Mechanical characterization of primary human mesenchymal stem cells via dual-beam optical stretching

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OVERVIEW

Optical stretching (OS) allows the mechanical behavior of whole tissue cells to be measured in suspension via interaction with dual unfocused laser beams. We are applying this technique to study primary adult human mesenchymal stem cells (hMSCs). Mechanical signatures could provide useful characteristics for studying and sorting these cells, enabling correlation with downstream mechanotransduction mechanisms. **Our research question is: can we identify and track characteristic mechanical markers of therapeutically useful hMSCs via optical stretching?**

Here we present the whole-cell deformability from a population of >1000 hMSCs as they are expanded *in vitro* to produce a therapeutically relevant quantity. We find that the mean time-dependent deformability is described well by an offset-power-law rheological model. **This experimental evidence for power-law behavior is the first to originate from a biomechanics measurement technique that does not involve physical contact with the cell.**

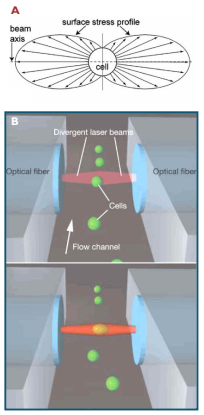

Figure 1. Optical stretching enables stiffness of adherent tissue cells to be evaluated in the absence of contributions from physical contact and direct influence of substratum chemomechanical properties. (**A**) Impinging photons create an outward surface stress on a cell membrane as they move from a medium of lower to higher refractive index; (**B**) tissue cells in suspension are positioned between two optical fibers and stretched one by one to build a data set of population mechanics [1,2].

Why deform *suspended* hMSCs?

1. Stem cells are often re-implanted in the suspended state; deformability could affect their movement through tissue.
2. Cells in suspension can be processed with higher throughput (similar to flow cytometry).
3. Suspension avoids factors like stress concentrations, adhesion site formation, and stress fibers that complicate other cell mechanics techniques.

APPROACH

Collection and preparation of human MSCs

Human MSCs were acquired from bone marrow aspirates and expanded on tissue culture polystyrene up to passage 8. For stretching experiments, cells at approximately 50% confluence were trypsinized, centrifuged, and resuspended at a cell density of 100-500 K/mL. The cells were injected into a hollow square glass capillary (80 μm ID, 160 μm OD) that was positioned between two single-mode optical fibers split from a 1064 nm fiber laser.

Optical stretching

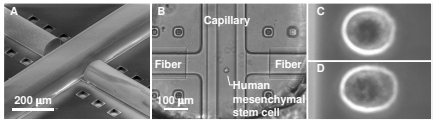

Figure 2. (**A**) Scanning electron micrograph and (**B**) phase contrast photograph of opposing optical fibers positioned to face a hollow glass capillary filled with cell suspension during operation. (**C**) and (**D**), human mesenchymal stem cell (hMSC) before and during irradiation.

We applied trapping power (0.2 W / 2 s), stretching power (0.9 W / 4 s), and trapping power (0.2 W / 2 s) per fiber to bring the cell to an equilibrium position; deform the cell; and allow recovery at the equilibrium position, respectively. The experiment was performed by stretching as many cells as possible in two hours; this duration was selected as a compromise to obtain a sufficiently large data set while minimizing the time these adherent cells spent in suspension at ambient temperature and uncontrolled pH.

ACKNOWLEDGEMENTS

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ADAPTATIONS ENABLING hMSC ANALYSIS

hMSC morphology presents challenges & opportunities

In the suspended state, hMSCs are larger than most cells previously analyzed by OS. While cells attached to substrata are spread out and feature prominent stress fibers in a state of mechanical tension, suspended cells lack stress fibers. In the suspended state, stiffness is largely dependent on an actin cortex just inside the plasma membrane [2]. OS can correlate morphological changes including blebbing with the mechanical response of hMSCs over time.

