) (Fig. 3B). These data suggest that pluripotent cells (both iPSCs) are more compliant than multipotent and unipotent cells, although the multipotent cell (hASC) is less compliant than the unipotent cell (fibroblast). Cells generally behave viscoelastically, meaning that they exhibit time-dependent strain in response to indentation. To compensate for viscous effects and represent mainly the elastic response of the

FIG. 2. Sampling regions of the cell colonies. **(A)** Schematic representing the sampling regimen for cell colonies and pseudo-colonies. Region a represents cells on the edge of the colony, region b represents the midpoint between the edge and center, and c is the center of the colony. Each scan region a, b, or c is composed of a 16×16 grid of probing points for a total of 256 sampled points within each region. **(B)** The heat maps for the various regions show that the cellular stiffness increases from the edge of each colony reaching a maximum value toward the center; black object at the left center is the atomic force microscope probe tip; white scale bar is 50 μm **(C)** The hASC pseudo-colonies showed similar relationships between edge and center cell moduli with the **(D)** hASC-iPSCs. **(E)** hASC-iPSC colony with differentiating cells preferentially at the periphery (black arrows). The black scale bar is 500 μm. Color images available online at www.liebertonline.com/ten.

cell to indentation, hASCs and iPSCs were probed at different indentation speeds ranging from 0.25 to 5 μm/s. The elastic modulus was monitored in response to the different indentation rates, and the corresponding apparent elastic modulus was extracted by the Hertz model. There was a consistent difference in the elastic modulus between hASCs and hASC-iPSCs at all indentation rates. There was also an increase in apparent modulus with increased indentation speed for both

cell types (Fig. 3C). It was found that indentation rates of 1 μm/s or less reduced the contribution of hydrodynamic drag on the cantilever while still being rapid enough to generate sufficient data points in a reasonable time frame. Therefore, indentation rates of 1 μm/s were used for all cell types to minimize viscoelastic effects. As a positive control the modulus of hASCs was compared to hASCs that were treated with CytoD to disrupt the actin cytoskeleton. As

FIG. 3. Cell stiffness results. **(A)** Force spectroscopy data showing representative lognormally distributed probability density functions for the moduli within regions of hASC-iPSCs, hASCs, Fib-iPSCs, fibroblasts, and hESCs colonies and pseudo-colonies. **(B)** Exhibited cell stiffness values, or elastic moduli, in order from least to most stiff: hASC-iPSC, hESC, fibroblast-iPSC, fibroblast, and hASC. Stromal cells (fibroblasts and hASCs) were stiffer than the pluripotent cells (hASC-iPSC, hESC, and fibroblast-iPSC). $^{\#}p < 0.05$ relative to hASCs and $*p < 0.05$ relative to IMR90 fibroblasts. **(C)** Apparent cell stiffness values extracted from different indentation speed experiments for hASC and hASC-iPSC. hASCs were relatively stiffer at all indentation rates. **(D)** hASC cell stiffness decreased after the application of actin inhibitor cytochalasin-D. $*p < 0.05$.

expected, hASCs were significantly stiffer than their CytoD-treated counterparts, suggesting that the major contributors to the measured elastic modulus were the actin filaments comprising the cytoskeleton (Fig. 3D).

Cytoskeletal differences between hASCs and hASC-iPSCs

Cell mechanics are highly mediated by the structural, or cytoskeletal, organization of the cell. Cells were stained for actin to compare cytoskeletal differences in cell mechanical properties of the most stiff (hASCs) and least stiff (hASC-

iPSCs) cells. In the hASC-iPSCs, actin was primarily found along the perimeter of the cell, whereas in the hASCs, actin was found throughout the interior of the cell (Fig. 4A–C). Microtubules were also stained to confirm the actin results (Fig. 4D–E). There appears to be many more actin filaments and microtubules qualitatively in the hASCs compared to the hASC-iPSCs, suggesting that the increased content of cytoskeletal components contributes to the higher cell stiffness. The apparent cell stiffness of hASCs was greatly reduced after application of actin inhibitor CytoD (Fig. 3D), further confirming the role of the cytoskeleton in the stiffness of the cell.

FIG. 4. Actin staining of hASC-iPSC colony and hASC pseudo-colony. **(A)** hASC-iPSC colony (edge indicated by dashed, white line), and **(B)** hASC pseudo-colony center and edge **(C)** stained for actin as an indicator of cytoskeletal organization and imaged using fluorescence microscopy. Actin was found primarily along the perimeter of the hASC-iPSCs, whereas actin was prevalent throughout the cell interior of the hASCs, at higher concentrations and at an even distribution, which may explain why hASCs were significantly stiffer than hASC-iPSCs. Similar results were seen in microtubule staining of **(D)** hASC-iPSCs and **(E)** hASCs. The scale bar is 30 μm . Color images available online at www.liebertonline.com/ten.