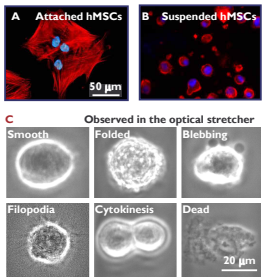

Figure 3. (**A**) Stress fibers (red) appear in attached hMSCs stained for actin; (**B**) Suspended hMSCs (fixed and adhered to poly-l-lysine-coated slides) have only an actin cortex. (**C**) We observe much variety in the morphology of suspended hMSCs, which exhibit folds, blebs, and/or filopodia and are sometimes undergoing cytokinesis when trypsinized. Cell appearance is divided approximately evenly between smooth, folded, and blebbing, with the other morphologies observed only occasionally.

Syringe rotation ensures cell availability

Syringe rotation counteracts cell sinking in the input syringe over time and increases the number of cells analyzed per experiment. Without syringe rotation, cell sinking (at an estimated terminal speed of 10 μm/s) resulted in few to no cells available after several tens of minutes due to syringe depletion. When using syringe rotation, however, the density of cells available for injection through the capillary to the trap is essentially unaffected over a 2 hr experiment.

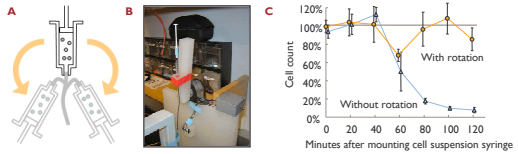

Figure 4. (**A**) Schematic and (**B**) photo of 6 RPM syringe rotator; (**C**) improvement in cell availability with syringe rotation.

RESULTS

Power law rheology discovered in suspended adherent cells by a non-contact technique

The average strain profile from all cells (n = 1246) was well fit with the fewest number of parameters by the equation $\varepsilon = At^{\alpha} + B$, where ε is strain, t is time, and A, α, and B are prefactor, exponent, and offset constants, respectively. **The fitted power-law exponents are identical within error for the creep (stretching) and recovery periods.** This finding complements experimental observations of power-law behavior for attached adherent cells [3-5], but without complications due to stress fibers, focal adhesions, and stress concentrations.

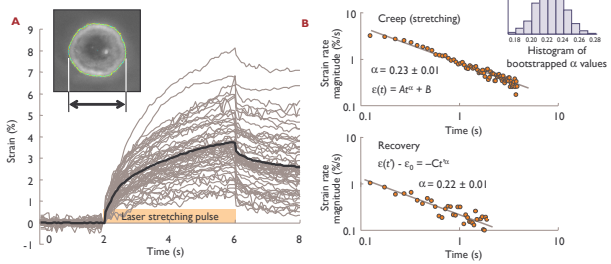

Figure 5. (**A**) From a representative passage of hMSCs (P3, three T-25 tissue culture flasks, 56 cells), individual and average strain in response to a step increase and decrease (0.2 W to 0.9 W to 0.2 W) in laser power per fiber at t = 2 s and 6 s. Inset, edge detection of cell shape via machine vision showing the dimension used as a metric for strain during irradiation. (**B**) Log-log plots of strain rate show a power law as a straight line slope of α-1 and demonstrate similar values of α for stretching and recovery. Inset, histogram of power-law exponents extracted via the statistical technique of bootstrapping, which allows parameter uncertainty to be estimated from large data sets (not necessarily Gaussian distributed) by the process of repeated sampling with replacement.

RESULTS (CONT'D)

hMSCs stiffen during first 90 min of detachment from substrate; recovery and power-law exponent unchanged

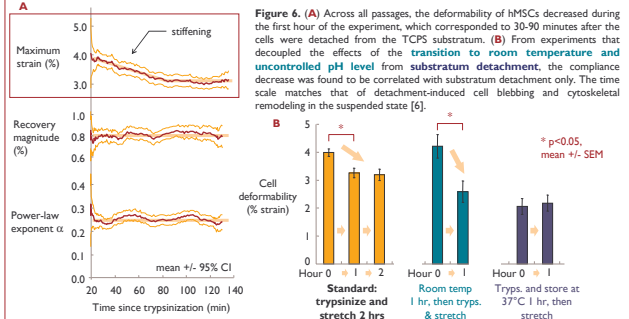

Figure 6. (**A**) Across all passages, the deformability of hMSCs decreased during the first hour of the experiment, which corresponded to 30-90 minutes after the cells were detached from the TCPS substratum. (**B**) From experiments that decoupled the effects of the **transition to room temperature and uncontrolled pH level** from **substratum detachment**, the compliance decrease was found to be correlated with substratum detachment only. The time scale matches that of detachment-induced cell blebbing and cytoskeletal remodeling in the suspended state [6].