Discussion

We were able to show a significant difference in cell stiffness between human fibroblasts, hASCs, hASC-iPSCs, fibroblast-iPSCs, and hESCs. These findings have substantial implications in the cell mechanics of uni-, multi-, and pluripotent cells, and create the development of a possible biomarker for stem cells. Elastic modulus, E , was used as the measure of stiffness, with higher moduli corresponding with stiffer materials. Elastic modulus describes the stiffness of a material, in this case the cell, and its resistance to elastic deformation. Elastic deformation occurs when a material returns to its original shape immediately upon removal of the applied stress; in pure viscous behavior, deformation is delayed in response to an applied load and the material does not completely recover its original shape. Cells have characteristics of both, a property called viscoelasticity, in which an initial elastic response to a load is followed by viscous, time-dependent deformation. Viscoelasticity is a characteristic of an amorphous material (the cell) and describes the movement of the cell components with the cytoplasm. To determine the elastic moduli of these cells, AFM nanoindentation readings were taken within the elastic deformation range of the cell.

Cell stiffness increased in the following order: hASC-iPSCs, hESCs, fibroblast-iPSCs, fibroblasts, and hASCs. The relatively lower stiffness of the reprogrammed iPSCs may be

due to active remodeling of multiple components of the cells internal structure, such as the filaments of the cytoskeleton—actin filaments, intermediate filaments, and microtubules. The overall apparent compliance of the pluripotent cells may denote the cells' unrestrained ability to assume several cell types and the associated material properties. The relatively larger stiffness of the fibroblast cell is expected, as the fibroblast commonly composes the main cell type of connective tissues, which provide considerable structural support in many parts of the body. An interesting finding here was that hASCs, which are multipotent cells, were stiffer than the differentiated fibroblast cell. This may be due to the fact that hASCs are relatively smaller cells than fibroblasts (widths of ~ 15 vs. $\sim 30 \mu\text{m}$, respectively), which increases the actin filament density in the hASCs compared to the fibroblast, and in turn perhaps increasing the stiffness.

Using the AFM as an instrument to read cell stiffness is a powerful tool, though there are factors that have to be taken into consideration when using such a technique on cells. One important element is adhesion of the AFM probe tip to the cell itself. Inaccurate stiffness readings would result when adhesion forces dominated during probing. One possible reason for this phenomenon is the presence of excess Matrigel atop the cells, creating a sticky layer. If adhesion appeared to be a problem during readings, those results were discarded and new cells were seeded and re-probed.

Stem cells have the unique characteristic of preferentially forming colonies. To obtain consistent cell stiffness readings between cell types, the traditionally noncolony forming fibroblasts and hASCs were plated into a cloning ring to induce high-density, colony-like seeding. Although there was a difference in the cell density achieved in these noncolonizing cells (4×10^5 cells/cm² for both hASCs and human fibroblasts, versus 7×10^5 cells/cm² for hESC and both iPS cell types), this difference was not substantial enough to account for the large, significant difference in elastic modulus between cell types. Fibroblasts are inherently larger cells than the hESCs, iPSCs, and hASCs ($\sim 30 \mu\text{m}$ in width and $\sim 70 \mu\text{m}$ in length for fibroblasts, compared to $\sim 15 \mu\text{m}$ in diameter for the stem cells studied here). This affects the maximum density attainable with the pseudo-colonies of fibroblasts, hence the lower number of cells per given area in comparison.

To investigate the extent of the affect of boundary conditions, cells at the center and at the periphery of the colonies were probed for cell stiffness. In the hASC pseudo-colony and iPSC colony, cells at the edge of the colonies exhibit slightly lower elastic moduli. This may indicate that the cells in the center of the colonies receive some structural support from neighboring cells and their corresponding cytoskeleton organization, as opposed to cells at the edge that only receive support from a few sides (seen during actin staining).

In the current highly interdisciplinary research environment, an AFM is not an unlikely piece of equipment for any researcher. Tools such as the AFM may provide an informative method for simplifying various measures in stem cell and, overall, biological research. Finally, the mechanobiological properties of stem cells may lead to additional strategies to promote either dedifferentiation during reprogramming, or to differentiate stem cells for cell-based regenerative medicine.

Author Contributions

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Disclosure Statement

No competing financial interests exist.

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