hMSC deformability under load is gamma-distributed

Cell deformability has previously been universally fitted to a log-normal distribution [4, 5]. Our large data set allows us to reject the log-normal distribution in favor of the (similarly shaped but distinct) gamma distribution, which is known to describe certain populations undergoing stochastic properties. It is not yet clear whether this claim can be generalized to all cell rheology techniques and cells or whether it is restricted to optical stretching of hMSCs.

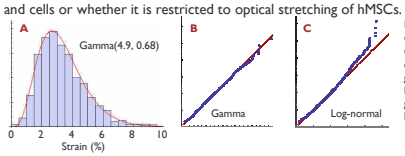

Figure 7. (**A**) Histogram of maximum strain during stretching, compared to a gamma distribution fit. (**B,C**) Probability plots comparing measured data to analytical gamma and log-normal distributions. The log-normal fit deviates at the tails, while the gamma fit better matches the data (straight line = correct fit).

Cytoskeletal inhibitors can modulate hMSC response

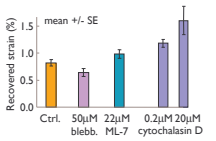

Figure 8. The addition of pharmacological cytoskeletal inhibitors can modulate hMSC mechanics as measured by optical stretching. Blebbistatin and ML-7, which inhibit myosin II and myosin light chain kinase, respectively, do not significantly alter the extent of recovery at these concentrations. Cytochalasin D, an inhibitor of actin polymerization, increases recovery in a concentration-dependent manner. Note that this recovery indicates mechanical consequences of drug-induced cytoskeletal modulation of the cell in its suspended state, in contrast to the well-studied dramatic changes accessible in the adherent state [7].

CONCLUSIONS

Here we show that adaptations of optical stretching can be implemented to enable mechanical characterization of dozens of hMSCs per 2 hr experiment and >1000 hMSCs overall. From this large data set we find power-law rheological behavior in suspended adherent cells upon creep and recovery and also obtain insight into the appropriate statistical analysis of hMSC populations. Ongoing work focuses on pharmacological modulation of hMSC structure and mechanics in the suspended state, including modulation toward downstream tissue lineages.

REFERENCES

1. J. Guck, R. Ananthakrishnan, H. Mahmood, et al., "The optical stretcher: a novel laser tool to micromanipulate cells," *Biophys. J.*, 81(2):767-784 (2001).
2. J. Guck, S. Schinkinger, B. Lincoln, et al., "Optical deformability as an inherent cell marker for testing malignant transformation and metastatic competence," *Biophys. J.*, 88(5):3689-3698 (2005).
3. B. Fabry, G.N. Maksym, J.P. Butler, et al., "Scaling the microrheology of living cells," *Phys. Rev. Letters*, 87:148102 (2001).
4. N. Desprat, A. Richert, J. Simeon, et al., "Creep function of a single living cell," *Biophys. J.*, 88(3):2224-2233 (2005).
5. S. Hiratsuka, Y. Mizutani, M. Tsuchiya, et al., "The number distribution of complex shear modulus of single cells measured by atomic force microscopy," *Ultramicroscopy* 109:937–941 (2009).
6. C.J. Harrison and T.D. Allen, "Cell surface morphology after trypsinisation depends on initial cell shape," *Differentiation*, 15(1-3) (1979).
7. W.R. Trickey, T.P. Vail, and F. Guilak, "The role of the cytoskeleton in the viscoelastic properties of human articular chondrocytes," *J. of Orthopaedic Research* 22:131-139 (2004).